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Tetrachromacy in a butterfly that has eight varieties of spectral receptors

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This paper presents the first evidence of tetrachromacy among invertebrates. The Japanese yellow swallowtail butterfly, *Papilio xuthus*, uses colour vision when foraging. The retina of *Papilio* is furnished with eight varieties of spectral receptors of six classes that are the ultraviolet (UV), violet, blue (narrow-band and wide-band), green (single-peaked and double-peaked), red and broad-band classes. We investigated whether all of the spectral receptors are involved in colour vision by measuring the wavelength discrimination ability of foraging *Papilio*. We trained *Papilio* to take nectar while seeing a certain monochromatic light. We then let the trained *Papilio* choose between two lights of different wavelengths and determined the minimum discriminable wavelength difference $\Delta\lambda$. The $\Delta\lambda$ function of *Papilio* has three minima at approximately 430, 480 and 560 nm, where the $\Delta\lambda$ values approximately 1 nm. This is the smallest value found for wavelength discrimination so far, including that of humans. The profile of the $\Delta\lambda$ function of *Papilio* can be best reproduced by postulating that the UV, blue (narrow-band and wide-band), green (double-peaked) and red classes are involved in foraging. *Papilio* colour vision is therefore tetrachromatic.

Keywords: colour vision; wavelength discrimination; spectral receptor; compound eye; ommatidium

1. INTRODUCTION

Two monochromatic lights appear differently coloured when their wavelength difference is larger than a threshold value, the minimum discriminable wavelength difference. The wavelength discrimination function $\Delta\lambda$ typically has minima or 'troughs' at particular wavelengths. These troughs usually are located in between the maxima of photoreceptor spectral sensitivities (Kelber *et al.* 2003). An analysis of the shape of the $\Delta\lambda$ function therefore allows us to make inferences about the number and spectral classes of photoreceptors used for wavelength discrimination.

In humans, very small $\Delta\lambda$ values, of approximately 1 nm, are found at approximately 500 and 600 nm (De Valois & Jacobs 1968). The existence of two such troughs is explained by the trichromacy of human colour vision, which is based on L-, M- and S-cones (Wandell 1995; Backhaus *et al.* 1998). The $\Delta\lambda$ function of dichromats exhibits only one trough (De Valois & Jacobs 1968). The colour vision system of honeybees (*Apis mellifera*) is also trichromatic, based on ultraviolet (UV), blue (B) and green (G) receptors (von Helversen 1972; Menzel & Backhaus 1989), and therefore their $\Delta\lambda$ function has two troughs, at approximately 400 and 500 nm (von Helversen 1972). The minimal $\Delta\lambda$ of honeybees, however, is approximately 4 nm, which indicates a coarser wavelength discrimination than that of humans. The colour vision of fishes, birds and turtles depends on four types of cone in their retina, with maximal sensitivity in the UV, B, G and red (R) wavelength regions (Goldsmith 1990; Neumeyer 1998). Their $\Delta\lambda$ functions accordingly have three troughs at approximately 400, 500 and 600 nm, where $\Delta\lambda$ is 2–3 nm (Neumeyer 1998).

Accumulating evidence indicates that many invertebrates have eyes with a large number of spectral receptor classes. The current champion is the mantis shrimp, whose eye has 16 different spectral receptor classes (Cronin & Marshall 2004; Cronin 2006). Butterflies also have a multiple receptor retina (Briscoe & Chittka 2001; Arikawa *et al.* 2004). For example, the eye of *Papilio xuthus* is known to contain at least six classes of receptors: UV, violet (V), B, G, R and broad-band (BB; Arikawa 2003; Kinoshita *et al.* 2006). The B and G classes can be further divided into two subclasses: narrow-band blue (NB) and wide-band blue (WB) for the B class (Kinoshita *et al.* 2006), and single-peaked green (SG) and double-peaked green (DG) for the G class (Bandai *et al.* 1992; Arikawa *et al.* 1999). Strictly speaking therefore, the eye of *P. xuthus* has at least eight different spectral receptors (figure 1).

The spectral receptors are embedded in ommatidia, the building blocks of compound eyes. The eye of *Papilio* is composed of approximately 12 000 ommatidia, each containing nine photoreceptors, R1–9. Although the basic structure of the ommatidia is identical, detailed anatomical studies revealed that there are at least three

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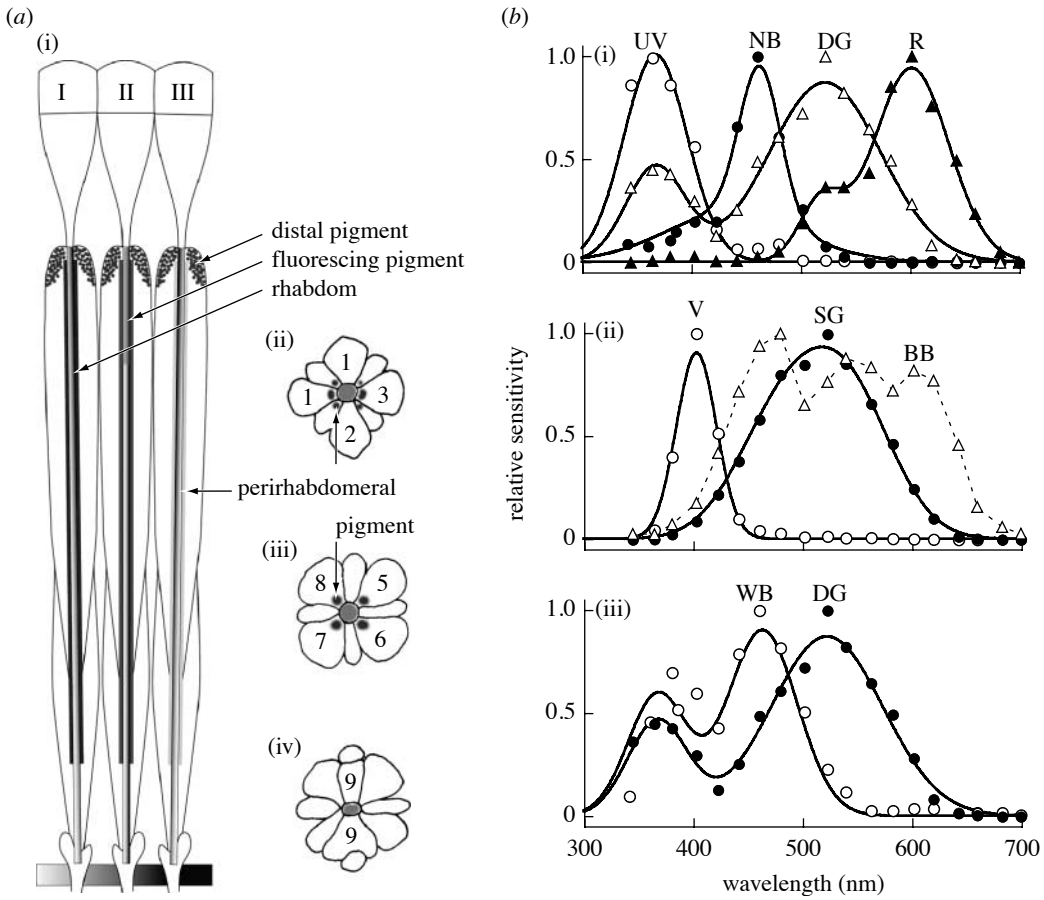

Figure 1. Three types of ommatidia in the compound eye of *P. xuthus*. (*a*) Scheme of the ommatidium: (i) longitudinal and (ii–iv) transverse sectional views (right). Each ommatidium contains nine photoreceptor cells (1–9). Type I, II and III ommatidia are distinguishable by characteristic pigmentation. (*b*) Spectral sensitivities of photoreceptors contained in each type of ommatidium: (i) type I, (ii) type II, and (iii) type III. Solid lines indicate approximated spectral sensitivity curves used in the calculations. Symbols are electrophysiological data. BB cells (dashed line) were not used for the model calculation.

ommatidial types, each with different combinations of spectral receptors. The ommatidia of types I, II and III bear four (UV, NB, DG, R), three (V, SG, BB) and two (WB, DG) receptor classes, respectively (figure 1). DG receptors can be distinguished into three anatomically distinct subgroups, R3–4 of type I, R3–4 of type III and R5–8 of type III (table 1). The spectral sensitivities of the first two subgroups are slightly different (Kinoshita *et al.* 2006), and the spectral properties of the R5–8 receptors of type III ommatidia are not yet characterized in detail. We therefore provisionally regard the three subgroups of DG receptors as physiologically identical.

Do *Papilio* butterflies use all spectral receptor classes for colour vision (Kinoshita *et al.* 1999)? Some may not participate in colour vision but rather serve for other visual functions, such as spatial and motion vision. Here we address this question by measuring the $\Delta\lambda$ function of foraging *Papilio*. We first trained *Papilio* to take nectar, by extending the proboscis while seeing a certain monochromatic light. We then showed two monochromatic lights, one of which the trained butterfly preferred by pointing to it with the extended proboscis. We thus determined the $\Delta\lambda$ value for various wavelengths and found that the $\Delta\lambda$ function has three troughs. Model calculations indicate that the $\Delta\lambda$ function is based on four receptor classes, i.e. the UV, B (NB+WB), G (DG) and R, meaning that the wavelength discrimination system of *Papilio* is tetrachromatic. Note that the excluded receptors, the V, SG and BB, are all included in type II ommatidia, indicating that this type of ommatidia are not involved in colour vision, at least in the relevant behaviour. In addition, the minimum $\Delta\lambda$ value of *Papilio* is approximately 1 nm, which is the best among animals studied so far and comparable to that of humans.

Table 1. Three types of ommatidia in the *Papilio* eye. (The spectral sensitivities of R9 cells are predictions based on Arikawa & Uchiyama (1996).)

	ratio (%)	photoreceptors				
		R1	R2	R3, R4	R5–R8	R9
type I	50	UV	NB	DG	R	R?
type II	25	V	V	SG	BB	R?
type III	25	WB	WB	DG	DG	DG?

2. MATERIAL AND METHODS

(a) *Animals*

We used newly emerged, spring-form females of the Japanese yellow swallowtail butterfly, *P. xuthus*, reared in the laboratory. The laboratory culture was derived from eggs laid by females caught in Kanagawa, Japan. The hatched larvae were fed on fresh citrus leaves under a light regime of 10 hour light : 14 hour dark at 28°C. The pupae were stored at 4°C for at least three months and allowed to emerge at 25°C. The day of emergence was defined as the post-emergence day-1.

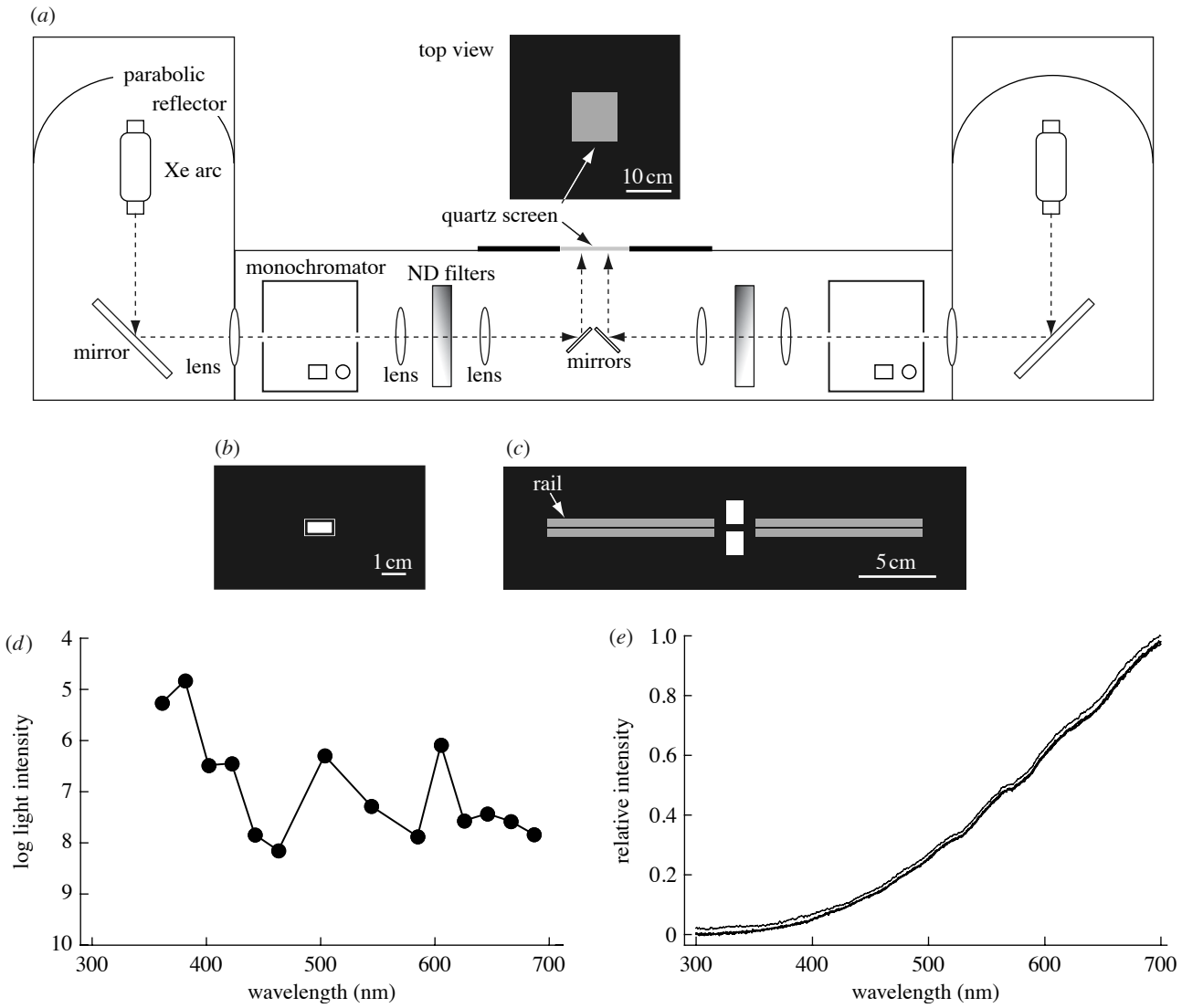

Figure 2. Experimental set-up for presenting monochromatic lights. (*a*) Light from a Xe arc was directed into a monochromator via a mirror. The monochromatic light, whose intensity was attenuated with ND filters, was reflected upwards to a quartz screen. (*b*) A single-windowed plate used to cover the screen during training sessions. (*c*) A double-windowed plate used to cover the screen during test sessions. Tethered butterflies were slid via two rails towards the windows from either side. (*d*) Action spectrum of the PER of *Papilio*. Light intensities required for eliciting 80% response were plotted against the wavelength. Modified from Koshitaka *et al.* (2004). (*e*) Spectrum of the room illumination.

(b) *Experimental set-up*

Behavioural experiments were performed on a bench ($W \times D = 160 \times 50$ cm^2) in a room at 28 ± 1°C. The bench top was covered with a black plate (figure 2*a*, inset), with a square hole (10×10 cm^2) in the centre. The hole was covered with a piece of frosted quartz glass, which served as a transparent screen. The bench top was illuminated by halogen lamps with luminosity 1000–1200 lux.

Monochromatic stimuli (360–680 nm) were provided by a 500 W xenon arc through a monochromator (Shimadzu SPG-120S, Tokyo, Japan) and a set of quartz neutral density filters (Sanso, Tokyo, Japan; figure 2*a*). In order to present two monochromatic stimuli simultaneously, we arranged two identical light beams beneath the bench. The monochromatic lights were reflected upwards to the quartz transparent screen from below. The lights were thus observable from above (figure 2*c*). The photon flux of each monochromatic light was measured at the screen surface with a radiometer (Model 470D, Sanso, Tokyo, Japan).

The behavioural experiments consisted of a training and a test session as described in the next section. During the training sessions, the quartz screen was covered with a black plastic plate with a hole (1.0×1.5 cm^2) lined with a piece of thin UV-transparent plastic plate (8.0×9.5 cm^2; single-windowed plate, figure 2*b*): the quartz screen was thus covered with the extra UV-transparent plastic plate. The plate has a gutter of 1 mm width along the inner hole in the black plate. This complicated arrangement was necessary to keep the sucrose solution reward at the edge of the window and to prevent spreading the nectar over the window. During the test sessions, the quartz screen was covered with a black plastic plate (9×30 cm^2) with two 1.5×2.0 cm^2 windows separated by a 15 mm gap, and two rails each pointing towards the windows from opposite directions (double-windowed plate, figure 2*c*). No reward was provided on the double-windowed plate.

(c) *Behavioural experiments*

Flower visit and proboscis extension are two consecutively occurring events during foraging behaviour in the field. Here, we used the proboscis extension as a measure of the wavelength discrimination ability. The wings of the butterflies were clipped together with a piece of cardboard. In the test sessions, we

moved the tethered butterflies towards the window by sliding on the bench top. We defined that the 'proboscis extension response (PER)' occurred when the butterfly spontaneously extended its proboscis and searched for nectar by probing around the window with the proboscis.

(i) *Training*

We started the training on post-emergence day-2. During the training sessions, we used a single-windowed plate. Each individual was trained at one wavelength; we used 21 wavelengths (360, 370, 380, 390, 400, 410, 420, 430, 440, 460, 480, 500, 520, 540, 550, 560, 570, 580, 600, 620, 640 nm, half bandwidth = 7.97–12.43 nm). We first brought a tethered butterfly close to the window illuminated with a certain wavelength of light, which was the training wavelength of the individual. There we fed the butterfly with 8% sucrose for 3–5 s, by manually extending the proboscis with a needle, and we continued the feeding process until the butterfly spontaneously recoiled the proboscis. We repeated the training typically for 10 days. Successful individuals performed PER by the second day of training. We used 533 butterflies, 101 of which were successfully trained.

(ii) *Test*

For the tests, we illuminated two windows separately with two independent light beams (figure 2). One of the windows was always illuminated with the wavelength used for the training (training light), while the other was illuminated either with the training light or with the light of a different wavelength (test light). The wavelengths of the test lights were 0, 1, 2, 5, 10 and 20 nm longer or shorter than the training wavelength. Using neutral density filters, the intensity of the two lights was adjusted so that they had the same brightness, calculated from the action spectrum of PER of *Papilio* (Koshitaka *et al.* 2004). We selected intensities that elicit PER in 80% of the tests (figure 2*d*), because this intensity was bright enough for most individuals to respond to the light as well as to recognize the difference in colour appearance, if any. We brought the tethered butterflies towards the two windows by sliding along the rail. The butterflies were thus allowed to see the two windows side by side. In order to cancel the effect of stimulus positions, we randomly changed the direction of approach by using two rail sets on opposite sides (figure 2*c*). The number of choices that each butterfly made in a test was in the range of 5–10.

When the butterflies did not perform PER towards the stimuli, we checked whether they retained their feeding motivation by presenting the training light at maximum intensity and giving a small amount of reward using the single-windowed plate. Only with such a motivation check, could we determine whether the butterflies recognized the stimuli. In addition to the motivation check, we fed the butterflies with a small amount of nectar once every three or four responses while showing the training light of maximum intensity using the single-windowed plate. This was important for maintaining their motivation throughout the tests. If the butterflies did not get any reward during the test sessions, even though they answered correctly, they readily learned that they never got any reward and stopped responding.

(d) *Model calculations*

To relate the shape of the $\Delta\lambda$ function to the receptor spectral sensitivities, we used a 'receptor noise-limited colour opponent model' (Vorobyev & Osorio 1998). This model is based on the assumption that visual thresholds are set by noise originating in the photoreceptors and that achromatic (intensity-based) cues are not used for colour discrimination. The model estimates a perceptual distance between the stimuli ΔS. When ΔS is less than a threshold distance, the stimuli corresponding to different wavelengths cannot be discriminated. This condition allows us to theoretically predict the $\Delta\lambda$ function. Without loss of generality, the threshold distance can be assumed to be equal to 1: in this case, the perceptual distance is measured in terms of the just noticeable distance.

In the case of tetrachromatic vision, the distance between stimuli is given as follows:

$$(\Delta S)^2 = \frac{(\omega_1\omega_2)^2(\Delta f_4-\Delta f_3)^2+(\omega_1\omega_3)^2(\Delta f_4-\Delta f_2)^2+(\omega_1\omega_4)^2(\Delta f_3-\Delta f_2)^2}{(\omega_1\omega_2\omega_3)^2+(\omega_1\omega_2\omega_4)^2+(\omega_1\omega_3\omega_4)^2+(\omega_2\omega_3\omega_4)^2} + \frac{(\omega_2\omega_3)^2(\Delta f_4-\Delta f_1)^2+(\omega_2\omega_4)^2(\Delta f_3-\Delta f_1)^2+(\omega_3\omega_4)^2(\Delta f_2-\Delta f_1)^2}{(\omega_1\omega_2\omega_3)^2+(\omega_1\omega_2\omega_4)^2+(\omega_1\omega_3\omega_4)^2+(\omega_2\omega_3\omega_4)^2}, \tag{2.1}$$

where Δf_i is the difference between the signals of a photoreceptor i and ω_i is the noise of receptor mechanism i. To describe the receptor signals f_i for stimuli that differ significantly from the adapting light, we used a logarithmic relationship between the receptor quantum catches q_i and the receptor signals (Vorobyev *et al.* 1998). The logarithmic model is a mathematical formulation of Weber's law, and for this model ω_i is equivalent to the Weber fraction of receptor mechanism i. The logarithmic model predicts that for any two stimuli ΔS does not depend on the intensity of these stimuli. However, the logarithmic model has an obvious limitation—it cannot be used for stimuli producing a zero quantum catch in one of the photoreceptors, because a logarithm of 0 is equal to negative infinity. To overcome this limitation, we assume that the f_i are given as follows:

$$f_i = \ln(1+q_i), \tag{2.2}$$

where ln is the natural logarithm and q_i is the normalized quantum catch of a photoreceptor i. Because the receptor noise-limited colour-opponent model assumes that signals that differ only in intensity from the background produce a zero colour opponent signal, receptor sensitivities must be normalized to background light, i.e. to the room illumination (figure 2*e*). For monochromatic light of wavelength λ and intensity I_0, the quantum catches are given as follows:

$$q_i(\lambda) = I_0 k_i P_i(\lambda), \tag{2.3}$$

where $P_i(\lambda)$ is the spectral sensitivity of photoreceptor i and $k_i = c/\int P_i(\lambda)I(\lambda)\mathrm{d}\lambda$. Here, $I(\lambda)$ is the light intensity distribution as a function of wavelength λ, and parameter c is chosen so that the k value for R (red) receptors k_R equals 1 ($k_\mathrm{R}=1$). We further assume that the intensity of the monochromatic stimuli I_0 is equal to 1, which implies that lights are not discriminated on the basis of intensity differences.

Because the threshold distance is set to 1, the minimum discriminable wavelength difference $\Delta\lambda$ can be found from the condition

$$\Delta S(\Delta\lambda) = 1. \tag{2.4}$$

For small $\Delta\lambda$

$$\Delta f_i = \frac{\mathrm{d}f_i}{\mathrm{d}\lambda}\Delta\lambda. \tag{2.5}$$

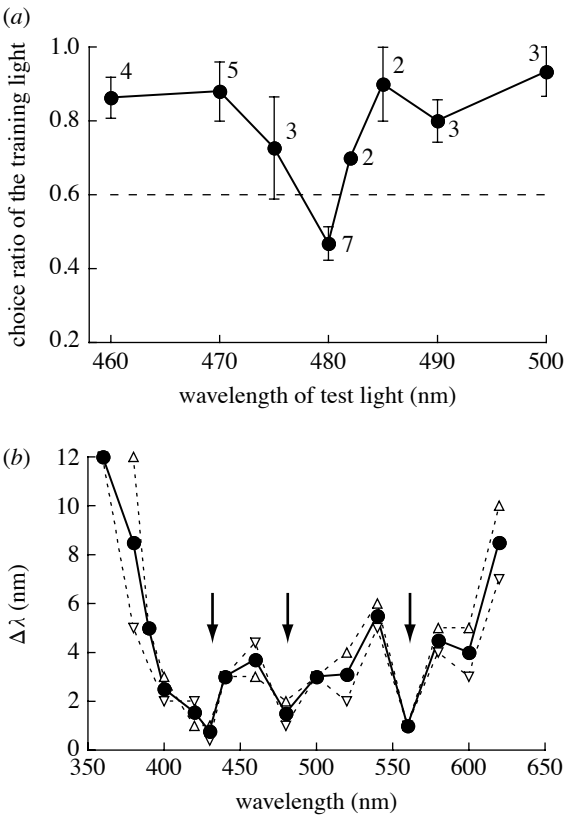

Figure 3. Wavelength discrimination. (*a*) Example of wavelength discrimination at 480 nm training light. The numbers at the data points indicate the number of butterflies tested at the wavelengths ($n=7$). Dashed line indicates the 60% criterion. Error bars are represented as mean$\pm$s.e. (*b*) The $\Delta\lambda$ function of foraging *Papilio*. The curve exhibits three high-discrimination regions (arrows) at 430, 480 and 560 nm, respectively. Up triangle, long wavelength side; down triangle, short wavelength side; circle, average.

Table 2. Parameters used in equation (2.8) to approximate receptor spectral sensitivities.

	A	λ^0	δ	B	λ^1	σ
UV	1.00	367.7	29.0	0	—	—
V	0.90	403.2	18.4	0	—	—
NB	0.73	462.9	18.7	0.24	436.8	60.2
WB	0.90	463.6	31.8	0.59	367.8	27.1
SG	0.88	541.5	41.0	0.55	47.9	41.1
DG	0.89	522.5	51.2	0.46	367.5	27.1
R	0.93	601.5	34.4	0.29	519.2	20.1

Substitution of equations (2.4) and (2.5) into equation (2.1) gives the minimum discriminable wavelength difference

$$\Delta\lambda = \sqrt{\frac{(\omega_1\omega_2\omega_3)^2 + (\omega_1\omega_2\omega_4)^2 + (\omega_1\omega_3\omega_4)^2 + (\omega_2\omega_3\omega_4)^2}{(\omega_1\omega_2)^2\left(\frac{df_4}{d\lambda} - \frac{df_3}{d\lambda}\right)^2 + (\omega_1\omega_3)^2\left(\frac{df_4}{d\lambda} - \frac{df_2}{d\lambda}\right)^2 + (\omega_1\omega_4)^2\left(\frac{df_2}{d\lambda} - \frac{df_3}{d\lambda}\right)^2 + (\omega_3\omega_4)^2\left(\frac{df_1}{d\lambda} - \frac{df_2}{d\lambda}\right)^2}} \tag{2.6}$$

$$\frac{df_i}{d\lambda} = \frac{k_i}{1 + k_i P_i}\frac{dP_i}{d\lambda}. \tag{2.7}$$

To calculate the derivatives of the photoreceptor spectral sensitivities $P_i(\lambda)$, the sensitivities were approximated as sum of two Gaussian functions

$$P_i(\lambda) = A_i \exp\left(-\frac{(\lambda-\lambda_i^0)^2}{2\delta_i^2}\right) + B_i \exp\left(-\frac{(\lambda-\lambda_i^1)^2}{2\sigma_i^2}\right), \tag{2.8}$$

where A_i, B_i, λ_i^0, δ_i, λ_i^1, and σ_i are parameters (see table 2) whose values were adjusted to provide a least-squares approximation of measured spectral sensitivities using the 'FindFit' procedure in MATHEMATICA v. 5 (Wolfram). Equation (2.8) reasonably well approximates the measured sensitivities (figure 1*b*).

To estimate ω_i, we used a method previously applied to model the behavioural spectral sensitivity of some animals (Vorobyev & Osorio 1998). Because the signal-to-noise ratio can be improved by the summation of signals of individual photoreceptors, the relative noise level is inversely proportional to the number photoreceptors of a given spectral type $\omega_i = \nu_i/\sqrt{\eta_i}$, where ν_i is the noise level of a single photoreceptor and η_i is the number of receptors of a type i. We assume that different cells have similar levels of noise and set the noise of the R receptor to 0.05, i.e.

$$\omega_i = 0.05\sqrt{\frac{\eta_R}{\eta_i}}. \tag{2.9}$$

The choice of the noise value for the R receptor to be 0.05 does not follow from actual measurements, but it is a value used in previous theoretical studies of animal vision (Vorobyev 2003; Schaefer *et al.* 2007). The number of photoreceptors of each type is based on the actual distribution of receptors in the eye shown in table 1.

3. RESULTS

(a) *Behavioural test*

Among the 21 wavelengths used for training, we could test individuals trained at 16 wavelengths: 360, 380, 390, 400, 420, 430, 440, 460, 480, 500, 520, 540, 560, 580, 600 and 620 nm. No individuals were successfully trained at 370, 550 and 570 nm and therefore could not be tested at these wavelengths. A few individuals were trained at 410 and 640 nm, but these individuals did not survive long enough to be tested.

We presented two light stimuli to the trained individuals, one at the training wavelength (training light) and another at a slightly different wavelength (test light). The wavelength of the test lights was changed in the range of -20 to $+20$ nm away from the training wavelength. Using the action spectrum of PER (figure 2*d*), we adjusted the intensities of the training and the test lights to an equal *Papilio*-subjective brightness.

Figure 3*a* shows a typical result obtained from individuals trained at 480 nm. The abscissa indicates the test light wavelength, whereas the ordinate is the choice ratio of the training light. When the two lights were at the same wavelength, at 480 nm in this case, the choice ratio was of course approximately 0.5. When the test light wavelength was 20 nm longer or shorter, the choice ratios were approximately 0.9, indicating that the butterflies clearly discriminated the two lights. As the wavelength difference became smaller, the choice ratio approached to 0.5, forming the typical V-shaped graph.

We assumed that butterflies discriminated two wavelengths when the correct choice ratio was larger than 0.6 (Giurfa *et al.* 1996; Takeuchi *et al.* 2006). We thus measured the width of the V-shaped graph at 0.6 choice

ratio (figure 3*a*) and plotted the values against the training wavelengths to draw a $\Delta\lambda$ function (figure 3*b*).

The $\Delta\lambda$ function exhibited three troughs at 430, 480 and 560 nm, respectively. At these wavelengths, butterflies discriminated a wavelength difference of only 1 nm. The $\Delta\lambda$ values strongly increase in the wavelength regions below 400 and above 600 nm.

(b) *Analysis of the $\Delta\lambda$ function and comparison with model predictions*

The *Papilio* retina is furnished with eight varieties of spectral receptors: UV, V, NB, WB, SG, DG, R and BB (table 1). It is unlikely that the BB receptor contributes to the wavelength discrimination, because its spectral sensitivity is practically flat in the blue–red region of the visible wavelength range. The remaining seven receptors belong to the following five classes: UV, violet (V), blue (NB and WB), green (SG and DG) and red (R). If these five classes were used for wavelength discrimination, the $\Delta\lambda$ function would have four troughs located between the peaks of photoreceptors (for review, see Kelber *et al.* 2003). We observe only three troughs, located between the peaks of the R and G, the G and B, the B and V or UV receptors (figure 3*b*). This may indicate that either the UV receptor or the V receptor does not contribute to wavelength discrimination.

To further investigate the contribution of the different photoreceptor types, we calculated the $\Delta\lambda$ function using a model (Vorobyev & Osorio 1998; Vorobyev *et al.* 1998). The parameters of this model, that is, the levels of noise in the photoreceptor channels, are fixed because we assumed that the noise is determined by the numbers of receptors of each type, and the R receptor noise was fixed at the value used in previous theoretical analyses of colour discrimination (see §2).

Figure 4*a–d* shows four examples of calculation. With the UV, V, NB+WB, SG+DG and R receptors, the curve has four troughs and therefore does not fit at all to the behavioural results (figure 4*a*). Exclusion of the UV receptor eliminates one trough located at the shortest wavelength, but the curve does not match especially in the short wavelength region (figure 4*b*). Removal of the V receptor instead of the UV receptor gives a reasonable fit (figure 4*c*). Further removal of the SG receptor improved the fit slightly (figure 4*d*).

4. DISCUSSION

(a) *Discrimination based on chromatic cues*

Wavelength discrimination is the ability to discriminate monochromatic lights using chromatic but not achromatic cues. Here, we presented two lights of different wavelengths whose intensities were adjusted according to the behavioural spectral sensitivity of *Papilio* (figure 2*d*). This method has been used in some animals to minimize the effect of discrimination via the light intensities (De Valois & Jacobs 1968; von Helversen 1972; Fratzer *et al.* 1994; Neumeyer 1998), although the method does not necessarily exclude achromatic cues (Kelber *et al.* 2003). On the other hand, because we know that a number of diurnal animals ignore intensity cues when tested with stimuli subtending relatively large visual angles (Brandt & Vorobyev 1997; Giurfa & Vorobyev 1998; Vorobyev & Osorio 1998), $\Delta\lambda$ functions likely reveal the properties of chromatic mechanisms irrespective of the method of adjusting the intensities. In addition, the present model, which excludes achromatic cues (see §2), well explains the behavioural spectral sensitivities of some diurnal species (Brandt & Vorobyev 1997; Giurfa & Vorobyev 1998; Vorobyev & Osorio 1998). Therefore, the fact that the model well fits to the *Papilio* $\Delta\lambda$ function suggests that *Papilio* discriminates monochromatic lights predominantly on the basis of chromatic cues.

(b) *Evidence for UV vision*

The present results clearly show that colour vision of *Papilio* includes the UV wavelength region. Although it is generally accepted that most insects can see UV, because their eyes are furnished with UV receptors (Menzel & Backhaus 1991), conclusive evidence can only be obtained from behavioural tests. Together with our previous measurement of the action spectrum of PER in *Papilio* (figure 2*d*), we conclude that foraging *Papilio* can use UV light as a signal from nectar sources.

(c) *Comparison of $\Delta\lambda$ functions*

Figure 4*e* shows $\Delta\lambda$ functions of honeybees (von Helversen 1972), goldfish (Neumeyer 1986) and humans (De Valois & Jacobs 1968) together with our present result of *Papilio*. Clearly, different animals have different $\Delta\lambda$ values. The smallest $\Delta\lambda$ values are found in humans and *Papilio*. The main cause of this difference is the difference in the criterion for discrimination: we used a 60% criterion, whereas von Helversen (1972) and Neumeyer (1986) both used a 70% criterion. In fact, applying a 60% criterion reduced the $\Delta\lambda$ values of honeybees and goldfish by 25 and 35%, respectively. Still, the smallest $\Delta\lambda$ value of honeybees at 490 nm is 3 nm, and that of goldfish at 500 nm is 2.6 nm, so the wavelength discrimination ability of *Papilio* is best among the animals tested so far. We have to note, however, that the measured $\Delta\lambda$ values will also depend on some experimental conditions, such as the bandwidths of the monochromatic lights, the stimulus sizes and the applied light intensities (Eskew *et al.* 1999). In fact, von Helversen (1972) and Neumeyer (1986) used interference filters, which may have resulted in larger $\Delta\lambda$ values than if they had used a monochromator supplying quasi-monochromatic light.

(d) *First tetrachromacy among invertebrates*

The colour vision of honeybees is known to be trichromatic, as it is based on a set of UV, B and G receptors (Menzel & Backhaus 1989). This well explains the $\Delta\lambda$ function with two troughs at approximately 400 and 500 nm (figure 4*e*), which correspond to the regions where the spectral sensitivities of the UV and B receptors, and B and G receptors overlap. The $\Delta\lambda$ function of goldfish has three troughs, at approximately 400, 500 and 600 nm, indicating that their colour vision system is tetrachromatic, based on a set of UV, B, G and R receptors (Neumeyer 1998). The $\Delta\lambda$ function of *Papilio* also has three troughs, at approximately 420, 480 and 560 nm, suggesting that four spectral classes of photoreceptors contribute. This is in fact the first clear evidence of tetrachromacy among invertebrates.

Which four out of the eight different spectral receptors (figure 1) are involved here? We first excluded the BB class, because its sensitivity covers almost the entire spectrum, from 450 to 650 nm (Arikawa *et al.* 2003). The trough at 420 nm is probably produced by the

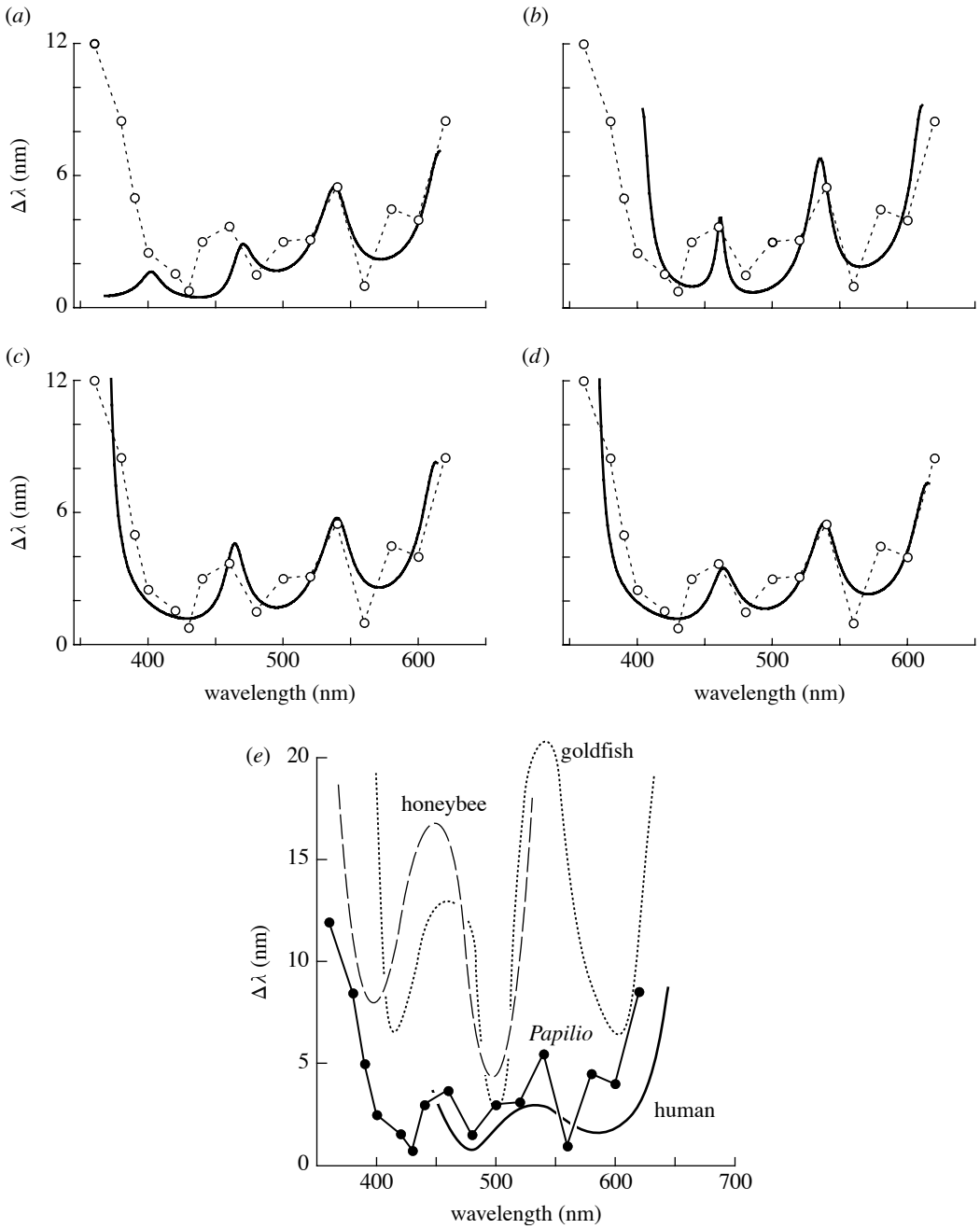

Figure 4. Model calculations and (*a–d*) and comparison of Δλ functions (*e*). Dotted lines and solid lines indicate the present behavioural data and model predictions, respectively in (*a–d*). (*a*) All spectral classes except for the BB are involved. (*b*) Like (*a*), but the UV receptor was removed. (*c*) Like (*a*), but the V receptor was removed. (*d*) Like (*c*), but the SG receptor was removed. (*e*) The Δλ functions of human (solid line; De Valois & Jacobs 1968), goldfish (dotted line; Neumeyer 1986), honeybee (dashed line; von Helversen 1972) and foraging *Papilio*.

combination of either UV/B or V/B. Model calculations show that a better fit can be obtained by eliminating the V receptor but not the UV receptor (figure 4*b*,*c*).

Note that the receptors that can be excluded—the BB and V receptors—are located exclusively in type II ommatidia (figure 1; table 1). The type II ommatidia also contain SG receptors. Interestingly, the model calculations using a combination of UV, NB+WB, DG and R receptors, so excluding the contribution of the SG receptor to wavelength discrimination, provide the best fit to the experimental Δλ function (figure 4*d*). This suggests that type II ommatidia are not involved in wavelength discrimination behaviour. We have to note that the Δλ function could be different when determined by using a behaviour other than foraging. At any rate, the present study provides an important clue for analysing the neuronal mechanisms underlying the colour vision of *Papilio*, and also shows how one might begin to understand the diversity of spectral receptor types in arthropod compound eyes including those of the well-studied *Drosophila* and stomatopods.

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