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POLARISATION-DEPENDENT COLOUR VISION IN *PAPILIO* BUTTERFLIES

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Summary

Butterflies of the genus *Papilio* have polarisation-sensitive photoreceptors in all regions of the eye, and different spectral types of receptor are sensitive to different e-vector orientations. We have studied the consequences of this eye design for colour vision in behavioural tests and find that *Papilio* spp. see false colours due to the polarisation of light. They discriminate between vertically and horizontally polarised light of the same colour in the contexts of oviposition and feeding. The discrimination depends on the spectral composition of the stimuli. In the blue and probably in the green range, discrimination does not depend on intensity. However, colour discrimination is influenced by polarisation. Thus, colour and polarisation processing are not separated in the visual system of *Papilio* spp. From these results, we propose hypotheses about which photoreceptors contribute to colour vision in *Papilio* spp. and what adaptational value such a system might have for the butterflies. Finally, we give examples for other eyes that have a similar structure.

Key words: colour vision, polarisation vision, butterfly, *Papilio* spp., Lepidoptera, vision.

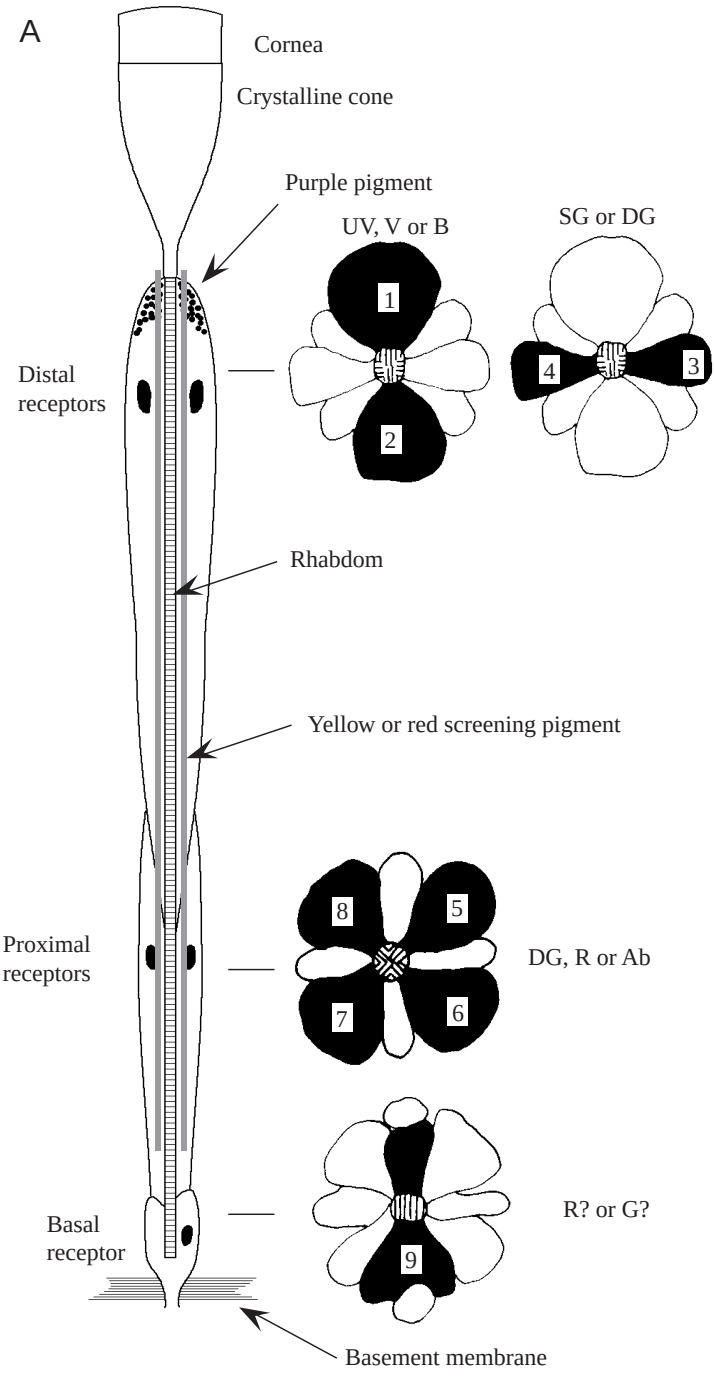
Introduction

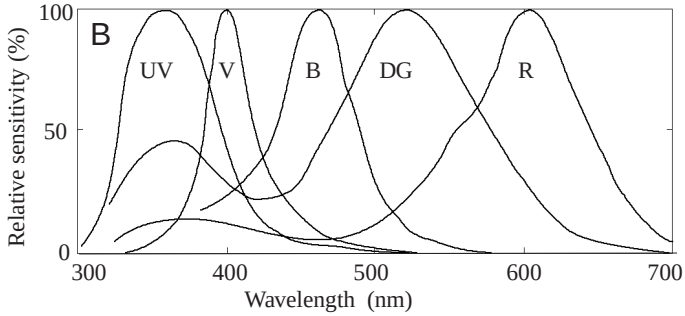
False colours due to polarisation – false polarisation due to colour

Light has three qualities: phase, wavelength (or frequency) and e-vector orientation or polarisation angle. Phase is not used by any animal, as far as we know, but both wavelength and polarisation angle are known to be exploited by a variety of animals. The wavelength spectrum of light is used predominantly for object detection and recognition. The best understood use of polarisation is the skylight compass of insects such as bees, crickets, flies and ants (Labhart and Meyer, 1999), but there are also many polarisation cues on earth: unpolarised light reflected by a non-metallic mirror-like surface becomes, to a certain degree, linearly polarised. The polarisation angle (δ) and the degree of polarisation depend on the microtexture of the surface, on the direction of the incoming light and on the spatial orientation of the reflecting surface. Generally speaking, the polarisation angle is parallel to the reflecting surface and perpendicular to the plane of incidence of the light. Horizontal surfaces therefore reflect horizontally polarised light (δ=90 °). Water-living insects use this to find ponds (Schwind, 1991). The light reflected by vertical surfaces is polarised vertically (δ=0 °) or obliquely (0 °<δ<90 ° or 90 °<δ<180 °) depending on the direction of the illuminating light and the angle of view.

To preserve a maximal amount of information, the intensity, the wavelength spectrum and the polarisation angle of light should be analysed independently. If an animal is able to discriminate two lights using the wavelength spectrum independently of stimulus intensity, it is said to have true colour vision (e.g. Menzel, 1979). If an animal can discriminate between two stimuli by means of the polarisation angle independently of stimulus intensity, it is said to have true polarisation vision (Nilsson and Warrant, 1999). For true colour vision, an animal needs at least two spectral types of receptors; for true polarisation vision, it needs at least two polarisation types of receptors. The polarisation receptors are characterised by the polarisation preference angle ϕ to which they are most sensitive and by the polarisation sensitivity, the ratio of the sensitivity to light polarised at angle ϕ to the sensitivity to light of the same intensity but polarised at ϕ+90 °.

For polarisation vision and colour vision to be independent, spectral receptor types should be insensitive to polarisation or share the same polarisation preference angle ϕ and the same polarisation sensitivity; polarisation receptors should be insensitive to colour, i.e. have equal spectral sensitivities. This is the case in the eyes of many insects that are able to use both colour and polarisation. They possess an eye region with several spectral types of receptor that are insensitive or only weakly sensitive to polarisation, and a different eye region with two polarisation types of receptor with different polarisation preference angles ϕ but with the same spectral sensitivity (e.g. Wehner and Bernard, 1993; Labhart and Meyer, 1999). If colour and polarisation processing were not separated in the way described above, there would be a false-colour problem due to polarised light and a false-polarisation problem due to coloured light (as described by Wehner and Bernard, 1993).





The eye of butterflies of the genus Papilio*: anatomy and physiology*

In each ommatidium of their apposition eyes, butterflies of the genus *Papilio* have nine photoreceptors (R1–R9) that combine spectral and polarisation sensitivities in a complicated manner (Fig. 1; Arikawa et al., 1987; Arikawa et al., 1999; Arikawa and Uchiyama, 1996; Bandai et al., 1992; Kitamoto et al., 2000). The receptors are arranged in three tiers. Two of the four distal receptors (R1 and R2) are maximally sensitive to vertically polarised light (ϕ=0 °) around 360 nm (ultraviolet or UV receptors), 400 nm (violet or V receptors) or 460 nm (blue or B receptors), and the other two distal receptors (R3 and R4) are maximally sensitive to horizontally polarised light (ϕ=90 °) of 520 nm (green or G receptors). All four proximal receptors (R5–R8) and the basal receptor (R9) can be maximally sensitive at 520 or 600 nm (red or R receptor). R5–R8 have oblique microvilli orientations and, thus, ϕ is 35 or 145 °, and R9 is maximally sensitive to vertically polarised light (ϕ=0 °). R1, R2 and R9 are long visual fibres terminating in the second optic neuropil, the medulla, whereas the other receptors are short visual fibres terminating in the first optic neuropil, the lamina (Matic, 1983; Ribi, 1987; Bandai et al., 1992; Arikawa and Uchiyama, 1996).

Since three physiologically characterised receptor types, the UV, V and B receptors, share only two anatomical positions, it is obvious that not all ommatidia possess a complete set of spectral types. In addition, the absorption spectra of the visual pigments are greatly modified by longitudinal and lateral filtering, and several receptors contain two pigments and, thus, have a broader sensitivity (A in Table 1; Kitamoto et al., 1998). There are therefore three distinct types of ommatidium rather randomly distributed over the retina, each with a different subset of receptor types (Table 1; Kitamoto et al., 2000).

We do not yet know how these ommatidia are used for vision. It is possible that the different types of ommatidium serve different visual subsystems such as motion vision, polarisation vision and colour vision. In that case, type 1 ommatidia (see Table 1) would be the best candidates for the colour vision system. They contain all except the V (and the broadband A) receptors. The distal receptors have

Fig. 1. (A) Diagram of an ommatidium of *Papilio* spp. (modified from Kitamoto et al., 1998). The ommatidium contains nine photoreceptor cells R1–R9. Photoreceptors R1–R4 are distal photoreceptors that contribute the rhabdomeral microvilli to the distal two-thirds of the rhabdom. Photoreceptors R5–R8 are proximal photoreceptors forming the rhabdom in the proximal one-third of the ommatidium. The basal photoreceptor, R9, contributes to the rhabdom at the region immediately distal to the basement membrane. UV, ultraviolet receptor; V, violet receptor: B, blue receptor; G, green receptors (two subtypes: SG, single-peaked green receptor; DG, double-peaked green receptor); R, red receptor; A, abnormally broadband receptor sensitive in the red and green ranges of the spectrum. (B) Relative sensitivities of five spectral types of photoreceptor.

Table 1. *Receptor types and characteristics of three different types of ommatidium randomly distributed in the retina of* Papilio xuthus

		Type 1		Type 2		Type 3	
Pigmentation:		Red		Red		Yellow	
Fluorescence:		No		Yes		No	
Photoreceptor	ϕ (degrees)	Colour type	PS	Colour type	PS	Colour type	PS
R1	0	UV	1.3–1.5	V	2	B	2
R2	0	B	1.3–1.5	V	2	B	2
R3, R4	90	DG	2?	SG	2	DG	2
R5, R7	35	R	2	A	2	DG	2
R6, R8	145	R	2	A	2	DG	2
R9	0	R or G	2	R or G	2	R or G	2

R1–R9, photoreceptors.

UV, ultraviolet receptor; V, violet receptor; B, blue receptor; G, green receptor; SG, green receptor missing a beta peak; DG, green receptor with a beta peak; R, red receptor; A, abnormally broadband receptor sensitive in the red and green range of the spectrum (still presumptive at this stage). For details on the pigments see Kitamoto et al. (2000).

ϕ, polarisation angle to which the receptor is maximally sensitive (0 ° is vertical, 90 ° is horizontal); PS, polarisation sensitivity calculated as the sensitivity to light polarised at angle ϕ divided by the sensitivity to light polarised at ϕ+90 °.

Data on polarisation sensitivities are taken from Bandai et al. (1992) and Arikawa and Uchiyama (1996).

curved microvilli and thus probably reduced polarisation sensitivity. Alternatively, it can be assumed that only a subset of receptors in all ommatidia serve colour vision, whereas the others are used for motion vision. Since the long visual fibres may constitute the colour vision system in flies (Strausfeld and Lee, 1991), this possibility has also been proposed for *Papilio xuthus* (Arikawa and Uchiyama, 1996). Two further arguments strengthen this hypothesis: if several ommatidia are considered, then these three receptors contain all five spectral types of photoreceptor and could possibly allow pentachromatic colour vision. Second, all long visual fibres have vertically oriented microvilli, and this would make colour vision independent of polarisation (see Wehner and Bernard, 1993). Arikawa et al. (Arikawa et al., 1999) suggested that R3 and R4, being green-sensitive in all ommatidia, would be useful for spatial vision, whereas R1 and R2 (being UV, V or B receptors) could constitute the colour vision system together with the red- or green-sensitive R5–R8.

Behavioural context of colour vision in Papilio *spp.*

Butterflies are thought to use colour in a variety of behavioural contexts: mate choice (Hidaka and Yamashita, 1975), feeding and oviposition. Both the Australian orchard butterfly *Papilio aegeus* and the Japanese yellow swallowtail *Papilio xuthus* use colour vision to detect and recognise food sources (Kelber and Pfaff, 1999; Kinoshita et al., 1999). Even though no direct tests for the dimensionality of colour vision have been performed, we can deduce from training experiments that they use at least three types of photoreceptor for colour discrimination: the B, G and R receptors (Kelber and Pfaff, 1999). Female *Papilio aegeus* lay eggs on the underside of the shiny leaves of Rutaceae plants. The choice of an oviposition substratum is partially guided by visual cues. In laboratory experiments with coloured paper, a stimulus is preferentially chosen when it provides a high green receptor quantum catch and low blue and red receptor quantum catches (Kelber, 1999b). It has recently been shown that, in the context of oviposition, polarisation cues can influence the choice behaviour, and it has been proposed that butterflies do experience false colours as a result of the polarisation of light (Kelber, 1999a).

In the present report, we provide further evidence for this finding. We have tested the influence of polarised light on choice behaviour in the contexts of oviposition and feeding by asking three questions. (i) Do butterflies have separate polarisation and colour vision pathways or does polarisation influence the colour vision system? (ii) If the latter is the case, does polarisation change the colour or the perceived intensity of light? (iii) Which receptors are involved? We will discuss the possible adaptive value of a combined polarisation-dependent colour vision system and other examples of animals in which polarisation and colour vision are not separated.

Materials and methods

Experimental design

Experiments were performed on free-flying butterflies, *Papilio aegeus* (oviposition tests) and *P. xuthus* (feeding tests). The general experimental design was similar in both experiments; we refer to the differences in separate sections. Animals were tested for colour and polarisation preferences in dual-choice tests. Two vertically oriented windows (4 cm×4 cm wide in feeding tests, 5 cm×5 cm in oviposition tests) in a black plate were evenly illuminated from behind. The light passed through a matt screen, a coloured filter, in some experiments through a neutral density filter and, finally, in most experiments through a polarisation filter. The animals approached the apparatus and chose one of the stimuli. Each approach resulted

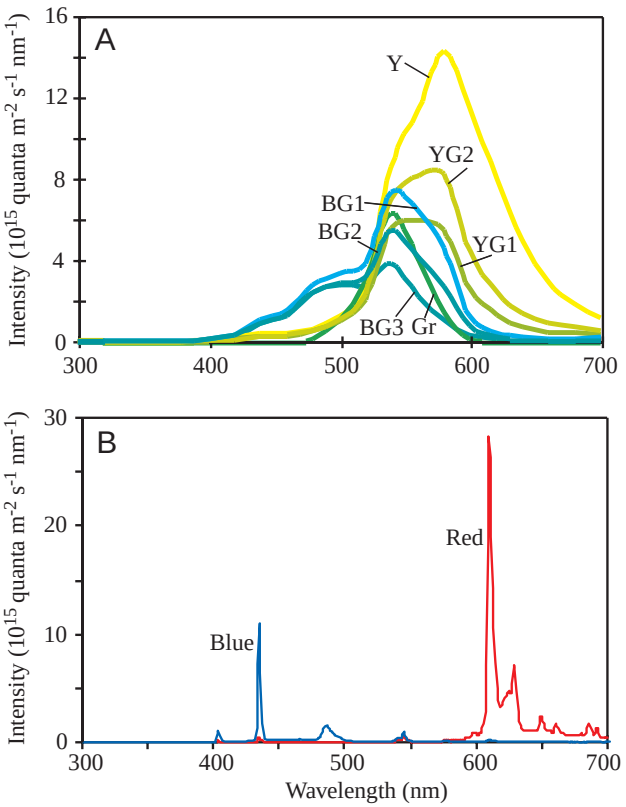


Fig. 2. The spectral distribution of the stimuli used in oviposition experiments (A) and in feeding experiments (B). Y, Gr, YG1 and YG2, BG1–BG3 are the abbreviations used for the colours in all further figures and the text.

in only one registered choice and counted as an independent data point. *G*-tests were used to determine whether choice frequencies differed significantly from random (with William's correction; Sokal and Rohlf, 1981).

The spectral distributions of all stimuli were measured using calibrated spectrophotometers (Ocean Optics, S1000 and S2000) and are given in Fig. 2. From these data and the known spectral and polarisation sensitivities of *Papilio* receptor types (Fig. 1B; Matic, 1983; Ribi, 1987; Bandai et al., 1992), we calculated the receptor quantum catches Q_i for each receptor type and stimulus using equations 1 and 2:

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

where λ is wavelength, $I(\lambda)$ is the spectral distribution of the stimulus light, $R(\lambda)$ is the spectral sensitivity of the receptor (see Fig. 1B) and $d\lambda$=10 nm. C corrects for the polarisation sensitivity of the receptor and is given by:

$$C = 1 - [1 - (1/PS)]\sin^2(\delta - \phi) , \quad (2)$$

where PS is the polarisation sensitivity ratio of the receptor in question (see Table 1), δ is the polarisation angle of the stimulus and ϕ is the polarisation angle to which the receptor responds maximally (see Table 1).

Oviposition experiments on P. aegeus *females*

For oviposition tests, caterpillars of *Papilio aegeus* were collected in Canberra, Australia, during the southern summer of 1998 and fed on leaves of *Choysia mexicana*. The adult females and their offspring were kept in an indoor cage (3 m×2 m×2 m) illuminated from above by six Osram Universal White tubes and six Osram Cool White light tubes under long-day conditions (14 h:10 h light:dark). In this cage, the animals fed from Petri dishes marked with blue or red tape and mated. The experiments were conducted on females that had not seen or come into contact with the larval food plant as adults. Usually, approximately 10 females were present during an experiment, but only approximately five of them approached the stimuli.

The stimuli were placed close to the feeders, and the lamp was switched on. Mated females approached and drummed with their forelegs on the colour filters, a behaviour performed only in the context of oviposition (Scherer and Kolb, 1987). Sometimes, a female curled her abdomen and eventually laid an egg on or close to one of the coloured filters before flying away to rest on the cage wall. Maximally, one drumming, curling and oviposition event occurred following one approach. Not all drumming events resulted in curling and oviposition. Animals were allowed to make multiple approaches during experiments since no influence of experience on the choice behaviour could be detected (Kelber, 1999b) and each choice was considered to be an independent event. Eggs were removed immediately.

All three reactions, drumming, abdomen curling and egg laying, were counted during experiments. Choice frequencies were very similar for all three reactions (see Fig. 3). Drumming was the first behavioural response to the stimuli and was examined in most experiments. Tests with different pairs of stimuli followed in a pseudo-random order, and stimuli were presented equally often in the right and left positions.

Four types of dual-choice test were performed: tests with lights of the same spectral distribution and intensity but differing in polarisation angle; tests with unpolarised lights of different colours (see Fig. 2A for spectral distributions and intensities); tests with two unpolarised lights of the same spectral distribution but different intensities; tests with two stimuli differing in both colour and polarisation angle.

It was assumed that oviposition is driven by a linear interaction between receptors. The model is described elsewhere in some detail (Kelber, 1999b), but the important facts are summarised in the Appendix. In brief, it is assumed that a linear interaction between the B, G and R receptors accounts for the choice behaviour and that a logit function accounts for the sigmoid nature of the choice behaviour. For modelling, the receptor quantum catches and the choice frequencies in dual-choice tests were used. The optimal model parameters were calculated using a least-squares algorithm. The model was used only to describe the response of butterflies to colour. It was not possible to test models based on the receptor characteristics in the three ommatidia types separately. For this purpose, exact data on all spectral and

polarisation sensitivities of all receptors in all ommatidia types would be required as well as a huge number of behavioural tests.

Feeding experiments on P. xuthus

Both sexes of the spring form of the Japanese yellow swallowtail butterfly *Papilio xuthus* L. were used for training. The pupae were grown on *Citrus* leaves in Yokohama, Japan, brought to Lund, Sweden, and stored at 10 °C until needed. The butterflies eclosed at 24 °C and were kept in a large flight cage (280 cm×160 cm×195 cm) under a 15 h:9 h light:dark regime. The cage was illuminated with 25 Osram Biolux light tubes from the sides and from above.

Animals were marked individually on the day of emergence and trained in a dual-choice situation to associate food (10 % sugar solution) with one stimulus (red or blue horizontally or vertically polarised light) and no reward with the other stimulus. The sugar solution was presented in a small container in front of the positive stimulus, whereas the container in front of the negative stimulus was empty. The training stimuli differed only in the polarisation angle of light. Each individual was allowed to feed twice every day, with the stimuli and feeder being rearranged between feedings to exclude position learning. Unrewarded tests were started after several days of training. No sugar water was present, and the positions of the two stimuli were altered in pseudo-random order.

During tests, the stimuli were either identical to the training stimuli or reduced in intensity by means of

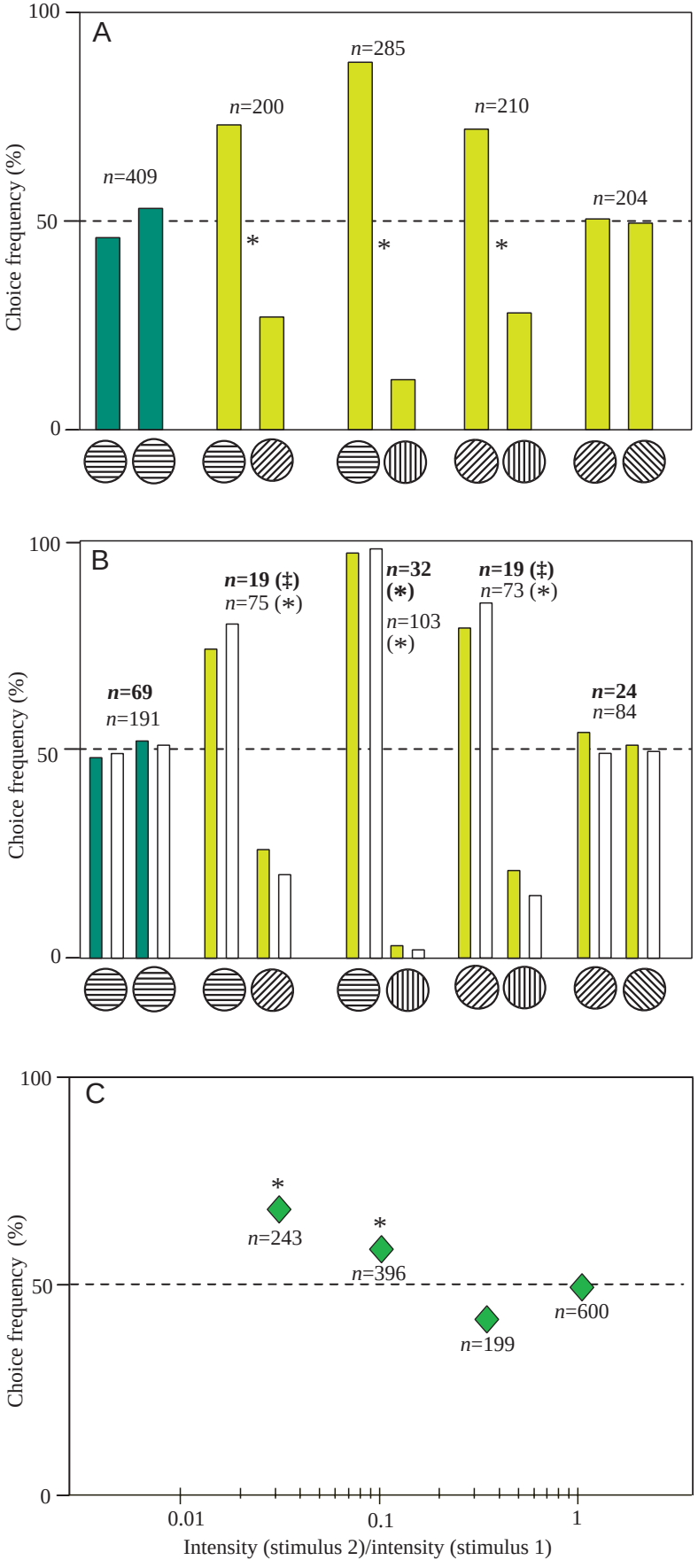


Fig. 3. Results of oviposition tests. (A) Choice distributions of the drumming reactions for different pairs of stimuli. Both stimuli of a pair had the same colour and intensity; YG2 was used in most tests, but BG1 was used in the left-hand test. Symbols on the abscissa show the polarisation angle of stimuli; from left to right: both stimuli horizontally polarised (δ=90 °), δ=90 ° *versus* δ=45 ° (obliquely polarised light), δ=90 ° *versus* δ=0 ° (vertically polarised light) and so on. *n* is the number of choices in each test. Asterisks mark choice distributions that differ significantly from chance (*G*-tests, $P<0.001$); the remaining choice distributions do not differ from chance ($P>0.1$). (B) Choice distributions of curling reactions (open columns) and egg-laying (coloured columns) during the same tests as in A. Bold values of *n* above the columns give the total number of eggs, values of *n* in normal type give the total number of curling reactions in each experiment. Asterisks for the respective behavioural reactions are as in A; double daggers mark choice distributions where *n* was too small to obtain a significant result ($P<0.1$, binomial test); unmarked choice distributions to not differ from chance (*G*-tests, $P>0.5$). (C) Choice frequencies of drumming reactions for the brighter of two unpolarised stimuli with equal spectral distribution. The colour Gr was used for the spectral distribution (see Fig. 1A). Asterisks are as in A.

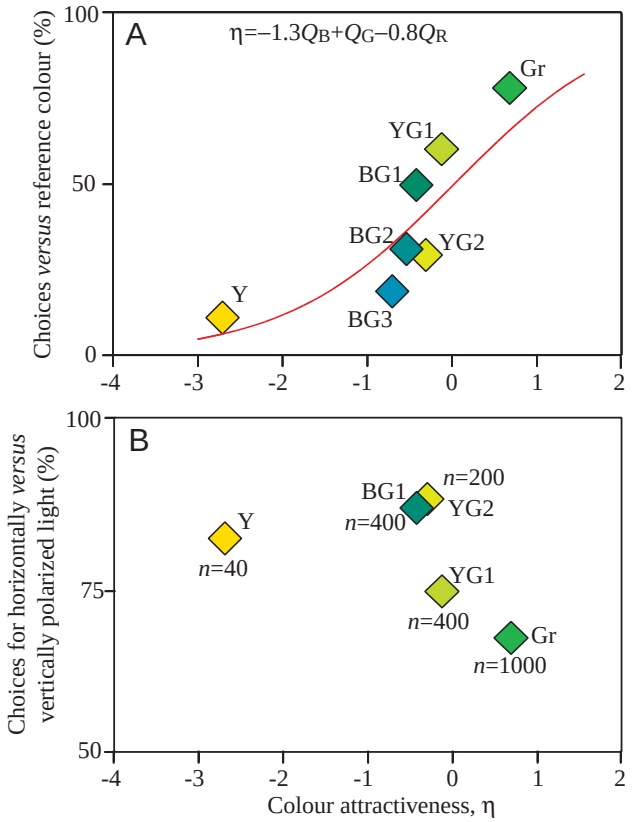


Fig. 4. Results of oviposition tests. (A) Choice frequencies for seven colours *versus* the reference colour BG1 (see Fig. 2A); 200 choices for each colour. The abscissa gives the attractiveness η of a colour, and the equation for the underlying receptor interaction is given; Q_B, blue receptor quantum catch; Q_G, green receptor quantum catch; Q_R, red receptor quantum catch. The red line is the fitted model curve. For further explanation, see text and Appendix. (B) Choices for horizontally polarised light *versus* vertically polarised light of the same colour and intensity as a function of colour attractiveness. *n* is the number of choices in each experiment, all choice distributions differ significantly from chance (*G*-tests, $P<0.001$).

neutral density filters (absorbing 70 % or 80 % of the light). Finally, the butterflies were also tested for their polarisation preference with stimuli of a colour that differed from the training colour. Animals were tested individually, and only one test was performed each day. Each landing in front of a stimulus followed by a proboscis extension towards the screen or the feeder was counted as a choice. Five choices were recorded from each animal during each test. The butterflies usually made these five visits within 1 min. The number of visits to the positive and negative stimuli was recorded for each individual butterfly. The test with the training stimuli was always presented first. Six animals that only chose the stimulus in either the right or left position in this test, independently of colour, were excluded from the data set and not tested further. Not all animals survived or were cooperative until all the tests had been performed, hence the smaller number of animals in some of the later tests. No behavioural differences were detected between males and females.

Results

Oviposition

Female *P. aegeus* were tested with two stimuli of the same green colour and the same intensity but different polarisation angles. They spontaneously preferred horizontally ($\delta=90°$) polarised light over vertically ($\delta=0°$) or obliquely ($\delta=45°$ or 135 °) polarised light of the same colour and obliquely polarised light over vertically polarised light (for the drumming reaction see Fig. 3A). Choices for two stimuli of equal polarisation angle did not differ from random (Fig. 3A). Animals did not discriminate between light polarised at 45 ° or 135 ° (Fig. 3A). For all three measured behavioural reactions, the choice distributions were similar. Fig. 3B gives the data for the curling reactions and egg-laying.

The strength of the preference for horizontally *versus* vertically polarised light depended on colour. In tests with a narrow-band green stimulus (Gr in Fig. 2), the animals chose the horizontally polarised light in only 67 % of choices (not shown, $N=1000$, $P<0.001$), whereas with a bluish (BG1) or yellowish (YG2) green, more than 80 % of the choices were for the horizontally polarised light (Fig. 3A, Fig. 4B). Obviously, the relative choice frequency for vertically and horizontally polarised lights depends on the colour of the stimulus. For this reason, we also tested for colour preferences (Fig. 4A).

Unpolarised light of each of seven colours was presented together with a reference colour (BG1). In Fig. 4A, choice frequencies are shown as a function of colour attractiveness η. A generalised linear model (see Appendix) was used to find an optimal model on the basis of receptor quantum catches for each colour. Since the UV and V receptors are not sensitive to the spectral range of the stimulus lights, they were excluded from the modelling procedure. The optimal model was the B-G-R model, the result of which is shown in Fig. 4A (red line). Models assuming an influence of only two spectral types of receptor did not describe the data adequately. Colours that induce a high quantum catch in the green receptor but low quantum catches in the blue and red receptors are highly attractive. This finding confirms earlier results (Kelber, 1999b). Fig. 4B shows that there is a tendency for the polarisation preference to be stronger in unattractive than in attractive colours.

The coloured stimuli used in the colour preference tests differed not only in spectral distribution but also intensity (Fig. 2). Did the relative choice frequencies differ because of the different intensity or because of the different spectral distribution of stimuli? We tested the butterflies with unpolarised light of different intensities but with the same spectral distribution (Gr). In these tests, the animals did not show a clear preference for either the brighter or the darker of two stimuli (Fig. 3C). Even when one stimulus was darker by a factor of 20, it received approximately 30 % of the choices. Note that the yellow stimulus (Y in Fig. 2A and Fig. 4A) was chosen less frequently than the green stimulus (Gr in Fig. 2A and Fig. 4A) even though it had a higher intensity at all

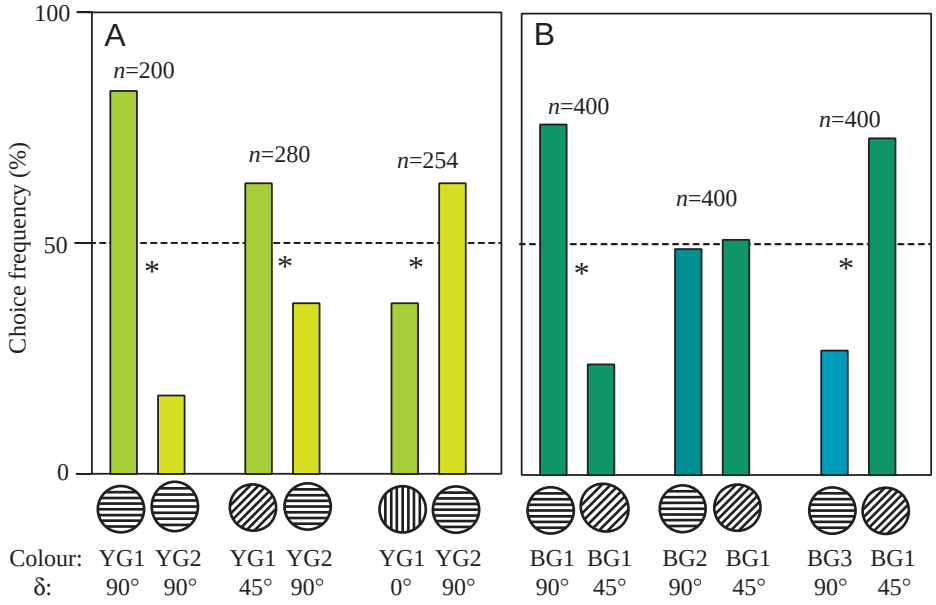


Fig. 5. Results of oviposition tests. (A) In three different tests, the same two colours were presented with the same or differing polarisation angles (δ). (B) In three tests, stimuli with 45° and 90° polarisation angles were presented, either both of the same colour or of different colours. In both sets of tests, choices depend on both colour and polarisation. *n* is the number of choices in each test; asterisks mark choice distributions that differ significantly from chance (*G*-test, $P<0.001$).

wavelengths. This provides further evidence that the animals did not choose the stimulus of highest intensity.

The butterflies could discriminate between stimuli differing in either colour or polarisation angle. To determine how strongly these two cues influence choice behaviour, both colour and polarisation were varied in two test series. In the first series, a pair of colours (YG1 and YG2) was presented with different combinations of polarisation angle. When both colours were presented with the same polarisation angle of 90°, there was a strong preference for YG1 (Fig. 5A, left pair of columns). The preference was weaker when YG1 had an oblique polarisation angle (45°); when YG1 was presented with a vertical polarisation angle (0°), the choice frequency for the other colour (YG2) was higher (Fig. 5A, right pair of columns). In the second series, one stimulus always had a polarisation angle of 45° and the same colour (BG1), and the second stimulus was always horizontally polarised, but varied in colour. When the colours of both lights were the same (left pair of columns), the horizontal polarisation angle was chosen, as before. When we changed the colour of the horizontally polarised light by introducing blue filters (BG2 and BG3), the choices became random (BG2) or animals preferred the obliquely polarised light (BG3) (Fig. 5B).

Feeding

Animals trained to associate vertically polarised blue light with food strongly preferred this stimulus in unrewarded tests with the training stimuli (Fig. 6A). This preference did not change even when either the rewarded or the unrewarded stimulus was reduced in intensity (Fig. 6B). Animals trained to vertically polarised red light had a weaker, but still significant, preference in tests in which both stimuli had equal light intensity, whereas animals trained to horizontally polarised red light did not learn the stimulus (Fig. 6A). Therefore, we show the choices for the vertical polarisation direction rather than the positive stimulus in Fig. 6B. In all animals trained to red stimuli, the choice behaviour depended strongly on intensity (Fig. 6B): when the intensity of the rewarded stimulus was reduced, it was chosen less frequently and *vice versa*.

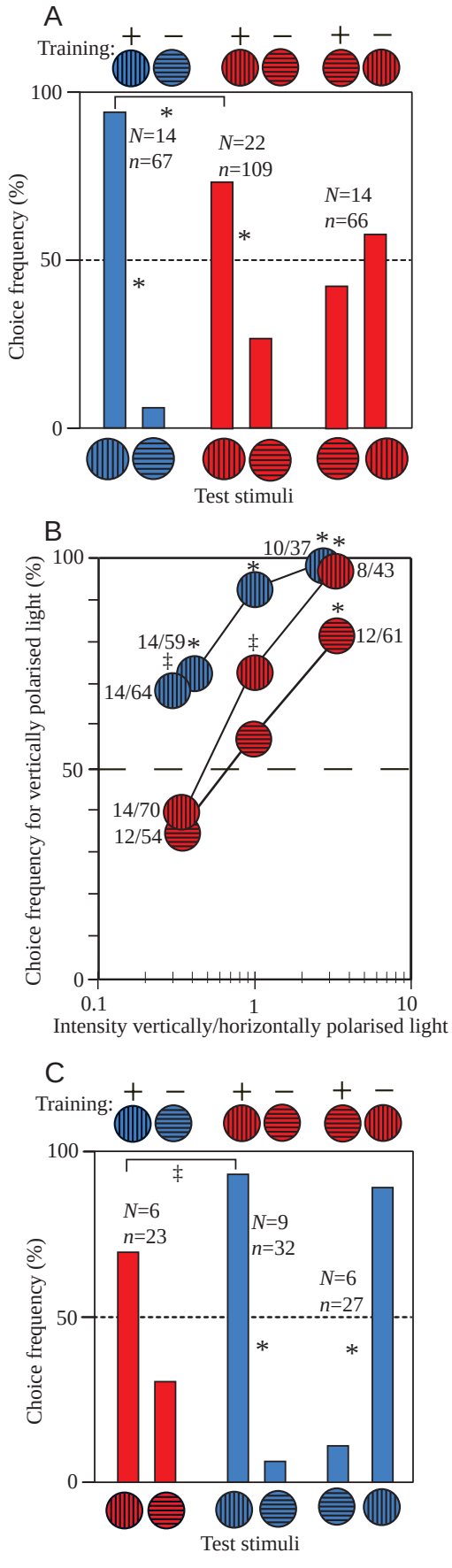
The outcome of tests with a colour different from the colour of the training colour is shown in Fig. 6C. Both groups of red-trained animals preferred the blue vertically polarised stimulus to the blue horizontally polarised stimulus. Interestingly, the preference for vertically polarised light was as strong as it had been in the blue-trained animals (compare Fig. 6C with Fig. 6A). Animals trained to blue vertically polarised light chose red vertically polarised light in 70% of cases (Fig. 6C), again a choice frequency similar to that of the animals that had been trained to this (red, vertically polarised) stimulus.

Discussion

Papilio spp. butterflies can easily discriminate between two polarised lights that differ only in their angle of polarisation. They do so for both oviposition and feeding; hence, we assume that the same photoreceptors are involved in both behaviours. Do the butterflies use separate sets of photoreceptors for polarisation and colour vision?

Do separate colour and polarisation vision systems exist?

For flies, it has been suggested that the central photoreceptors, R7 and R8 (the long visual fibres), provide input to the colour vision system (Strausfeld and Lee, 1991). Adopting this view for butterflies, photoreceptors R1, R2 and R9 would be used for colour vision (Arikawa and Uchiyama, 1996). If the butterflies used only these photoreceptors for colour vision, they would see an intensity difference and no colour or polarisation difference between horizontally and vertically polarised lights.



Two sets of photoreceptors could be suitable for a true polarisation vision system: the green receptors R3–R9 in type 3 ommatidia, which are sensitive to 0°, 35°, 90° and 145° polarisation angles, or the red receptors R5–R9 in type 1 or type 2 ommatidia (see Table 1). Both alternatives would allow discrimination between polarisation angles of 45° and 135°. Butterflies did not discriminate between these angles in the context of oviposition (Fig. 3). For a polarisation-sensitive system involving R or G receptors, we would expect best discrimination in the red or green range and bad discrimination in the blue range. The opposite is the case (Fig. 6). Thus, colour influenced the discrimination of stimuli differing only in polarisation angle. Oviposition experiments showed that the polarisation angle influenced the choices of colours as well (Fig. 5A).

Therefore, we assume that a separate polarisation system based on the green or red receptors is very unlikely to explain our results. We then have to assume that polarisation and colour are processed in the same visual pathway.

Which photoreceptors are involved: colour loci in B-G-R colour triangles

Which receptors build the colour-polarisation vision system of *Papilio* spp.? We start with the simple part. The blue-sensitive receptors R1 and R2 are certainly involved. They have an inhibitory influence on colour choices in oviposition behaviour (Fig. 4A; Kelber, 1999b) and they must guide choices for blue stimuli in the feeding response (Fig. 6). R1 and R2 are sensitive to vertically polarised light. Do blue lights of different polarisation angle appear to butterflies as if they had different spectral compositions or different intensities? The PS value of blue receptors (in ommatidia of types 2 and 3) is close to 2 (Table 1). Animals trained to vertically polarised blue light could have learned the stimulus with a higher blue receptor quantum catch. However, the butterflies chose the correct polarisation angle even though the light intensity was reduced by a factor of more than 3 (Fig. 6B). In this test, the blue receptor received more photons from the horizontally polarised light. Therefore, the blue receptor quantum catch cannot have been the choice criterion: other receptors must have been involved.

We have seen that discrimination between different

Fig. 6. Results of feeding experiments. Three groups of animals were trained to discriminate stimuli differing only in the polarisation angle. Asterisks mark choice frequencies differing significantly from chance or from each other (*G*-tests; **P*<0.0001; ‡*P*<0.005). *N* gives the number of animals in each test, *n* gives the number of choices. (A) Tests with the training stimuli (see symbols above and below the diagram, + marks the rewarded pattern, – marks the unrewarded pattern). (B) Choice frequencies for the vertical polarisation angle as function of the intensity ratio between the vertically and horizontally polarised lights. Hatched circles give the polarisation angle and the colour of the training stimulus. (C) Choice frequencies for vertically and horizontally polarised lights of a colour different from the training colour (as in Fig. 6A, see symbols above and below the diagram for training and testing stimuli).

polarisation angles is quite independent of intensity in the blue range (Fig. 6B). The oviposition experiment with green lights of different intensities gives an indication that intensity is not the main cue in the green range: the animals preferred the green (Gr) over the yellow (Y) stimulus even though it had a lower intensity at all wavelengths (Fig. 1, Fig. 4A). They did not prefer the stimuli of lower intensity when they had the same spectral distribution as more intense ones (Fig. 3C). If the discrimination of polarisation angle depended on intensity only, the discrimination should have been independent of colour. Since this was not the case, we assume that, to the eyes of *Papilio* spp., lights with different polarisation angles appear to have different colours rather than different intensities.

We have chosen to show the colour loci of the stimuli in a colour triangle. We use triangles representing the blue, green and red receptors. This is a projection of the possibly five-dimensional colour space of *Papilio* spp. onto two dimensions, disregarding the ultraviolet and violet receptors and the intensity. This allows us to visualise the influence of polarisation on colour under different assumptions (Fig. 7). Fig. 7A shows the colour loci in a triangle assuming that only the long visual fibres are involved. This means that all three receptor types have $\phi=0°$. Polarisation angle has no influence on colour. Thus, we can rule out the possibility that long visual fibres are the basis of colour vision in *Papilio* spp.

In the second colour triangle (Fig. 7B), we test the assumption that, in addition to R1 and R2, the red and green receptors R5–R8 are involved in colour vision (as suggested by Arikawa et al., 1999). Since the animals did not discriminate between polarisation angles of 45° and 135°, we assume that signals from R5–R8 are summed and not compared, resulting in a slightly higher sensitivity to vertically polarised light than to horizontally polarised light. Under these assumptions, discrimination of polarisation angles depends on colour, as we have seen in the experiments (Fig. 4B, Fig. 6A). However, we cannot explain the discrimination between vertically and horizontally polarised red or yellow lights.

Finally, we have proposed that the receptors of only one of the three types of ommatidia might be involved. The most probable candidates are ommatidia of type 1 (Table 1). R1 and R2 of these ommatidia have curved microvilli and thus reduced PS values. Fig. 7C shows the colour loci in the triangle. This triangle shows different colour loci for all pairs of stimuli. There are many more possibilities for which receptors are involved and for how they are weighted. As an example (not

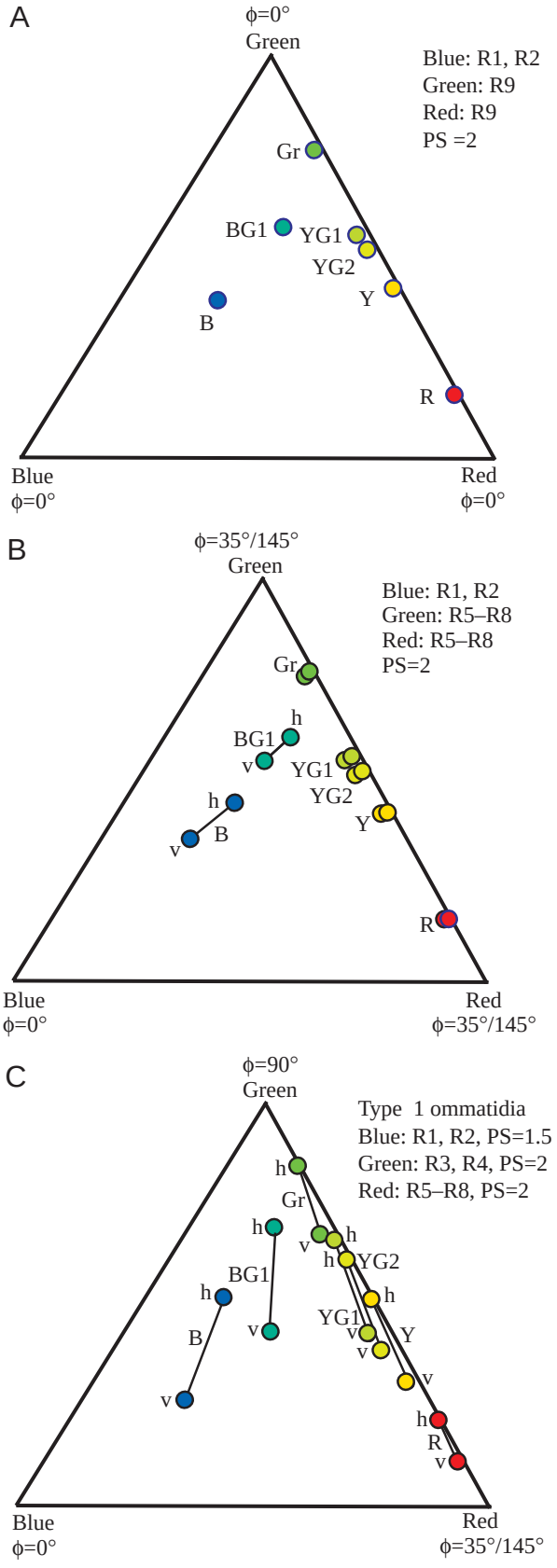


Fig. 7. Colour triangles for the blue, green and red receptor colour space of *Papilio* spp. on the basis of different assumptions about the receptors involved and their polarisation preference angles ϕ. The anatomical receptor types assumed to be involved are listed on the right, and assumed values of ϕ are given at the corners of the triangles with the respective receptor names. For receptor nomenclature, see Fig. 1 and Table 1. Coloured circles show the colour loci of the stimuli used in oviposition and feeding tests (B, blue used in feeding tests; R, red used in feeding test; for the other colours and for the spectral curves, see Fig. 2). Two connected circles show the position of stimuli with the same spectral distribution (hence the same colour of the circle) but different polarisation angle δ (vertical, v; horizontal, h). PS, polarisation sensitivity (see Table 1).

shown), it is possible that all receptors in all types of ommatidia contribute, to some degree, to colour vision.

From the data we have gathered so far, we cannot draw a final conclusion on which receptors are involved. Our behavioural data provide evidence that polarisation and colour are processed in the same visual pathway, that polarisation can induce a change in perceived colour and that intensity has an additional, but smaller, influence. Our colour triangle considerations suggest that both short and long visual fibres are involved. In further experiments, we will test whether butterflies prefer vertically polarised light to horizontally polarised light in the feeding reaction, irrespective of colour, and we will train the animals to polarised lights of spectral colours and test them with lights of changed wavelength and polarisation angle. This should allow us to determine which photoreceptors interact and how this interaction occurs for colour-polarisation vision in the eye of *Papilio* spp. butterflies.

Ecological relevance

What is the consequence of polarisation-dependent colour vision for the butterflies in their ecological context? Is it just the result of evolutionary constraints on the visual system of *Papilio* or does it have an adaptive value for the animals? Other insects, such as bees and flies, have twisted rhabdoms in the part of their eyes used for colour vision (see Labhart and Meyer, 1999), and other Lepidoptera have strongly curved microvilli (Eguchi, 1999; Warrant et al., 1999). In both cases, the receptors are insensitive or only very weakly sensitive to the polarisation angle of light, and false colours are avoided. Why have butterflies of the genus *Papilio* preserved polarisation sensitivity in the entire eye?

Polarisation may be behaviourally relevant in the context of oviposition. Females of many species of *Papilio*, including both species used in this study, lay eggs on the shiny leaves of plants in the Rutaceae or *Citrus* family. Shiny leaves reflect partially polarised light (e.g. Land, 1993; Shashar et al., 1998). Horizontally oriented leaves reflect horizontally polarised light, whereas vertically oriented leaves reflect obliquely or vertically polarised light depending on the position of the sun and the observer. To our eyes, the shiny leaves reflect white light and thus have a less saturated colour but the same hue (see Wehner and Bernard, 1993). To an approaching *Papilio* female, the shiny leaves of a *Citrus* bush should have different colours depending on their orientation: a horizontally oriented leaf should look more green whereas a vertically oriented leaf should look more blue-green or reddish. Horizontally oriented leaves should therefore be more attractive to an approaching female, whereas vertically oriented leaves should be less attractive. We do not yet know whether butterflies use the colour difference as a cue to detect leaf orientation, whether they prefer to lay eggs on horizontally oriented leaves and whether it is of advantage to lay eggs on horizontally oriented leaves. As a whole, a plant with shiny leaves should look more colourful than one that does not reflect polarised light. This could help the females to choose the larval food plant from a distance.

The polarisation-sensitive colour vision system could therefore be a ‘matched filter’ for optimal oviposition sites. In contrast, many flowers have structures on the surface that avoid mirror reflections (see Wehner and Bernard, 1993). To a butterfly flying over a meadow, and thus with a changing angle of view for the different plants, as a result of the polarisation, the grass and shiny green leaves provide a background of changing colour spots. Even in a meadow with highly polarising grass, the colour-constant flowers should stick out quite clearly. To understand the ecological relevance of polarisation-dependent colour vision, we need to learn more about the occurrence and properties of reflected light in the natural habitat of the butterflies.

Polarisation-sensitive colour vision and colour-dependent polarisation vision

How common are polarisation-dependent colour vision systems similar to that of *Papilio aegeus* and *P. xuthus*? Anatomical and physiological data suggest that other animals could also possess such a system. This has been overlooked so far, because PS values of 2 are low compared with those of the photoreceptors used to detect the polarisation patterns of the sky (Labhart and Meyer, 1999) or of water surfaces (Schwind, 1991; Bartsch, 1991). Many insects have twisted rhabdoms (e.g. bees and many Diptera) or strongly curved microvilli (e.g. sphingid moths; Eguchi, 1999; Warrant et al., 1999). But even if low PS values were of little use for polarisation vision, they could have a strong influence on colour vision.

In contrast, many other Lepidoptera have preserved some polarisation sensitivity in the entire eye. Anatomical studies on *Pieris rapae*, *P. brassicae* and *P. protodice* (Pieridae) have shown that straight and untwisted microvilli are present in all ommatidia (Kolb, 1978; Shimohigashi and Tominaga, 1991). In two nymphalid species, polarisation-sensitive photoreceptors have been detected electrophysiologically in the entire eye (Kinoshita et al., 1997). Again, different spectral types of receptors have different polarisation preferences.

Similar cases seem to exist in other insect orders. In flies, photoreceptors R7 and R8 are thought to be the basis for colour vision (e.g. Strausfeld and Lee, 1991). In tabanid flies, the rhabdoms of R7 and R8 are not twisted. Even outside the dorsal rim, they must have PS values greater than 1, which should make colour vision polarisation-dependent (Smith and Butler, 1991). Anatomical data from ladybirds (*Coccinella septempunctata*; Coccinellidae, Coleoptera) show clearly aligned microvilli in all receptors and different directions of alignment in the blue and green receptors (Lin, 1993). Waterstriders (*Gerris palludum*; Gerridae, Heteroptera) have polarisation-sensitive blue and green receptors with mean PS values of 4. The green receptors are sensitive to polarisation angles of 90 ° or 0 °, whereas all the blue receptors have their highest sensitivity for polarisation angles of 90 ° (Bartsch, 1991). It remains to be shown whether tabanid flies, beetles and waterstriders have a colour vision system and whether they use receptors with different polarisation preferences for this task.

Recently, the counterpart of polarisation-dependent colour

vision has been found: polarisation vision is wavelength-dependent in *Daphnia pulex* (Novales Flamarique and Browman, 2000). *Daphnia* spp. have four spectral types of photoreceptor sensitive in the ultraviolet, blue, green and red regions of the spectrum. Under white illumination, they swim perpendicular to the polarisation angle of light. *Daphnia magna* does this even when the illumination is restricted to long wavelengths. *D. pulex*, in contrast, is disoriented when the illumination is restricted to wavelengths above 515 nm and orients at 90° (thus swimming parallel to the polarisation angle of light) when only wavelengths above 570 nm are present. The most probable explanation is that, in *D. pulex*, two spectral types of receptor (red and green receptors) with orthogonal microvillar directions interact for polarisation vision (Novales Flamarique and Browman, 2000). This should also make their colour vision polarisation-dependent. The only known behaviour for which *Daphnia* spp. uses colour vision is phototaxis (e.g. Storz and Paul, 1998). It remains to be determined how and why colour and polarisation are used in combination by *Daphnia pulex* but separately by *D. magna*. The visual system of *D. pulex* may be another example of a 'matched filter' – a system adapted to serve a very specific behavioural task with minimal costs.

The mechanisms of polarisation vision are poorly understood in vertebrates. Hypotheses about salmon polarisation vision also predict an interaction between colour and polarisation (Novales Flamarique et al., 1998).

We suggest that wavelength-dependent polarisation vision and polarisation-dependent colour vision are more common than one might have expected. Natural scenes contain highly polarised light reflected by leaves and other shiny surfaces, making the polarisation-dependence of colour vision a relevant problem. Since many animals have evolved ways to get around this problem, we propose that there must be advantages to the animals that have retained the polarisation-dependence of colour vision. Polarisation-dependent colour vision may have evolved as a cheap solution to a very specific problems. We must study the light environments of animals in more detail to understand the possible advantages and consequences of these systems better.

Appendix: the linear modelling procedure

The modelling procedure follows the theory of generalized linear models (McCullagh and Nelder, 1989). The choice frequency C_c for a colour is given by:

$$C_c = F(\eta) . \quad \text{(A1)}$$

This model formula has two parts, the linear predictor η and the link function F. In this particular case, η is the colour attractivity, and it is assumed to be the result of a linear interaction between different receptor types:

$$\eta = \Sigma x_i Q_i , \quad \text{(A2)}$$

where Q_i represents the quantum catches of the respective receptors (see Materials and methods, equations 1 and 2) and x_i represents the positive or negative coefficients of the quantum catches. The values of C_c lie between 0 (for a colour that is never chosen) and 1 (for a colour that is always chosen); the error follows a binomial distribution and depends only on the number of registered choices N. A logit function is used as the appropriate sigmoid link:

$$F = 1/(1 - e^{\eta}) . \quad \text{(A3)}$$

The results would be very similar if a different sigmoid link function were chosen (see McCullagh and Nelder, 1989; Francis et al., 1993). The model was fitted with coefficients x_i optimized using a least-squares algorithm.

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