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ACTION SPECTRUM OF FORAGING BEHAVIOR OF THE JAPANESE YELLOW SWALLOWTAIL BUTTERFLY, *PAPILIO XUTHUS**

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This paper describes the action spectrum of foraging behavior of a butterfly, *Papilio xuthus*. We first established an experimental protocol to evaluate learning and discrimination of monochromatic light by the butterflies. We trained butterflies to feed on sucrose solution at the window illuminated with certain monochromatic light produced through a monochromator. After confirming that they learned the monochromatic light, after 10 days of training, we tested the butterflies one by one. We presented training wavelengths for each individual at different intensities, and recorded whether they perform foraging behavior under freely-flying as well as tethered conditions. Freely-flying butterflies responded to light by visiting the window and searching for nectar around it, whereas tethered butterflies responded by extending their proboscides towards the window. The light intensity required to elicit 50% response for each tested monochromatic light was plotted. The resulting action spectrum for the visit was rather flat with the maximum sensitivity a 420 nm, whereas the spectrum for the proboscis extension had prominent peaks at 380, 500 and 600 nm. The difference in action spectra indicates that the visit and the proboscis extension are controlled by two independent mechanisms at least in part.

Keywords: Compound eye – color vision – monochromatic light – nectar guide – spectral sensitivity

INTRODUCTION

The Japanese yellow swallowtail butterfly, *Papilio xuthus*, feed on nectar provided by flowers of various colors. In order to see whether they see colors or not, we trained butterflies to feed on sucrose solution on a paper patch of certain color and tested their color discrimination ability. The trained butterflies successfully selected patches of the color with which they were trained among several patches of different colors including different shades of grays. The result indicates that the butterflies have the ability to discriminate visual stimuli depending on their chromatic contents irrespective of their brightness: they have color vision [1]. We also demonstrated that the butterflies are color constant, i.e. the color discrimination ability of butterflies

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was basically unchanged even if the color patches were illuminated with colored lights [2].

For proper interpretation of these behavioral results, the brightness of color patches must be measured accurately. The brightness is, of course, not for humans but for butterflies: we have to know the butterfly-subjective brightness somehow. In the previous studies, we calculated the *Papilio*-subjective brightness (B_i) of a paper i by

$$B_i = \int_{325}^{700} I(\lambda)\, R_i(\lambda) S(\lambda)\, \mathrm{d}\lambda$$

where $I(\lambda)$ is the irradiance spectrum of illumination, $R_i(\lambda)$ is the reflectance spectrum of color paper i, and $S(\lambda)$ is the sensitivity spectrum of the compound eye of *Papilio xuthus* determined by electroretinogram (ERG) recording [1]. Use of the ERG-determined $S(\lambda)$ for this purpose is considered to be acceptable but not ideal, because the retinal sensitivity does not necessarily provide linear spectral input to the color vision system. More reasonable function is the action spectrum of the foraging behavior itself.

Therefore, we here measured the action spectrum of the foraging behavior of *Papilio xuthus* by using a series of monochromatic light ranging from 360 nm to 680 nm. We carried out two separate experiments for two aspects of foraging behavior, i.e. the proboscis extension behavior towards the monochromatic light and the visit of freely flying butterflies to the monochromatic light.

MATERIALS AND METHOD

Animals

We used newly emerged spring form females reared in the laboratory. The laboratory stock was 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 fresh citrus leaves under a light regime of 10 h light: 14 h dark at 28 °C. The pupae were stored at 4 °C for at least for 3 months and allowed to emerge at 28 °C prior to the experiments. The day of emergence was defined as the post-emergence day-1.

Experimental setup

Behavioral experiments were performed on a bench ($150 \times 50 \times 20$ cm^3) set in a room (temperature = 28 °C, humidity = 60%). The bench top was made of black plastic plate (Fig. 1a, inset). In the center the plastic bench top has a hole of 10×10 cm^2, which is covered by a piece of frosted quartz glass served as a transparent projection

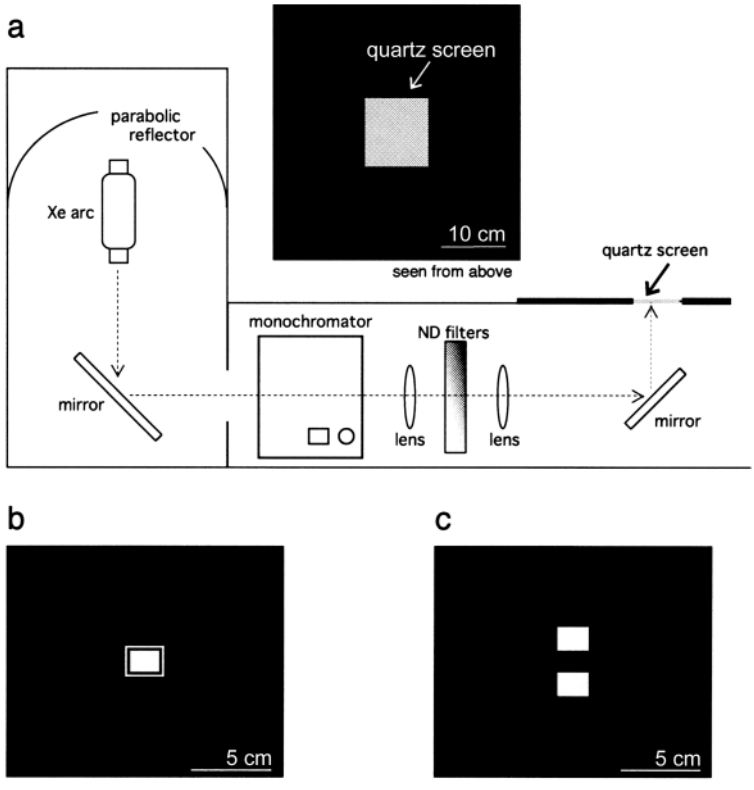

Fig. 1. a) Optics for presenting monochromatic stimulus. Inset shows the top view of the bench, which in the center has a hole of 10×10 cm^2 covered with a piece of frosted quartz glass. At the end of the optics the light was reflected by mirror 2 up towards the quartz glass from below. b) Single-windowed plate used during the training sessions. c) Double-windowed plate used during the test sessions

screen. The bench top was illuminated with four 300 W halogen bulbs at the luminosity of 1,500–2,000 lux at the surface.

Monochromatic stimuli (ranging between 360 nm and 680 nm) were provided by a 500 W Xenon arc through a monochromator (Shimadzu) and a set of quartz neutral density filters. The optics was set beneath the bench, and the monochromatic light whose intensity was adjusted was reflected up towards the quartz transparent screen from below. The light was thus observable from above (Fig. 1). The photon flux of each monochromatic light was measured at the screen surface by a radiometer (Model 470D, Sanso, Tokyo, Japan), which was used to calibrate the behavioral results afterwards.

Behavioral experiments consist of two sessions, the training and the test (see next section). During the training sessions, the quartz screen was covered with a piece of black plastic plate (15 × 18 cm^2) with a window of 1.5 × 2.0 cm^2 in the center (sin-

gle-windowed plate, Fig. 1b). The window was surrounded by a gutter of 2 mm width in which we put sucrose solution for reward. During the test sessions, the screen was covered with a black plastic plate (15×18 cm^2) with two 1.5×2.0 cm^2 windows separated with 15 mm gap (double-windowed plate, Fig. 1c). A cage ($W \times D \times H = 50 \times 50 \times 50$ cm^3) was placed on the bench when we carried out the tests of visit using freely flying butterflies.

Behavioral experiments

Visit and proboscis extension are two consecutively occurring events of foraging behavior in the field. Here visit was defined as the behavior of freely flying butterfly landed on the floor of the cage, the bench top, and extended its proboscis towards the window from which the stimulus light could be seen. For observing proboscis extension, we used butterflies tethered by clipping their wings with cardboard. We moved clipped butterflies sliding on the bench top towards the window. The butterfly spontaneously extended its proboscis and searched for sucrose solution by touching around the window with the proboscis, which we defined as the proboscis extension.

Training

We used a single-windowed plate (Fig. 1b) for trainings. We started the training on the post-emergence day-2. Protocol of the training is summarized in Fig. 2. One individual was always trained at one wavelength of light. We first brought a starved naive butterfly close to the window illuminated with a monochromatic light. There we fed the butterfly on 8% sucrose solution for 3–5 sec, and repeated such short feeding

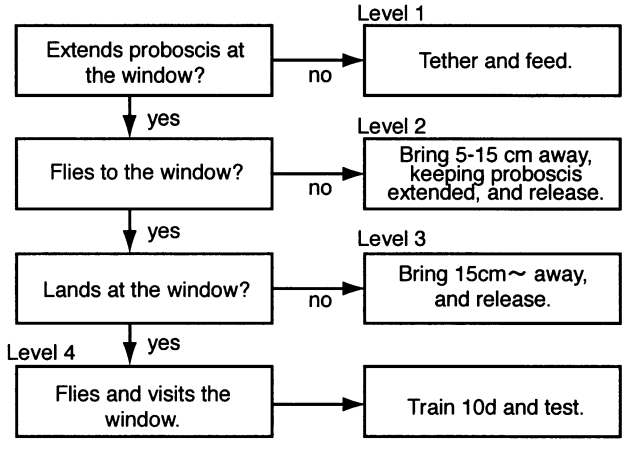

Fig. 2. Flow chart of training. Level 2–4 individuals were used for tests. See text for details

until the butterfly stopped taking sucrose solution, i.e. until the butterfly became full. When the butterfly became full, we stopped the training of the day. We repeated such training typically for 10 days, during which the response of butterflies usually changed. The response can be divided into four levels: level 1, no positive response; level 2, proboscis extension; level 3, proboscis extension plus approach (no landing); level 4, spontaneous visit. Most successful individuals reached the level 4 in a few days. For level 1 individuals, we brought them at the window and manually extended the proboscis using a needle to feed them on sucrose. For level 2, we let them perform proboscis extension at the window, and then brought back into the air at the distance of 5–15 cm from the window keeping the proboscis extended towards the window, and released. If they landed and took sucrose at the window, we let them take sucrose for several seconds, and repeated it until they became full. If not, we again brought them close to the window and uncoiled the proboscis manually. For level 3, we fed the butterflies manually at the window, and released from a distance of about 15 cm from the window. If the butterflies landed at the window, we let them continue. When the butterflies did not land, we again forced them to take sucrose at the window. For level 4 individuals, we let them visit the window and take sucrose, chasing once every 5 sec from the window.

Test

We used level 2–4 individuals for the tests. We illuminated light to one of the two windows of the double-windowed plate (Fig. 1c), and other window was kept un-illuminated. Butterflies were subjected to select one of the windows. By using a double-windowed plate, we could be certain that the butterflies did not respond to the window itself. Position of illuminated window was changed randomly in order to cancel the bias of stimulus position. Proboscis extension was tested for all individuals, whereas visit was tested only with level 4 individuals. We tested butterflies one by one.

Protocol of the test is summarized in Fig. 3. When butterflies did not respond to presented stimuli at any circumstances, we checked their motivation by presenting the maximum intensity of training wavelength and gave a small amount of reward: only by doing such motivation check, we can be sure whether the butterflies did recognize the stimuli or not (Fig. 3a). In addition to the motivation check, we fed the butterflies with small amount of sucrose solution once every 3 or 4 responses showing the training monochromatic light of maximum intensity by using the single-windowed plate. This was particularly important for maintaining their motivation throughout the tests. If they did not get any reward during the test sessions even though they answered correctly, they readily learned that they never get any reward and stopped responding.

On the first day of the test, we checked whether the butterflies perform foraging behavior within 30 sec responding to the light of trained wavelength at the maximum intensity. After confirming the performance of expected behavior, either visit or pro-

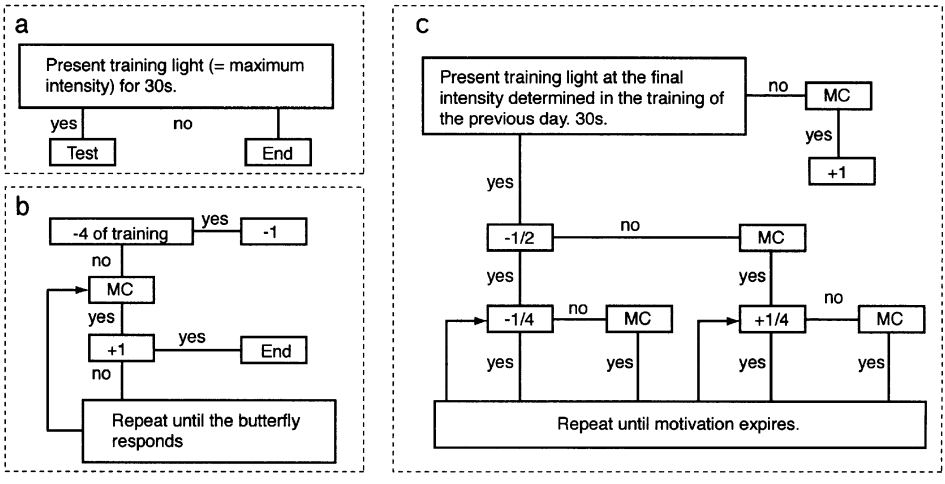

Fig. 3. Flow chart of tests. Performance was judged in 30s. If butterflies responded to the light within 30s, we recorded it as positive. Otherwise, negative. a) Protocol of checking motivation. This motivation check (MC) was done when necessary in the tests. Only we are sure that the butterflies maintain their motivation for foraging behavior, we continued tests. b) Test for day-1. c) Test for day-2 or later. Max, maximum intensity of monochromatic light of training wavelengths; MC, motivation check; Numbers with plus or minus (e.g. –4, +1/4), intensity change of light in log unit (e.g. 4 log unit darker or 1/4 log unit brighter. Comparison with the previous test light otherwise specified). See text for details

boscis extension, we presented the same wavelength of light at the intensity of 4 log units reduced (= 1/10,000 of the maximum). If the butterfly still performed foraging behavior to the dim light, we further reduced the intensity another 1 log unit, and tested the behavior: no butterflies responded to light of 5 log units reduced intensity. If the butterfly did not respond to 4 log units reduced light, we increased the light intensity stepwise at the interval of 1 log unit to determine, roughly, the threshold intensity that elicit foraging behavior (Fig. 3b).

On the second day and subsequent days of tests, we started by presenting the threshold intensity we determined for the individual by the test of the day before. When the butterfly responded to the light of threshold intensity, we further reduced the intensity by 0.5 log unit and checked the response. If the light of threshold intensity did not elicit response, we increased the intensity by 0.25 log unit. By repeating such trials, we determined the threshold intensity at the sampling rate of 0.25 log unit for each individual (Fig. 3c).

We measured the threshold intensity for each individual at least for 3 times, and we used at least 3 individuals for each test wavelength. We thus plotted the percent response versus photon flux. The photon flux required for eliciting 50% response was plotted versus wavelength to obtain the action spectrum of foraging behavior.

RESULTS

Among about 600 individuals we used, 111 individuals were (more or less) successfully trained to one of the 17 different test wavelengths.

Figure 4 shows the log intensity-response functions for visits (Fig. 4a) and proboscis extension (Fig. 4b). In all wavelengths tested, the slope of these curves seems rather constant with the dynamic range of about 1–1.5 log unit.

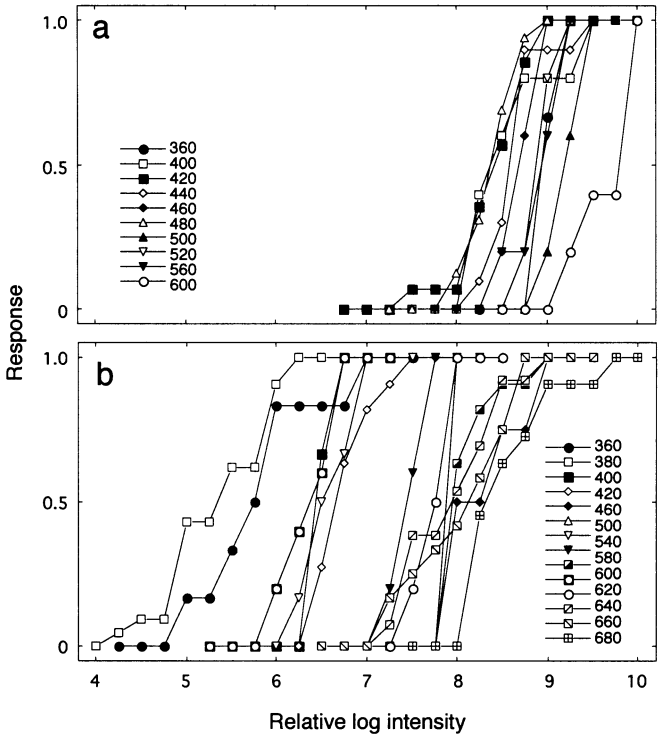

Fig. 4. Response-log stimulus intensity functions of visit (a) and proboscis extension (b) at different wavelengths

We calculated the relative photon number to elicit 50% response at different wavelengths for both visit and proboscis extension. Reciprocals of the photon numbers were plotted as sensitivities versus wavelength of light. Figure 5 shows the resulting action spectra. Most conspicuously, these action spectra have different profiles. For visits, the spectrum is rather flat with the maximum sensitivity at 420 nm. On the other hand, the spectrum for proboscis extension expresses three prominent peaks at 380, 500 and 600 nm. In addition to the difference in the peak wavelengths, it turned out that visit requires more light compared to proboscis extension under the present experimental condition.

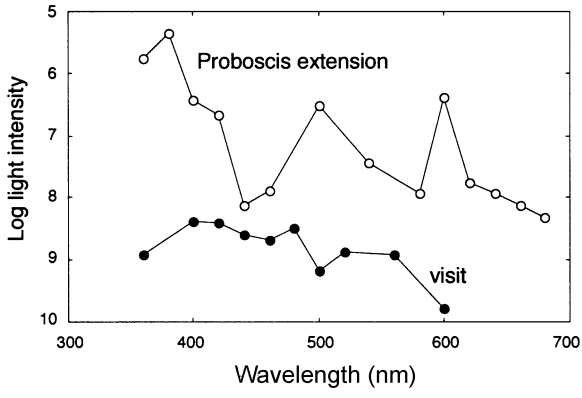

Fig. 5. Action spectra of visit (filled circles) and proboscis extension (open circles). Light intensities required for eliciting 50% response were plotted against wavelengths

DISCUSSION

Kinoshita et al. [1] demonstrated that *Papilio xuthus* has true color vision when searching for food. They used laboratory-reared summer-form females for all of their tests. In the study they presented four colored patches of blue, green, yellow and red to newly emerged individuals kept for 2 days after emergence without food, and recorded the color of the disk which the butterfly visited for the first time in their life as their innate color preference. It thus appeared that the summer-form females prefer yellow and red over blue and green [1]. But the preference is not identical for spring-form females: spring-forms rather prefer blue and learn blue more quickly over the other three colors (Kinoshita, personal communication). This observation seems to be supported by the present results. The action spectrum of visits has the primary peak in the blue wavelength region, indicating that the spring-form females are more efficiently attracted by blue when searching for food, at least in the very early stage of their adult life.

The action spectrum measured by proboscis extension has the primary and the secondary peaks in the ultraviolet (380 nm) and the red (600 nm) wavelength regions, respectively. These two wavelength regions are basically covered by UV receptors and red receptors among five different types of spectral receptors in the *Papilio* eye [3]. Although the existence of UV receptors in the retina strongly indicates that *Papilio* can see UV, there have been no direct behavioral evidence for the actual use of UV light so far. High efficiency of UV light for eliciting their proboscis extension supports the idea that they use UV-involved visual pattern upon searching for food.

Difference in action spectrum between visit and proboscis extension indicates that there are two separate systems each controlling these different levels of foraging behavior. In the field, butterflies search for flowers from a certain distance, of probably several meters. When they find a candidate, they visit to it, and after confirm-

ing the flower is a good source of nectar, they extend their proboscis to receive food. How do butterflies see their surroundings in the course of the sequence of the foraging behavior? Judging from the optical structure of their compound eyes, the spatial resolving power is not particularly high: the resolution limit has been assumed to be about 1° at most, which is much worse than humans whose resolution limit is about 1/60°. Visual angle of 1° roughly corresponds to the item of 1 cm diameter at a distance of 50 cm. Note that many flowers are too small to be precisely detected by the visual system of flying butterflies. Butterflies most likely view the rough structure of vegetation from a distance, using the system with the 'visit action spectrum' and approach to some appropriate target. Many flowers possess a color pattern called nectar guide, which involves absorption/reflection of UV light. We know that the nectar guides are actually used by butterflies [5]. Once they are close enough to certain flowers, they shift the search mode to the system with the 'proboscis extension action spectrum', and detect the characteristic nectar guide to locate the food precisely.

Sensitivity in the green wavelength region appeared to be not particularly high in both action spectra. The action spectrum of foraging behavior of a nymphalid species also expresses decreased sensitivity in green [6]. Green light is not strongly connected to foraging behavior in the case of butterflies. In fact, butterflies have to spend longer time to learn green as a food source [1]. Instead, green is the strongest sign of leaves for female butterflies as the potential candidate of food plant for larvae on which they lay eggs. In fact the action spectrum of oviposition behavior of *Pieris brassicae* [7] expresses high sensitivity in the wavelength range of 500–600 nm.

ACKNOWLEDGEMENTS

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