



Influence of visual stimuli on host location in the butterfly, *Eurema hecabe*

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Abstract

The influence of visual stimuli 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 models of different colours were presented to females, they landed and deposited eggs predominantly on the yellow-green model. However, the size of the model was not important. When females were presented with simple square models and a model on which small squares were assembled, they predominantly chose the assembly model. This suggests that female discern the pattern which resembles the leaf of their main host plants, which consists of many small leaflets. However, the whole image of a leaf of the host plant was not an essential cue for host location, as females also chose the square with the central space more frequently than that without the space. We suggest that the long contour of a complicated form plays a role in female host location.

Introduction

Various sensory cues are essential for insects to locate and reach the host plants properly and then perform the appropriate behaviour on those plants (Bernays & Chapman, 1994). When insects locate host plants from far away and approach them, visual and olfactory cues play essential roles (Prokopy & Owens, 1983). After landing, insects recognise the species and the quality of a plant to decide what behaviour they should perform (Renwick & Chew, 1994).

In the coevolution between pollinators and flowers, plants are generally selected to facilitate the flower visitation by pollinators (Barth, 1985). It follows that entomophilous flowers are likely to have a conspicuous form and scent and are prominent from a distance. The complicated form of the flowers is also an attractive visual cue for pollinators. Pollinators prefer the long contour of complicated figures and the long boundary between areas of different colours (e.g., Hertz, 1930; Ilse, 1932; Vaidya, 1969). Honeybees also use the contour length in associative learning (Lehrer, 1999).

In the coevolution of herbivores and food plants, on the other hand, 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 many herbivores evolved a metabolism that detoxifies some secondary metabolites, they feed on the plants of specific taxa. The contact chemical sense is, therefore, crucial for phytophagous insects to recognise whether the plant is an appropriate host or not (Renwick & Chew, 1994).

Visual and olfactory cues are also important for phytophagous insects to land on appropriate food plants effectively. Gravid females alight non-randomly on plants with leaves of similar shape to those of food plants (Papaj, 1986; Mackay & Jones, 1989). Some experimental studies indicate that the size, direction and arrangement of leaves also have roles in the recognition of an appropriate food plant (e.g., Harris et al., 1993; Foster et al., 1997; Rojas & Wyatt, 1999). However, little attention has been paid to the role of contour length of leaves in the host recognition by phytophagous insects, especially ovipositing

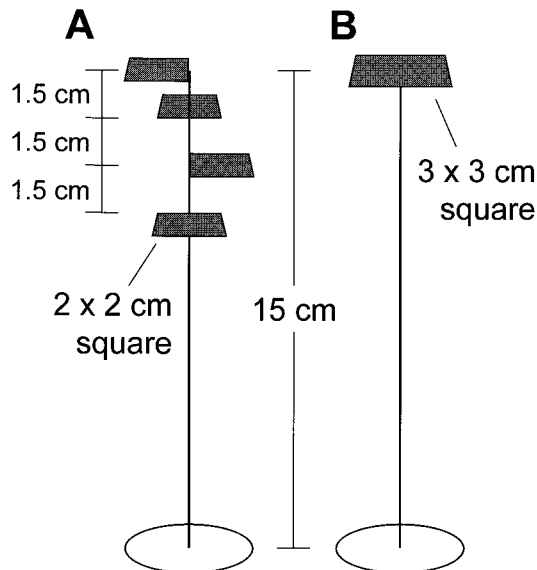


Figure 1. Plant models. (A) The model used in Experiments 1 and 2. Four squares of the same colour are attached on a model. (B) The model used in Experiments 3 to 5. The 3 × 3 cm square on the top is replaced by the squares of different sizes and morphology according to the purpose of experiments.

females. Since the organs of some food plants have, like flowers, a complex morphology, it is conceivable that insects that feed on those plants use contour length in host location. We examined this hypothesis, using the Japanese pierid butterfly *Eurema hecabe*, whose host plants have complex leaves.

The primary aim of the present study is to investigate whether host location of gravid females depends on the contour length of ovipositional substrates. As we have no information on other visual cues which might play a role in the oviposition behaviour of *E. hecabe*, the effects of leaf colour and leaf size were also investigated.

Materials and methods

In the mainland of Japan, *E. hecabe* is multivoltine with seasonal wing-pattern (Kato & Sano, 1987) and overwinters in adulthood. This species uses some plants belonging to the Fabaceae. In Japan, the larvae of this butterfly are frequently found on the sericea lespedeza, *Lespedeza cuneata* (Fabaceae: Papilionoideae) and the silk tree, *Albizia julibrissin* (Fabaceae: Mimosoideae) (Kato, 1994). A choice test in the laboratory revealed that gravid females oviposited mainly on those plants (Kato et al., 1992).

Lespedeza cuneata is a herbaceous perennial and has compound leaves that are composed of leaflets (1.0–1.5 cm in length, 0.2–0.6 cm in width). *Albizia julibrissin* is a deciduous arbor and has compound leaves (5–15 cm) which are composed of elliptic leaflets (1.0–1.7 cm in length, 0.4–0.6 cm in width).

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.

Experiment 1

Methods

Plant models. Four kinds of plant models having blue, green, yellow and red leaves, respectively, were made to investigate which colours gravid females prefer. The plant models consisted of four square leaves (2 × 2 cm) of the same coloured paper (Hi-New Colour, Shikoku Paper Co., Ltd.) (Figure 1A). The coloured paper was treated with 1 ml cm⁻² of the leaf extract, which was obtained by soaking 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 preliminary experiments revealed that when coloured papers not treated with the extract were presented, gravid females did not actively perform the flight behaviour for host location. The spectral reflectances of coloured paper treated with the extract were examined with a spectrophotometer (UV-260, Shimadzu) equipped with an integrating sphere assembly (ISR-260, Shimadzu) (Figure 2A).

Observations. Distilled water was sprayed onto the plant model to help the female detect the leaf extract (Honda, 1986; Nishida & Fukami, 1989). Blue, green, yellow and red 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 coloured paper on the floor. The cage was illuminated with two 30 W fluorescent tubes in an air-conditioned room (at 25 °C). The females were given

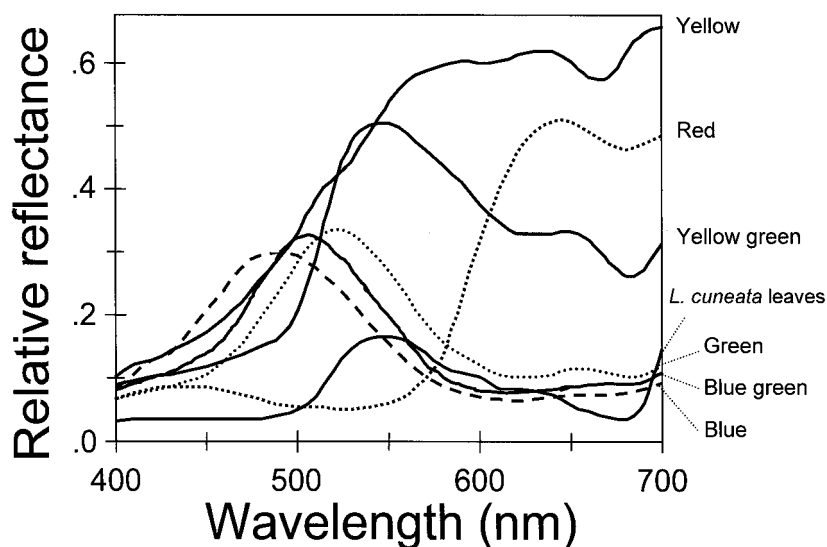


Figure 2. Spectral reflectances of the different coloured models and the leaves of *L. cuneata*.

10% sucrose solution by proboscis extension half an hour before the experiments. After the females were released into the acrylic cage, their behaviour was monitored with an 8 mm video camera for two hours (Sony, Hi8). To eliminate the influence of interaction among females, only one female was released at one time 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 changed to eliminate the influence of position. After each test the deposited eggs were counted, and the landings of a female on each model were examined from the video-record. Eleven females were used in the tests, and the test for each female was carried out two hours 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 for the chi-square test (Heterogeneity chi-square analysis) (Zar, 1999). If the frequency distribution was not heterogeneous among females, the chi-square analysis was applied to the pooled data. When the frequency distribution was significantly heterogeneous among females, the data were analysed by the Friedman test, where the models were ranked for each female by the frequency of landings and the randomness of the distribution of rank over models was evaluated by the randomisation method (see also Zar, 1999). When the Friedman test showed significant

treatment effects, we carried out multiple comparisons for nonparametric randomised block (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, the presence or absence of oviposition after each landings 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

Nine out of 11 gravid females landed on the green model most frequently, whereas the other two females landed mostly on the yellow model (Figure 3). Heterogeneity chi-square analysis showed that the distribution of landing over four models was significantly heterogeneous among females ($\chi^2 = 287$, $df = 30$, $P < 0.001$). The Friedman test showed that females alighted on models non-randomly ($F_{3,30} = 6.87$; $P = 0.001$). Multiple comparison showed that the green model was chosen more frequently than the blue and red models ($P < 0.02$).

The logistic regression analysis showed that the oviposition rate was significantly different among plant models as well as among females. This analysis estimated that the oviposition rate on the green model was 2.2 times higher than that on the blue model (Wald test, $P < 0.05$), and that the oviposition rate on the yellow model was also 2.4 times higher than that on

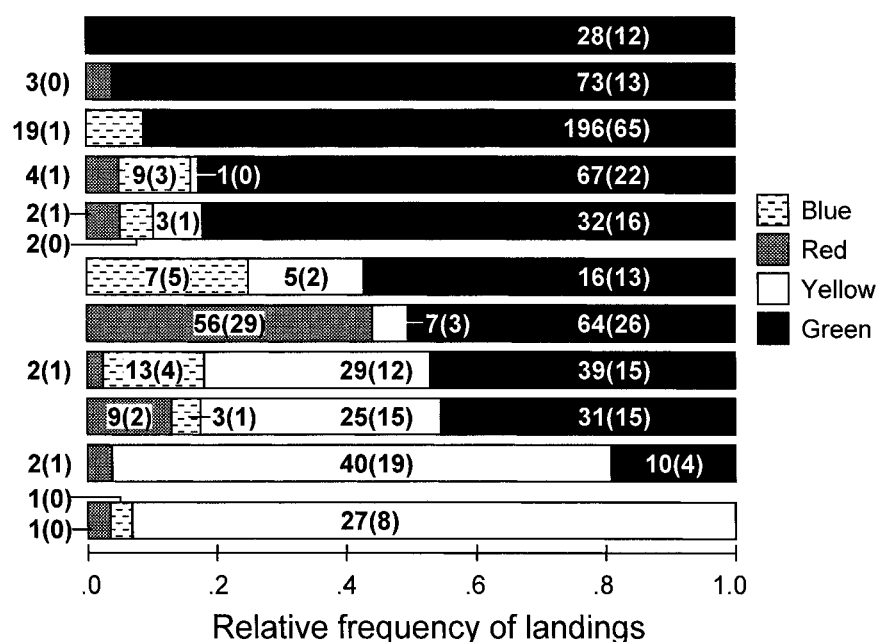


Figure 3. Frequencies of landing on the different colour models. Different bars represent the data of different females. The numerals in and beside bars represent the number of landings. The numerals in the parentheses represent the number of deposited eggs.

the blue model (Wald test, $P < 0.05$). No significant difference was detected between other models (Wald test, $P > 0.05$).

Experiment 2

Methods. To investigate ovipositional preference for colour in detail, we made blue-green and yellow-green models that have a hue close to that of the green model which was chosen the most frequently in Experiment 1. Those models were made in the same manner as in Experiment 1 (Figures 1A and 2). The spectral reflectances of leaves of *L. cuneata* were compared with those of coloured models. Eight females were observed in the same manner of Experiment 1 except that the blue-green and yellow-green models, instead of blue and red models, were presented to a gravid female together with the green and yellow models.

Results. Although all of the 8 females landed predominantly on the yellow-green model (Figure 4), the distribution of landing was significantly heterogeneous among them (Heterogeneity chi-square analysis, $\chi^2 = 75.9$, $df = 21$, $P < 0.001$). The Friedman test showed that gravid females alighted on models non-randomly ($F_{3,21} = 18.2$, $P < 0.001$). Multiple comparisons showed that the yellow-green model was chosen

more frequently than the yellow and blue-green models ($P < 0.005$). The yellow-green model that was chosen predominantly had spectral reflectances similar to those of *L. cuneata* (Figure 2).

Logistic regression analysis showed that the oviposition rate was significantly different among plant models as well as among females. It was estimated that the oviposition rate on the yellow-green model was 3.8 times higher than that on the yellow model (Wald test, $P < 0.05$), and that the oviposition rate on the green model was 3.3 times higher than that on the yellow model (Wald test, $P < 0.05$). No significant difference was detected between other models (Wald test, $P > 0.05$).

Experiment 3

Methods. We made three plant models that had square leaves of different sizes to investigate whether gravid females had a preference for leaf size (Figure 1B). The leaves of those models were made with yellow-green papers of different sizes (0.75×0.75 , 1.5×1.5 , 3×3 cm). Eight females were observed in the same manner as in Experiment 1 except that three models were located at the apexes of the regular triangle in the acrylic cage.

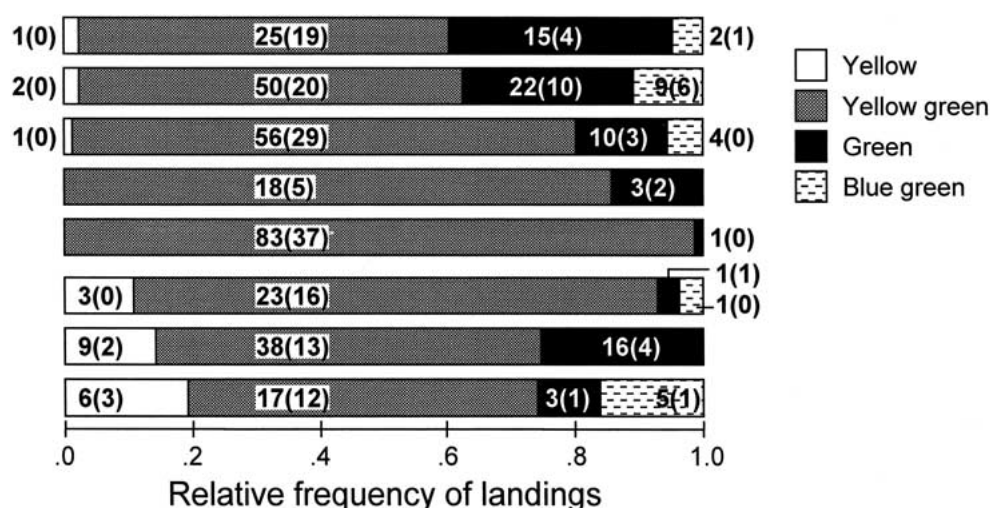


Figure 4. Frequencies of landing on the different colour models. Different bars represent the data of different females. The numerals in and beside bars represent the number of landings. The numerals in the parentheses represent the number of deposited eggs.

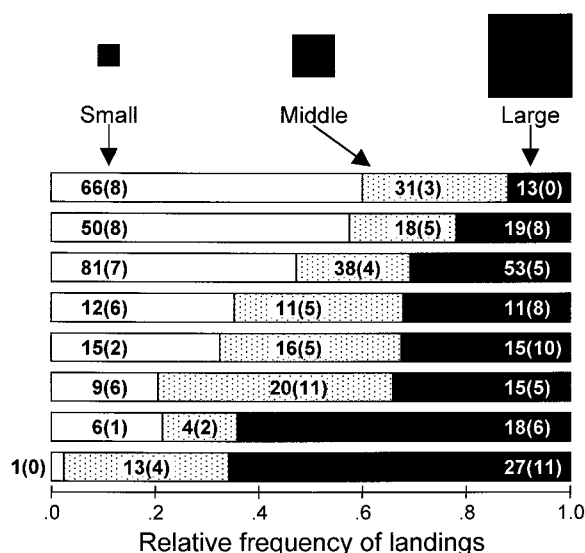


Figure 5. Frequencies of landing on the models of different sizes. Different bars represent the data of different females. The numerals in and beside bars represent the number of landings. The numerals in the parentheses represent the number of deposited eggs.

Results. The distribution of landing was significantly heterogeneous among the females (Figure 5; Heterogeneity chi-square analysis, $\chi^2 = 97.1$, $df = 14$, $P < 0.001$). The Friedman test showed that the females did not alight at significantly different frequencies among the three models ($F_{2,14} = 0.27$, $P > 0.7$). The logistic regression analysis showed that the oviposition rate was different between the small model (0.75×0.75 cm) and the large model (3×3 cm) as

well as among females. The oviposition rate on the large model was estimated to be 1.8 times higher than that on the small model (Wald test, $P < 0.05$).

Experiment 4

Methods. To investigate the influence of contour length, we prepared the following three models that have the yellow-green leaf of different patterns. For one model, sixteen squares of 0.75×0.75 cm were attached on a piece of transparent plastic sheet of 3.6×3.6 cm in a 4×4 array with 0.2 cm space (see legend of Figure 6). The other two models were square and designed to eliminate the influence of size. One of the two squares was a 3×3 cm square which had the same area as the total area of the sixteen squares of 0.75×0.75 cm, and the other was a 3.6×3.6 cm square which had the same area as the whole area of the complicated model including spaces. Nine females were observed in the same manner as in Experiment 3.

Results. Eight of the 9 females landed predominantly on the model of 16 squares, whereas the remaining female more frequently alighted on the 3.6×3.6 cm square model (Figure 6). The distribution of landing was significantly heterogeneous among the females (Heterogeneity chi-square analysis, $\chi^2 = 38.3$, $df = 16$, $P < 0.002$). The Friedman test showed that those models were alighted non-randomly ($F_{2,16} = 12.7$, $P < 0.001$). Multiple comparisons showed that the model of 16 squares was chosen more frequently

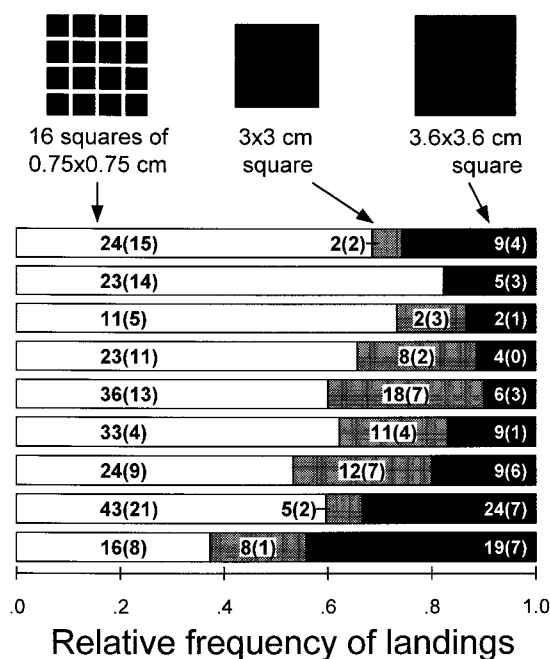


Figure 6. Frequencies of landing on the models of different contour length. Different bars represent the data of different females. The numerals in and beside bars represent the number of landings. The numerals in the parentheses represent the number of deposited eggs.

than the square models ($P < 0.02$). The oviposition rate was not different among the three models (Wald test, $P > 0.05$).

Experiment 5

Methods. The sixteen-square model was chosen predominantly in Experiment 4, probably because this model is similar to the compound leaf of food plants. It is unknown, however, whether the long contour or the similar whole image was attractive to gravid females. To reveal the influence of contour length, we made a model that had a 3×3 cm yellow-green square with a 2×2 cm square hole at the centre (see legend of Figure 7). This model was presented to a gravid female with the model that had a 3×3 cm yellow-green square with no holes. Thirteen females were observed in the same manner as in Experiment 1 except that the two models were located at opposite corners of the acrylic cage. Statistical analyses were also carried out in the same manner as in Experiment 1 except that the Wilcoxon signed-rank test (Zar, 1999) was used when the distribution of landings was heterogeneous among females.

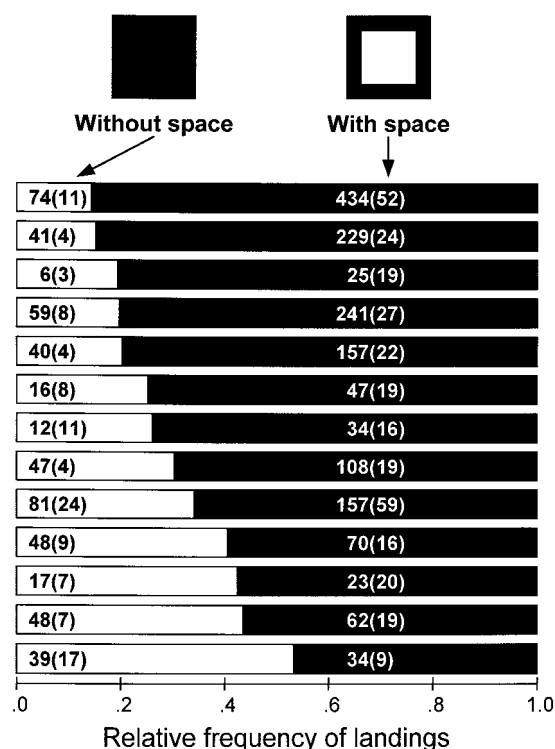


Figure 7. Frequencies of landing on the models of different contour length. Different bars represent the data of different females. The numerals in bars represent the number of landings. The numerals in the parentheses represent the number of deposited eggs.

Results. Twelve of the 13 females landed on the model with the hole at the centre more frequently than the model without a hole (Figure 7). The distribution of landing was significantly heterogeneous among females (Heterogeneity chi-square analysis, $\chi^2 = 125$, $df = 12$, $P < 0.001$). A Wilcoxon signed-rank test showed that the model with the hole was chosen significantly more frequently than the model without a hole ($T_{12} = 1$, $P < 0.001$). The oviposition rate was not different between the two models (Wald test, $P > 0.05$).

Discussion

The results of Experiments 4 and 5 revealed that during oviposition, gravid females of *E. hecabe* preferred the complicated figures that had the long contours (Figures 6 and 7). As silhouettes of long contours are made by the compound leaves of major food plants (*L. cuneata* and *A. julibrissin*), the preference for the long contours would be one of the essential visual cues for gravid females to alight on the appropriate food

plants. Although the small size of leaflets is also a common visual cue in these host plants, Experiment 3 showed that the small figure is not a sufficient cue for host location. While previous studies had reported that the contour length is an essential cue for pollinators to locate and learn the nectar sources (Hertz, 1930; Ilse, 1932; Vaidya, 1969; Lehrer, 1999), the present study is the first report on the preference of phytophagous insects for contour length in food plant location.

Some previous studies concluded that leaf pattern is not essential for gravid females to locate the host plants, although the preference of contour length was not investigated (Prokopy et al., 1983; Renwick & Radke, 1988; Harris et al., 1993; Foster et al., 1997; Rojas & Wyatt, 1999). It is possible, however, that some of those insects showed a preference for contour length, especially when the host plants had the organs of complex morphology. Städler (1974) reported that female *Choristoneura fumiferana* (Tortricidae) deposited more eggs on a needle-shaped model, which resembled the host plant more closely, than on a broad-leaf model. It is possible that the preference of *C. fumiferana* for the needle-shaped model would result from its preference for contour length, since this needle-shaped model had a longer contour than the broad-leaf model. However, it is not obvious how the contour length influences the host location of *C. fumiferana*, as Städler (1974) did not determine whether the whole image or the contour length of needle-shaped model was responsible for the preference for the needle-shaped model. Further studies should reveal that the preference for the contour length of host plants is prevalent among the phytophagous insects.

The results of Experiments 1 and 2 revealed that the females of *E. hecabe* alighted selectively on the yellow-green model that had a spectral reflectance similar to that of leaves of *L. cuneate*. The colour is likely another essential cue for locating the appropriate host plants, since the leaf colour is different among species and dependent on the leaf quality (Prokopy & Owens, 1983; Bernays & Chapman, 1994). The proportion of oviposition following landing was also different among colour models. Females oviposited more frequently per landing on the green and yellow models in Experiment 1, and on the yellow-green and green models in Experiment 2. In those experiments the oviposition rate was high on the models where females landed more frequently. It follows that colour stimuli would influence the reproductive drive after landing.

The oviposition rate was higher on the large model than on the small model, although the frequency of landings was not different among the models of different sizes (Experiment 3). Since we sometimes observed that females failed to oviposit on the small model because their bending abdomen could not reach the leaf paper, we concluded that the small leaf did not restrain their reproductive drive but made it physically difficult for them to oviposit. No difference in the oviposition rate was detected among the models of different contour lengths. It follows that the leaf morphology would have less influence on the reproductive drive after landing than colour stimuli.

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