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Color discrimination at the spatial resolution limit in a swallowtail butterfly, *Papilio xuthus*

Yuichi Takeuchi*, Kentaro Arikawa and Michiyo Kinoshita†

School of Advanced Sciences, The Graduate University for Advanced Studies, Hayama 240-0193, Japan

*Present address: Department of Zoology, Graduate School of Science, Kyoto University, Kitashirakawa-Oiwake, Sakyo, Kyoto 606-8502, Japan

†Author for correspondence (e-mail: kinoshita_michiyo@soken.ac.jp)

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Summary

Spatial resolution of insect compound eyes is much coarser than that of humans: a single pixel of the human visual system covers about 0.008° whereas that of diurnal insects is typically about 1.0°. Anatomically, the pixels correspond to single cone outer segments in humans and to single rhabdoms in insects. Although an outer segment and a rhabdom are equivalent organelles containing visual pigment molecules, they are strikingly different in spectral terms. The cone outer segment is the photoreceptor cell part that expresses a single type of visual pigment, and is therefore monochromatic. On the other hand, a rhabdom is composed of several photoreceptor cells with different spectral sensitivities and is therefore polychromatic. The polychromatic organization of the rhabdom suggests that insects can resolve wavelength information in a single pixel, which is an ability that humans do not have. We first trained the Japanese yellow swallowtail butterfly *Papilio xuthus* to feed on sucrose solution at a paper disk of certain color. We then let the trained butterflies discriminate disks of the training color and grey disks each presented in a Y-maze apparatus. *Papilio* correctly selected the colored disk when the visual angle was greater than 1.18° for blue, 1.53° for green or 0.96° for red: they appeared to see colors in single pixels to some extent. This ability may compensate their rather low spatial resolution.

Key words: color vision, detection, rhabdom, compound eye, *Papilio xuthus*.

Introduction

The spatial resolution of a visual system is basically determined by the photoreceptor array in the retina. The central part of the human retina, the fovea, is equipped with densely packed cone photoreceptors, placed with an interval of little more than 2 μm at a distance of about 17 mm from the nodal point of the eye, which thus corresponds to a visual angle of about 0.0084°. However, the cone photoreceptors are not uniform in terms of their spectral sensitivity: they are either short, middle or long wavelength-sensitive. The polymorphic cones form the physiological basis of trichromatic color vision, i.e. the sense of color is produced if cones with different spectral sensitivities are stimulated simultaneously and their signals are processed with so-called spectrally opponent interneurons (Wandell, 1995) at a more central level. Therefore if the visual target is as small as the limit of our spatial resolution, we cannot detect the color of the target (Wandell, 1995).

The compound eyes of insects consist of a number of functional units called ommatidia. In many diurnal insects, such as bees and butterflies, each ommatidium contains several photoreceptor cells that construct together a single photoreceptive organelle, the fused rhabdom. The fused rhabdom acts as a single optical waveguide, which receives light *via* its dioptric apparatus, a facet lens and crystalline cone, from a limited spatial area. The visual angle covered by an ommatidium, 1–3° in most cases, corresponds to a single pixel of the visual field of insects: a rhabdom is therefore equivalent to a cone outer segment of vertebrate retina (Land and Nilsson, 2002). However, rhabdoms strikingly differ from cone outer segments because a rhabdom is usually made up of the photosensitive organelles of two or more different spectral photoreceptors. Colocalization of multiple spectral photoreceptors in a single rhabdom suggests that it is basically possible to analyze wavelength information within a single pixel. The rhabdom therefore is polychromatic (Gribakin, 1975; Menzel, 1979).

How small can a visual target be to still allow insects to detect its color? This question has been previously addressed in honeybees and in bumblebees. Honeybees were found to detect objects if their visual angle is larger than 5° (Giurfa et al., 1997; Giurfa et al., 1996), whereas the minimal angle for visual detection was found to be 3° in bumblebees (Spaethe and Chittka, 2003; Spaethe et al., 2001). Color discrimination

was lost at larger visual angles, around 15°, thus showing that target detection at smaller angles was achromatic. Here we determined the minimal visual angle at which color detection is possible for the Japanese yellow swallowtail butterfly, *Papilio xuthus*. This butterfly has ultraviolet (UV), violet (V), blue (B), green (G), red (R) and broad-band (BB) receptors in the retina. These spectral photoreceptors are embedded in fixed combinations in three types of ommatidia. Types I, II and III ommatidia contain four (UV, B, G, R), three (V, G, BB) and two (B, G) classes of spectral photoreceptors, respectively (Arikawa et al., 2003). *Papilio* butterflies use these receptors to see colors while foraging: they can be trained to approach a paper patch of a certain color by feeding them while they observe the colored stimuli (Kinoshita et al., 1999). The trained butterflies distinguish the training color from a range of different colors as well as from a series of grays. By training butterflies to a colored target, we were able to measure the minimum angular size that a visual target should subtend for the butterflies to detect its color in a Y-maze apparatus.

Materials and methods

Animals

We used spring-form males of *Papilio xuthus* L. The butterflies were taken from a laboratory stock culture derived from eggs laid by females caught in the field around the campus of Yokohama City University, Yokohama, Japan. The hatched larvae were reared on citrus leaves at 25°C under a light regime of 10 h:14 h light:dark. The pupae were stored at 4°C for at least 3 months, and then allowed to emerge at 25°C. The day of emergence was defined as the post-emergence day 1.

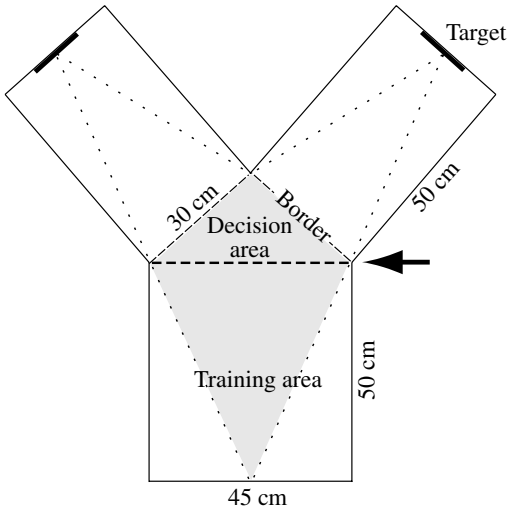

Fig. 1. Top view of Y-maze apparatus. The height of the Y-maze is 45 cm. The training and decision areas can be separated by inserting a removable board between two areas (arrow). The floor and the end walls of the two arms were covered with black plastic sheet. The other walls and the ceiling were covered with a 3 mm mesh plastic net. The tested butterflies were able to see two targets simultaneously while they were in the grey area.

Apparatus

Behavioral experiments were carried out in a Y-maze apparatus (Fig. 1). The Y-maze consists of a training area, a decision area, and two 30 cm wide arms. The distance of the targets to the imaginary border between the decision area and the arm was 50 cm. The training and decision areas were separated by a removable black acrylic board. The floor and the end walls of both arms were made of wooden board covered with a black plastic sheet. Stimuli were presented on the bilateral black end walls of the arms. The ceiling and walls of the apparatus were covered with a white plastic net of 3 mm mesh to allow the butterflies to freely enter the arms.

The Y-maze was illuminated with 10 halogen lamps (300 W) and six fluorescent tubes (40 W) to make the light flux at the floor of the Y-maze approx. 3000 lux. The illumination contained virtually no light shorter than 400 nm. Such UV-suppressed illumination has been found not to affect the color choice behavior of *Papilio xuthus* (Kinoshita and Arikawa, 2000; Kinoshita et al., 1999). The temperature was set at approx. 30°C.

Stimuli

We used as stimuli disks of chromatic (colored) and achromatic (grey) paper presented on black background paper. The papers used in this study were all printed by an inkjet printer (Seiko Epson PM800C, Tokyo, Japan) on super fine paper (Seiko Epson MJA4SP1), except for the black paper for the background. Reflectance spectra of the paper were measured with a spectrometer (S2000, Ocean Optics, Inc., Dunedin, FL, USA) that was calibrated against a MgO-coated surface as the reference (Fig. 2). Papers of human blue, green and red were used as training colors.

In order to demonstrate that butterflies discriminate two stimuli based on differences in chromatic content, they must be equal in terms of their brightness. We calculated the *Papilio*-subjective brightness of a given paper *i*, B_i, under the present conditions by:

$$B_i = \int_{400}^{700} I(\lambda)S(\lambda)R_i(\lambda)d\lambda \,, \quad (1)$$

where $I(\lambda)$ is the illumination spectrum, $S(\lambda)$ is the spectral sensitivity determined by mass recording of photoreceptor potentials by electroretinographic (ERG) recording (Arikawa et al., 1987), and $R_i(\lambda)$ is the reflectance spectrum of paper *i* (Fig. 2). The wavelength range was set from 400 nm to 700 nm, for the halogen lamps used in the present study emitted virtually no light with wavelengths shorter than 400 nm. The wavelength interval for the calculation ($d\lambda$) was 0.25 nm.

If butterflies discriminate two stimuli based on the relative responses of a particular class of spectral photoreceptors, the butterflies should be unable to discriminate a chromatic stimulus from an achromatic stimulus that activates the same photoreceptor class at equivalent brightness levels. To test this possibility, we prepared a set of four grey papers for each training color (Table 1, Fig. 2). We calculated the quantum catch *Q* of the spectral photoreceptors for each paper based on

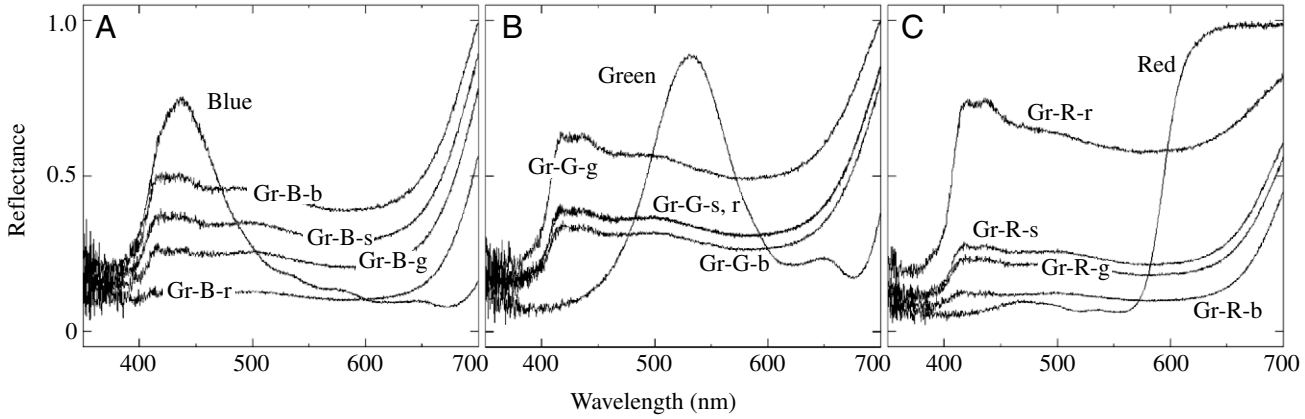

Fig. 2. Reflectance spectra of chromatic (color) and achromatic (grey) papers used in the present experiments. The spectra are shown relative to the maximal reflectance in a set of the training color and the corresponding four different greys. (A) Blue and four corresponding grey papers: with the same subjective brightness (Gr-B-s), and the same quantum catch for the blue (Gr-B-b), green (Gr-B-g) and red (Gr-B-r) receptors. (B) Green and four corresponding grey papers. (C) Red and four corresponding grey papers. For the calculated number of quantum catch, see Table 1.

the assumption that the butterflies use B (460 nm), G (520 nm) and R (600 nm) receptors under the present illumination conditions (Kinoshita and Arikawa, 2000; Kinoshita et al., 1999). The UV and violet receptors were excluded here because the illumination contained virtually no UV light. The BB receptors were also excluded based on the tentative assumption that the receptors are not involved in color vision because of their extremely broad sensitivity (Arikawa et al., 2003). The quantum catch, Q, of each spectral photoreceptor was calculated by:

$$Q_{\mathrm{B}} = \int_{400}^{700} 1.00\, I(\lambda) S_{\mathrm{B}}(\lambda) R_{\mathrm{i}}(\lambda) \mathrm{d}\lambda\ , \quad (2)$$

$$Q_{\mathrm{G}} = \int_{400}^{700} 0.89\, I(\lambda) S_{\mathrm{G}}(\lambda) R_{\mathrm{i}}(\lambda) \mathrm{d}\lambda\ , \quad (3)$$

$$Q_{\mathrm{R}} = \int_{400}^{700} 0.29\, I(\lambda) S_{\mathrm{R}}(\lambda) R_{\mathrm{i}}(\lambda) \mathrm{d}\lambda\ , \quad (4)$$

where $S_{\mathrm{B,G,R}}(\lambda)$ is the spectral sensitivity of B, G or R receptors determined by intracellular recording (Arikawa et al., 1987). The numbers indicate the relative sensitivity at 460 nm (B), 540 nm (G) and 600 nm (R) calculated from the spectral sensitivity function determined by ERG recording (Arikawa et al., 1987).

Training

Newly emerged butterflies were individually marked on a wing and each butterfly was kept in a separate box. On the day of emergence (day 1), no food was provided. On day 2, we started to train butterflies. We put a blue, green or red paper disk on the black floor, covered the whole floor with a piece of anti-reflection glass, and put some drops of 6% sucrose solution on the glass at the location of the colored disk. We then released one butterfly in the training area. If the released butterfly had not visited the disk by itself after a few minutes, we captured it and uncoiled its proboscis using a needle towards the drop of sucrose solution to let the butterfly take the reward. After the manual feeding, virtually all of such butterflies became able to visit the disk by themselves and to take sucrose. While they were taking sucrose, we chased the butterflies from the feeding site by waving hands or by blowing wind from outside of the cage. They then had to visit the disk spontaneously and repeatedly to get the reward: this procedure promoted their learning. The butterflies stopped feeding spontaneously after taking a certain amount of sucrose: they had probably become satiated. Each butterfly was trained to only one color.

We performed this training session once a day and repeated the training for 3 days. From post-emergence day 5, we presented the training colored disk vertically on a black acrylic

Table 1. *Characterization of the chromatic and achromatic papers*

		Quantum catch		
Paper ID	Subjective brightness	B	G	R
Blue	5.2	7.5	3.5	1.9
Gr-B-s	5.5	5.6	5.4	5.5
Gr-B-b	7.3	7.4	7.1	7.2
Gr-B-g	4.0	4.0	3.8	3.9
Gr-B-r	2.0	1.9	1.9	2.0
Green	6.9	5.8	10.5	6.5
Gr-G-s	6.6	6.7	6.4	6.5
Gr-G-b	5.5	5.6	5.4	5.4
Gr-G-g	10.4	10.7	10.2	10.1
Gr-G-r	6.5	6.6	6.4	6.5
Red	6.4	2.9	5.1	16.1
Gr-R-s	6.4	6.5	6.2	6.3
Gr-R-b	3.0	3.0	2.9	3.0
Gr-R-g	5.5	5.6	5.4	5.5
Gr-R-r	16.6	17.2	16.5	16.0

Subjective brightness and quantum catch were calculated using Eqn 1–4. For example, Gr-B-s is Grey paper with equal subjective brightness to the Blue paper; Gr-B-b is Grey paper with quantum catch equal to that of blue receptors with the Blue paper (underlined numbers).

board placed between the training and decision areas. The board was equipped with a plastic trough immediately below the training disk, such that the butterflies could take the sucrose reward from the trough while exposed to the colored disk in front of them. Here also we chased the butterflies away from the disk for several times until they stopped feeding spontaneously. We performed the training session once a day and repeated it for 4 more days. To avoid a possible association of the disk size with the reward, we changed the size of the disks (30 or 50 mm in diameter) after every second visit throughout the training period.

Pretest

On day 9, we performed pretests. The purpose of the pretests was to select appropriate individuals for the tests. We presented a disk (diameter 30 or 50 mm) of the training color vertically at the end of one arm and a grey disk of the same size at the end of the other arm (Fig. 1); the distance of 50 cm to the inspection point means that the disks of 30 mm and 50 mm diameter cover a 3.4° and 5.7° visual angle, respectively. We then released a trained butterfly into the training area that was separated by the board from the decision area. We removed the board to let the butterfly fly into the decision area, and further into one of the arms: the targets were invisible until the board was removed. When the butterfly first crossed the imaginary line between the decision area and one of the arms (Fig. 1), we recorded the behavior as a choice made by the butterfly. Most butterflies that entered into an arm finally reached the disk and extended the proboscis, indicating that they were actually performing foraging behavior, although no reward was provided during the pretests. We then chased the butterfly away from the arm back into the training area, and put the board back. After a pause of 30 s, we again removed the board and allowed the butterfly making another choice without reward. After every two visits, we inserted a board with a disk of the training color and the feeding trough between the training and decision areas, and fed the butterflies for 3 s: this was to keep and to check their motivation to feed at the disk of the training color. We repeated the pretest 6 times for one individual using the same size of disks. The positions of two disks were changed alternately after every second choice. We selected butterflies that made at least 5 correct choices in the pretests.

Test

Tests were started from day 10 post-emergence. We used disks of 6, 10, 15, 30 and 50 mm diameter. We presented a disk of the training color in one arm and a grey disk in the other arm. The sizes of the two simultaneously presented disks were always the same. As in the pretests, the butterflies were not rewarded at the end of the arms, but their motivation was checked and kept by feeding them for 3 s after every second visit at the board inserted between the training and decision areas.

We released a butterfly in the training area, and then removed the board separating the training area from the decision area. When a butterfly crossed the imaginary line between the decision area and the arm having the disk with the training color, we recorded the event as a correct choice. When a butterfly crossed the border of the arm presenting the grey disk or did not enter either of both arms after 2 min, we checked whether the individual was motivated for foraging. To this end, we inserted a board between the training and decision areas with a colored and a grey disk attached to it: motivated butterflies immediately visited the colored disk with their proboscises extended. Only when the motivation was confirmed, did we accept the previous negative responses as meaningful negative choices, and continued the tests with the same individual. One session of testing consisted of 6 consecutive choices as in the pretests. The sides of the two disks simultaneously presented were alternated after every second choice. We performed at least five test sessions using each of five sizes of disks for each individual.

Analysis

We scored the choice of the arm with the colored disk as 1, and the choice of the arm with the grey disk as 0. We omitted the cases in which butterflies did not enter either of the two arms after 2 min because this behavior does not fit into the classification ‘correct’ *vs* ‘incorrect’ choice. We analyzed the binomial data by using the generalized linear mixed effect model with restricted maximum likelihood estimation (REML-GLMM) to assess which parameters in this experiment affected the choice behavior. We treated disk size, training color, type of grey, combination of color and grey presented simultaneously as the fixed effects, and individual as the random effect when the REML-GLMM analysis was performed. To determine the minimum visual angle that was discriminated for each training color, we fitted curves for each by using the generalized linear model (GLM). We used the analysis software JMP version 5.0.1 (SAS Institute Inc. Cary, NC, USA).

Results

Choice behavior

About 100 male individuals were used in the present study, 52 of which passed the pretests. Data from the tests were collected from 39 individuals. The other 13 butterflies died for unknown reasons or received serious injuries on the wings and/or the legs at early stages of the tests. The injured butterflies could not properly fly in the cage or could not stay on the trough to receive the sucrose reward.

We presented disks of 6, 10, 15, 20, 30 and 50 mm in diameter at the distance of 50 cm in the Y-maze of Fig. 1. The butterflies made decisions not necessarily at the exact border between the decision area and the arm, but somewhere else in the decision chamber or even in the training area while they were flying during the tests. The longest possible distance where the targets are simultaneously visible from the butterflies in the apparatus is 106 cm (see Fig. 1). Actual decisions are therefore made between 50 cm and 106 cm.

When Giurfa et al. measured the minimum detectable visual angle in honeybees (Giurfa et al., 1996), they manipulated the distance range more strictly by making a hole through which the bees entered the decision area, which produced a 10 cm

distance range. They tested the bees with three different disk diameters presented in arms of variable length to confirm that the bees' detection was independent of either disk diameter or arm length. They eventually analyzed the results obtained by using the combination of a longer arm and a larger disk, which makes the effect of the distance range to the visual angle of the target smaller. Unfortunately such manipulations of distance range are so far not applicable to *Papilio xuthus*, because of the difference in foraging habits of *Papilio* and honeybees. *Papilio* requires a larger area to perform free-flying foraging behavior and in preliminary experiments never entered a hole made in the separating wall between the training and decision areas. It was also extremely difficult to let the butterflies fly into arms longer than 60 cm.

The disks of 6, 10, 15, 20, 30 and 50 mm in diameter when viewed from a 50 cm distance correspond to visual angles of 0.7, 1.1, 1.7, 2.3, 3.4 and 5.7°, respectively. When viewed from a 106 cm distance, the visual angles are 0.3, 0.5, 0.8, 1.1, 1.6 and 2.7°, respectively. The former set of numbers represent the maximum values of visual angles if the disks were discriminated in the present condition. We therefore used these numbers for analyses as the maximum estimated visual angles.

Fig. 3 demonstrates the results of the tests. Each panel presents the data of individuals trained to blue (Fig. 3A), green (Fig. 3B), or red (Fig. 3C) color. Data were collected from 13 individuals for each color. The four lines in each panel correspond to the choice selection between the colored disk and four different grays (see Fig. 2 and Table 1).

Blue- and red-trained butterflies discriminated the colored target from the four grey intensities almost perfectly when the visual angle was larger than 3.4°. The correct ratio gradually decreased as the visual angle became smaller, and reached a random level (0.5), when the visual angle was about 1.0° (Fig. 3A,C). Discrimination by the green-trained butterflies was rather variable.

Parameters that affect the correct choice

Choice behavior may be affected by several independent parameters. We performed the REML-GLMM analyses by incorporating five parameters, which were disk size, color, grey intensity, color and grey presented simultaneously and individually. Table 2 shows the χ^2 and the probability (P) values for each of these parameters. Clearly, the correct choice ratio strongly depends on the disk size (P<0.001) and also on the disk color (P=0.026).

Minimum disk size for correct choice

Fig. 4 shows the relationship between angular subtense and correct choices for the three training colors determined by GLM analysis. The regression curves for blue and red are rather similar but that for green is different, as expected.

In the present work we assumed that the butterflies discriminated the presented disks when the correct choice ratio was larger than 60% (see Giurfa et al., 1996). Each regression curve crosses the 60% criterion line at 1.18° for blue, 1.53° for green or 0.96° for red (Table 3).

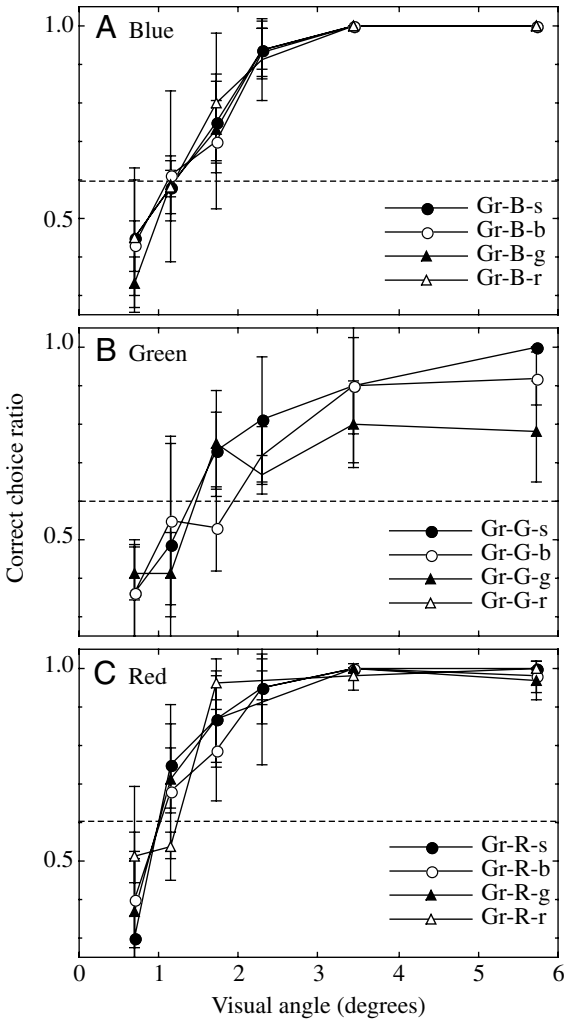

Fig. 3. Correct choice ratio for different sizes of target. Results of the blue- (A), green- (B) and red- (C) trained butterflies. The correct choice ratio is close to 0.5 (dotted lines) when the visual subtended angle of the disk is smaller than about 1.0°. Values are mean ± s.d. For an explanation of Gr-s, Gr-b, Gr-g and Gr-r, see Fig. 2 and Table 1.

Discussion

Spatial resolution of the Papilio eye

In order to compare the present results of color-based target detection with the monochromatic spatial resolution, we first estimate the spatial resolving power of the *Papilio* eye based on the theoretical analyses of the visual acuity in insects (Land, 1981; Land, 1997; Land and Nilsson, 2002; Snyder, 1979;

Table 2. *Results of REML-GLMM analysis*

Parameter	df	χ^2	P
Disk size	1	260.62	<0.001
Training color	2	7.30	0.026
Type of grey	3	4.92	0.178
Color-grey combination	6	1.91	0.928
Individual	27	20.53	0.808

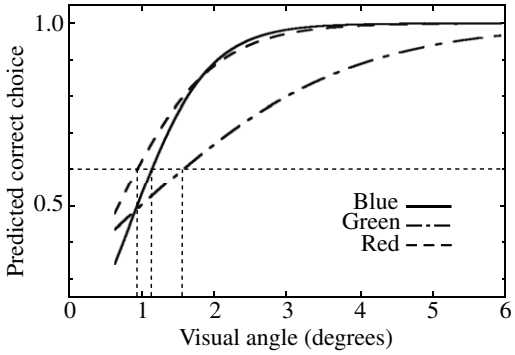

Fig. 4. Relationship of the correct choice ratio and the visual angle of the target predicted by the GLM. Estimated equation: $P=\exp(\beta_0+\beta_1 x)/[1+\exp(\beta_0+\beta_1 x)]$. x, visual angle; β_0, intercept; β_1, regression coefficient. Blue: $\beta_0=-1.78$, $\beta_1=1.84$. Green: $\beta_0=-0.57$, $\beta_1=0.64$. Red: $\beta_0=-0.96$, $\beta_1=1.43$.

Snyder and Menzel, 1975). The best sampling frequency that a compound eye can resolve, ν_s, is that for which there is one receptor unit for each half cycle of the grating. It is given by:

$$\nu_s = 1/(2\Delta\phi) , \quad (5)$$

where $\Delta\phi$ is the interommatidial angle. The interommatidial angle in the frontal region of the eye of *Papilio xuthus* is about 1.0° (Shibasaki et al., 2006), so that ν_s is about 0.5/degree. This indicates that *Papilio* can theoretically resolve two 1° dots separated by a spatial interval of 1°. However, the effective cut-off frequency of the optics, ν_{opt}, is affected by the ommatidial acceptance angle, $\Delta\rho$, which is expressed by:

$$\Delta\rho = \sqrt{(d/f)^2 + (\lambda/D)^2} , \quad (6)$$

where d is the rhabdom diameter, f is the focal length of the lens, D is the facet diameter and λ is the wavelength of light. Assuming that d is 2 μm (Arikawa et al., 1999), f is 70 μm (Nilsson et al., 1988) and D is 25 μm (Arikawa and Stavenga, 1997) for *Papilio xuthus*, $\Delta\rho$ should be about 1.95° for 500 nm light. The value is close enough to the electrophysiologically determined $\Delta\rho$ of a *Papilio* ommatidium, which is about 1.9° (Shibasaki et al., 2006) see also for *Papilio aegeus* (Horridge et al., 1983). Note that the $\Delta\rho$ is about as twice as much as the value of $\Delta\phi$ (1.0°). This relationship, $\Delta\rho=2\Delta\phi$, is derived by assuming $\nu_s=\nu_{opt}$ (Land, 1997). Therefore we tentatively conclude that the spatial resolution of *Papilio* is around 1.0°.

Table 3. *Estimated color detection limit*

	Visual angle (degrees)
Blue	1.18 (1.06–1.29)
Green	1.53 (1.24–1.78)
Red	0.96 (0.77–1.11)

Estimated visual angles (95% confidence limits in parentheses) that provide 60% correct choice for three colors.

Discrimination and eye structure

In the present work we found that the minimum angular subtense of a colored target detectable for a foraging *Papilio* is at 1.18° for blue, 1.53° for green or 0.96° for red, which are angles close to the spatial resolution predicted from the interommatidial angle, 1.0°. As described in the Results, the numbers are a maximum estimate: butterflies may have discriminated the targets from a point further away from the point used for the calculation above. Assuming that they discriminated the targets at 77 cm, the mid point of 50 and 106 cm, the angles are 0.76° for blue, 1.03° for green or 0.68° for red. Therefore the butterflies appeared to be able to see the color of targets whose size is close to the spatial resolution limit, which never happens in humans. This ability may compensate their poor spatial resolution, which is about 100 times coarser than our own (Land, 1997).

The ability of discriminating colors of targets of visual angle around 1° should be attributed at least in part to the polychromatic organization of the rhabdom. The eye of *Papilio* consists of three types of ommatidia, each containing 2–4 classes of spectral photoreceptors contributing to the rhabdom. Multiple spectral detectors with overlapping receptive fields are required for wavelength discrimination in general, and the polychromatic rhabdom of *Papilio* fulfils the requirement.

Of course the present results do not provide any direct evidence that the color discrimination is in fact possible with only a single ommatidium, because we did not precisely stimulate single ommatidia. Rather, the butterflies were flying when making decisions, so the targets must have been stimulating multiple ommatidia successively. The *Papilio* eye is furnished with six classes of spectral receptors embedded in the ommatidia in three distinct combinations, i.e. type I, UV, blue, green, red; type II, violet, green, broad-band; type III, blue, green. Note that type I is the only type containing the three classes of spectral receptors considered here. This does not necessarily mean that type I ommatidia are exclusively responsible for the task of color discrimination. Spatial and temporal scanning could have important roles, but the underlying mechanisms are fully unknown.

The limit angle of color discrimination is significantly larger for the green target than for blue and red targets (Table 3). The reflectance spectrum of the green paper (Fig. 2B) matches with the main sensitivity band of the green receptors of *Papilio* (Arikawa, 2003). It is therefore likely that the green targets have specifically stimulated the green receptors. In the *Papilio* compound eye, all of the six classes of spectral receptors are basically distributed with some spacing due to the random array of the three types of ommatidia (Arikawa and Stavenga, 1997). The array of green receptors is an exception, because the R3 and R4 of all ommatidia are green receptors. Assuming that the complete hexagonal lattice of the R3–4 green receptors is part of the color discrimination system, green colors should be discriminated best. This, however, is not the case and therefore the R3–4 green receptor system may not be directly involved in color discrimination. In fact anatomical studies demonstrated that photoreceptors other than R3 and R4 make

elaborate mutual connections in the lamina, which is presumably crucial for color vision (Takemura and Arikawa, 2006; Takemura et al., 2005). The R3–4 system is probably used for other aspects of vision, such as motion detection. We have to note that there is another group of green receptors, the R5–8 of type III ommatidia, that is used for color vision (Arikawa, 2003; Takemura and Arikawa, 2006). However, we have so far no direct behavioral evidence to identify all spectral receptor classes that are involved in the color vision of *Papilio*.

Comparative aspects

Free-flying honeybee foragers are able to detect colored targets with a visual angle larger than 5°, which corresponded to a visual field covered by seven ommatidia (Giurfa et al., 1996). Why is the minimal target size for color detection so large in honeybees? This cannot be attributed to the structure of the honeybee eye, because the interommatidial angle $\Delta\phi$ is also $\approx$1° (Land, 1997), and a rhabdom is also polychromatic with two or three classes of spectral photoreceptors (Spaethe and Briscoe, 2005; Velarde et al., 2005; Wakakuwa et al., 2005). A possible cause is the difference in the way of flight: honeybee flight is quite stable, with a small range of zigzag movement. Honeybees sometimes even hover in front of flowers, while butterflies fly with larger zigzag movements, which will result in more ommatidia to contribute for target scanning.

In fact, the best performance of target detection of honeybees at 5° is possible only when the background grey and the target color stimulated the green receptors differently, i.e. the pair of grey and color created green contrast. Otherwise the target must be larger than 15° to be discriminated. A 15° target is extremely large: a 131 mm target viewed from 50 cm, which corresponds to 55 ommatidia (Giurfa et al., 1996). Here we presented the targets on a black background to maximize the image contrast. The grey disks we used were designed to have the same subjective brightness of the colored target or to stimulate a specific type of spectral photoreceptor (blue, green or red receptors) as the colored targets do (Fig. 2, Table 1), but owing to the difference in the way of presentation, we cannot simply compare our results on *Papilio* with those of honeybees. Under the present conditions, the discrimination behavior of butterflies was not affected by the densities of grey (Table 2), indicating that the butterflies use multiple classes of spectral photoreceptors to analyze the chromatic information contained in the targets, rather than depending on contrast of a specific class of spectral photoreceptors.

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