Color discrimination on orientation of female *Eurema hecabe* (Lepidoptera: Pieridae)

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Abstract

The influence of color discrimination on female host location was investigated in *Eurema hecabe* (Lepidoptera: Pieridae). Mated females with no oviposition experience were presented with artificial plant models treated with the methanol extract of the host plant, *Lespedeza cuneata* (Fabaceae). When gray models of different intensity were presented to females, they landed and deposited eggs more frequently on the light gray model. However, when a yellow green model was presented to females with the gray, they landed and deposited eggs predominantly on the yellow green model. In the latter experiment, most females did not visit the gray models frequently, although one of the gray models had a similar intensity to that of the yellow green model. This result supports that *E. hecabe* distinguish the yellow green model by color.

Key words: Lepidoptera; Pieridae; host-finding behavior; visual cues; color discrimination

INTRODUCTION

Various sensory cues are essential for insects to locate and reach the host plants properly and then perform the appropriate behavior on those plants (Bernays and Chapman, 1994). When insects locate host plants from far away and approach them, visual and olfactory cues play essential roles (Prokopy and Owens, 1983). After landing, insects recognize the species and the quality of a plant to decide what behavior they should perform (Renwick and Chew, 1994). In the coevolution of herbivores and foodplants, the plants are selected to prevent herbivores from feeding or ovipositing (Futuyma, 1986). It follows that most plants produce secondary metabolites that hinder the normal development of herbivores. Since in many herbivores the metabolism that detoxifies some secondary metabolites has evolved, these herbivores feed on plants of specific taxa. The contact chemical sense is, therefore, crucial for phytophagous insects to recognize whether or not the plant is an appropriate host (Renwick and Chew, 1994).

Visual cues are also important for phytophagous insects to land on appropriate foodplants effectively. The experimental study indicates that the Japanese pierid butterfly *Eurema hecabe* also uses visual cues to locate their foodplants. Females of *E. hecabe* visit and lay eggs predominantly on artificial plant models that have a similar color (yellow green) and leaflet morphology to the appropriate foodplants, even when all models are treated equally by the chemicals of foodplants (Hirota and Kato, 2001). However, the previous study is insufficient to prove that *E. hecabe* discriminate color by hue to locate the foodplants, since the preference to lightness is not evaluated using gray models (Kinoshita et al., 1999).

The primary aim of the present study was to investigate whether gravid females of *E. hecabe* discriminate color by hue in host location. We evaluated the preference of gravid females to some gray models, and compared it with the preference to a yellow green model.

MATERIALS AND METHODS

Insect. In the mainland of Japan, *E. hecabe* is multivoltine with seasonal wing-pattern (Kato and Sano, 1987) and overwinters in adulthood. This species uses some plants belonging to Fabaceae. In the mainland of Japan, the larvae of this butterfly are frequently found on the sericea lespedeza, *Lespedeza cuneata* (Fabaceae: Papilionoideae) and the

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silk tree, *Albizia julibrissin* (Fabaceae: Mimosoideae) (Kato, 1994). The choice test in the laboratory revealed that gravid females oviposited mainly on those plants (Kato et al., 1992). In the present study, females were caught in a field in Mitaka, Tokyo, Japan. They were allowed to lay eggs on *L. cuneata* in the laboratory. Larvae were reared on the leaves of *L. cuneata* at 25°C under a photoperiod of L16 : D8. After adult eclosion, females were allowed to mate with males. Mated females were kept individually in plastic cups (11 cm in diameter, 5 cm in depth) with 10% sucrose solution until they were used in the following experiments. Those females were not used repeatedly for different experiments.

Plant models. Five kinds of plant models that have white, light gray, dark gray, black and yellow green leaves were made to evaluate the color discrimination of gravid females. The yellow green leaf had quite similar spectral reflectances to the leaves of *L. cuneata*, and was selected dominantly by gravid females in a previous study (Hirota and Kato, 2001). The light gray leaf looks like the yellow green leaf when they are observed using a monochrome video camera. The plant models had a 3×3 cm square leaf of colored paper (Hi-New Colour, Shikoku paper Co., Ltd.) (Fig. 1). The surface structures were the same among the papers of different colors. The colored paper was treated with 1 ml/cm² of the leaf extract, which was obtained by dipping 1 kg of fresh young leaves of *A. julibrissin* in 3 *l* of methanol for one month at room temperature under darkness, according to the method of Honda (1986). The spectral reflectances of colored paper treated with the extract were examined with a spectrophotometer (UV-260, Shimadzu) equipped with an integrating sphere assembly (ISR-260, Shimadzu) (Fig. 2). The spectral reflectances of leaves of *L. cuneata* were compared with those of plant models.

Observations. Distilled water was sprayed onto the plant model to help the female detect the leaf extract (Honda, 1986; Nishida and Fukami, 1989). Four kinds of models were placed at intervals of about 20 cm in an acrylic cage (30×30×30 cm), which had a screened window at one side and a sheet of brown colored paper on the floor. To evaluate the preference for lightness, white, light gray, dark gray and black models were presented to ten females. To evaluate the preference for hue, the
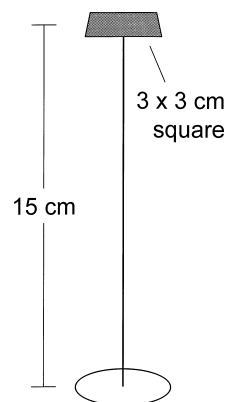
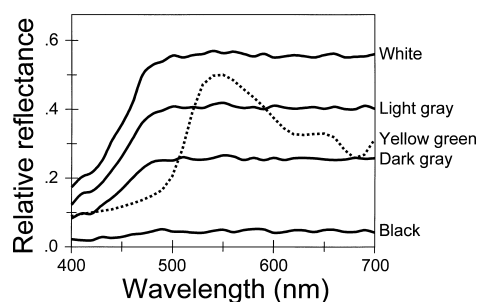

Fig. 1.   Plant models.

Fig. 2.   Spectral reflectances of the plant models.

white, light gray, black and yellow green models were presented to sixteen females. The cage was illuminated with two 30 W fluorescent tubes in an air-conditioned room (at 25°C). The females were given 10% sucrose solution by proboscis extension half an hour before the experiments. After the females were released into the acrylic cage, their behavior was monitored with an 8 mm video camera for two hours (Hi8, Sony). To eliminate the influence of interaction among females, only one female at a time was into the same cage. At intervals of 30 min, the plant models were sprayed with distilled water, and their location within the cage was randomly replaced to eliminate the influence of position. After each test the deposited eggs were counted, and the landings of a given female on each model were examined from the video-record. For each female the test was carried out during 12:00–18:00 and repeated for two days.

Statistical analyses. The frequency distribution of landings over plant models was compared among females to evaluate whether the data of different females can be pooled (Heterogeneity chi-square analysis) (Zar, 1999). When the frequency

distribution was not heterogeneous among females, the log-likelihood ratio test was applied to the pooled data (Zar, 1999). When the frequency distribution was significantly heterogeneous among females, the data were analyzed by the Friedman test and the multiple comparison for nonparametric repeated measures data (Zar, 1999).

Logistic regression analysis was also carried out by StatView software (SAS Institute, Inc., 1998) to evaluate whether the frequency of oviposition after landing (oviposition rate) was different among the models. In this analysis, whether or not each landing was followed by oviposition was set as the dichotomic dependent variable, and the kinds of plant models and the females who performed the landing and oviposition were selected as nominal individual variables.

RESULTS

When the white, light gray, dark gray and black models were presented to ten females, the preference to those models was different among females (Fig. 3). Heterogeneity chi-square analysis showed that the distribution of landing over four models was significantly heterogeneous among females ($\chi^2 = 682$, df=27, $p < 0.001$). The Friedman test showed that females alighted on models non-randomly ($F_{3,27} = 7.91$; $p < 0.003$). Multiple comparison showed that the light gray model was chosen more frequently than the black models ($p < 0.005$). The logistic regression analysis did not show a significant difference in oviposition rate among models.

When the white, light gray, black and yellow green models were presented to 16 females, most females landed on the yellow-green model at more than 80% of all landings (Fig. 4). The distribution of landing was homogeneous among females (Heterogeneity chi-square analysis, $\chi^2 = 537$, df=45, $p < 0.001$). The Friedman test showed that females alighted on models non-randomly ($F_{3,45} = 90.04$; $p < 0.001$). Multiple comparison showed that the yellow green model was chosen more frequently than the other models ($p < 0.005$), and that the white and the light gray models were chosen more frequently than the black model ($p < 0.02$). The logistic regression analysis showed that the oviposition rate was significantly different among plant models as well as among females. Using this analysis we estimated that the oviposition rate on the yellow green model was 2.8 (1.9 to 4.2, 95% confidence limit) times as high as that on the white model, and 1.7 (1.2 to 2.6, 95% confidence limit)
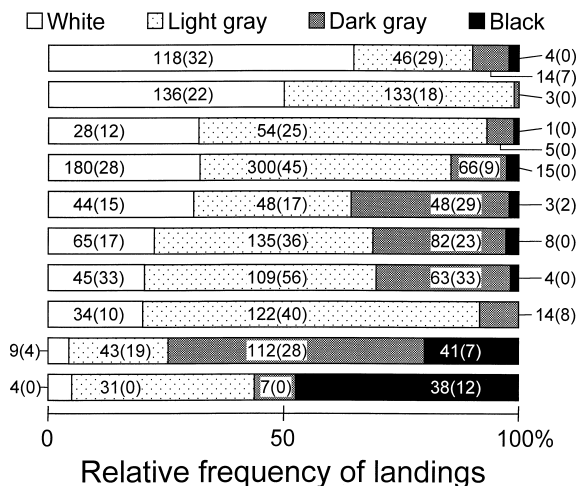

Fig. 3. Frequencies of landing on the gray models of different intensities. Different bars represent the data of different females. Numerals in and beside bars represent the number of landings. Numerals in the parentheses represent the number of eggs deposited.
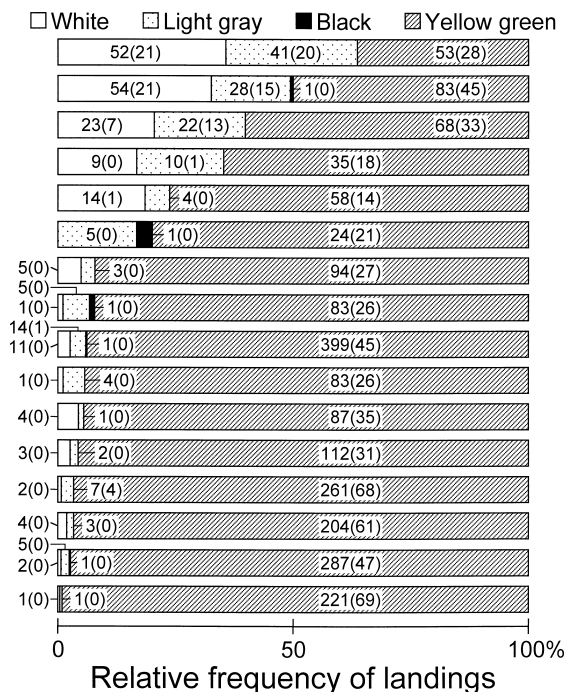

Fig. 4. Frequencies of landing on a yellow green model and three gray models of different intensities. Different bars represent the data of different females. Numerals in and beside bars represent the number of landings. Numerals in the parentheses represent the number of eggs deposited.

times as high as that on the light gray model (Wald test, $p < 0.01$). The significant difference was not detected between other models (Wald test, $p > 0.05$).

DISCUSSION

Females of *E. hecabe* had a heterogeneous preference to the gray models of different lightness. Females visited and oviposited on the light gray model most frequently, although their preferences were significantly different among females. When the yellow green model was presented to females, however, most did not visit the gray models predominantly. All females visited and oviposited on the yellow green model the most frequently. Although we also performed some multiple choices for ovipositing females, no model was selected as frequently as the yellow green model of the present study. This result indicates that females of *E. hecabe* discriminate the color by hue for host location.

When the yellow green model was presented with the gray models, the proportion of oviposition following landing was also different among color models. The oviposition rate was high on the yellow green model where females landed more frequently. A similar result was observed in the experiment where the yellow green model was presented to females with yellow, green and blue models (Hirota and Kato, 2001). It follows that the hue would influence reproductive drive after landing.

Monochromatic light is also a cue for behavior. *Pieris brassicae* performed the drumming and ovipositing behavior for the monochromatic lights of 500–600 nm, whereas feeding behavior was stimulated by the monochromatic lights of other wavelengths (Scherer and Kolb, 1987). However, the whole pattern of spectral reflectance is more important as an attractant for ovipositing females. *Delia radicum* (Diptera: Anthomyiidae) preferred the radish color that has a peak reflectance around 540 nm (Prokopy et al., 1983). However, *D. radicum* did not prefer the green and red cabbage leaves, which reflect a broad range of wavelengths including 540 nm intensively. A similar phenomenon was observed in the present study. Although *E. hecabe* females preferred the yellow green model that also was around 540 nm, they did not prefer the white model that reflected 540 nm more

strongly than the yellow green. This result suggests that the ovipositing behavior was suppressed by other wavelengths including the white model.

Kelber (2001) discussed how the inputs from different types of photoreceptors influenced the decision making of insects. His statistical model suggested that in *Papilio aegeus* and *Pieris brassicae* a green-sensitive receptor facilitated the oviposition, but a blue- and red-sensitive receptor suppressed it. In *P. rapae* a red-sensitive receptor was not related, although a blue- and green-sensitive receptor had a similar influence on *P. aegeus* and *P. brassicae*. The same physiological process would relate to the ovipositing behavior of *E. hecabe*. Unfortunately we can not apply the Kelber's model to *E. hecabe* because of the lack of information on the sensitivity of photoreceptors of *E. hecabe*. Since *Lycaena heteronea* have different photoreceptor distribution from the cogeneric species, *L. rubidus*, the data of related species (e.g. *Pieris* butterflies) are not always informative (Bernard and Remington, 1991). Further study is necessary to investigate the photoreceptor of *Eurema hecabe* and understand the physiology of host location by visual stimuli.

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