Mem. Fac. Sci. Kyoto Univ. (Ser. Biol.), 16: 87-94, Dec., 1999

Ethological Studies on the Flower-visiting Behavior of *Luehdorfia* Butterflies (Lepidoptera; Papilionidae) II. Effects of the Size, Shape and Odor of Flowers

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(Received November 5, 1999)

Abstract Experiments were conducted to test whether *Luehdorfia* butterflies distinguish the size, shape and odor of flowers. The results of the experiments suggested: 1) larger flowers attract more butterflies, 2) total margin lengths might be more important than total area of the flower, 3) the butterflies might recognize small flowers in mass, 4) the odor of flowers attracts butterflies only when the flower color is inappropriate, thus, 5) blue-violet reflection is of primary importance while the odor is of minor importance in the flower-visiting behavior of *Luehdorfia* butterflies.

Key words Flower-visiting behavior, *Luehdorfia*, Papilionidae, Visual cue, Olfactory cue

Introduction

Butterflies exhibit color sense in feeding and mating. Small tiger swallowtails, *Luehdorfia puziloi* and *L. japonica*, appear once a year in early spring. They feed mainly on purplish flowers blooming in sunny patches of deciduous broadleaf forests.

In the previous study (Fujii 1999), I tested the following hypothesis: *Luehdorfia* butterflies might distinguish flower color and prefer one or several color(s) when feeding. The results of color preference tests and spectroscopic analyses suggested:

(1) Butterflies locate nectar sources in response to the colors of flowers.

(2) Blue-violet is most attractive in both species, while yellow (or bee-purple) is less attractive only in *L. puziloi*. Red is not attractive to both species.

(3) Reflection spectra of bluish papers are similar to those of bee-blue flowers (or purplish to humans) such as *Erythronium japonicum*: they have a peak reflection wavelength around 450 nm.

(4) *Luehdorfia* butterflies visit purplish flowers frequently in their natural habitat, because these flowers reflect blue-violet around 450 nm.

However, *Luehdorfia* butterflies might also distinguish size, shape and/or odor of flowers when searching for nectar sources as is true in honey bees (e.g. Frisch 1914; Hertz 1929, 1930, 1931; Manning 1956; Gould 1986). To test these hypotheses, I conducted the following experiments.

Materials and Methods

Materials

Females of *Luehdorfia japonica* were collected in Tatekawa, Yamagata in 1985. Eggs were obtained from these females. Eggs of *L. puziloi inexpecta* were collected in Aomori from April to May 1985 and 1987. Larvae of both species were reared in the laboratory. Pupae were stored in a refrigerator till spring. Naive adults from these pupae were used in the experiments.

Experiments

The experiments were carried out in an outdoor cage, in Hiraka, Aomori, from 3 to 6 May 1986 (Series 1, 2 and 4) and from 30 April to 2 May 1988 (Series 3; for the structure of the cage see Fujii 1999). The weather was fair for the duration of the experiments. I used 30-50 naive adults in each experiment. Behavior was recorded with 8 mm video cameras, and analyzed afterward.

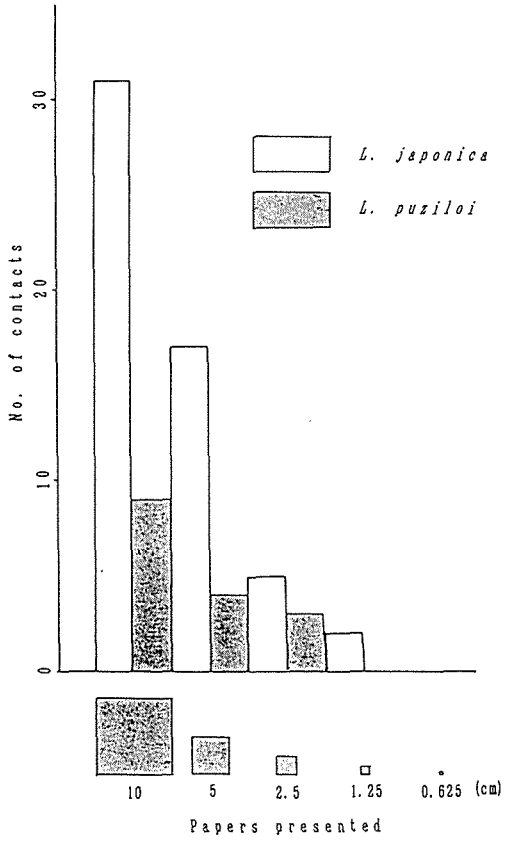

Fig. 1. Results of experiment Series 1.

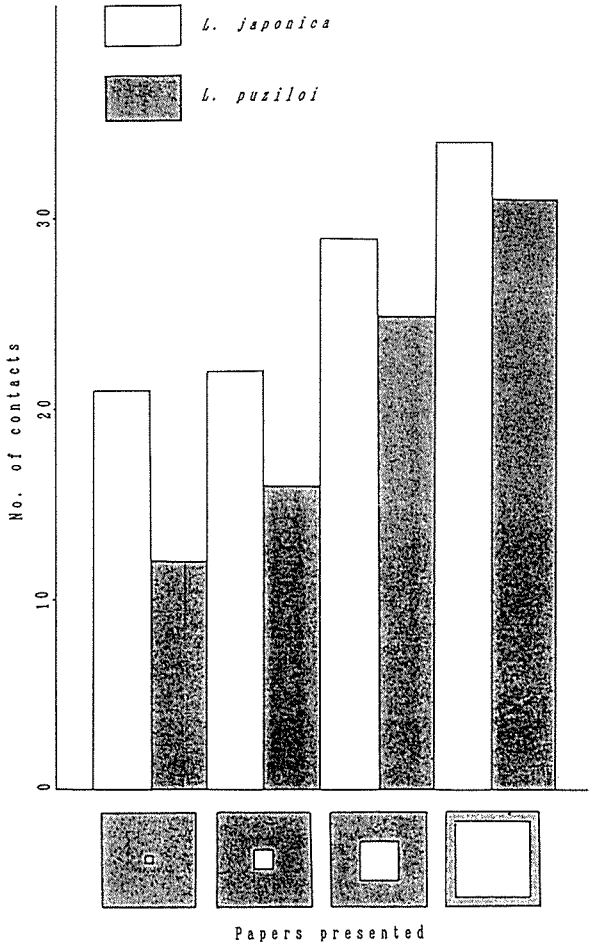

Fig. 2. Results of experiment Series 2.

Series 1

To test the effect of size, 5 square papers (10 × 10, 5 × 5, 2.5 × 2.5, 1.25 × 1.25, 0.625×0.625 cm^2) were arranged on the ground in order of descending size. Each paper was half the length and a quater the area of the preceding paper (Fig. 1). Vivid blue papers were used because *Luehdorfia* had been strongly attracted to this colored paper in the previous study (Fujii 1999). The frequency of contacts was tabulated.

Series 2

As the result of Series 1 was not in accord with my hypothesis (see Results), I arranged this experiment to know which was of primary importance, blue area or marginal length of blue paper. I prepared 5 × 5 cm^2 papers each with a different size (0.5 × 0.5, 1 × 1, 2 × 2, and 4 × 4 cm^2) white square in the center (Fig. 2). The papers were attached to the white square paper (15×15 cm^2) and fixed to the ground. If blue area is important for *Luehdorfia* to locate nectar sources, the blue paper with smallest blank should attract the most butterflies.

Series 3

The results of Series 1 and 2 suggest that larger flower attracts more butterflies (see Results). However, *Luehdorfia* butterflies also visit smaller flowers like violets in natural habitats. As the size of *Viola* is 1 cm^2 or less, *Viola* should be less attractive for *Luehdorfia* from the results of Series 1 and 2. How can adults of *Luehdorfia* find smaller flowers in natural habitats? *Viola* flowers usually bloom in groups in a rather limited area. Butterflies may perceive them as a mass. To test this hypothesis, 4 clusters of blue square papers were presented to *Luehdorfia*. I numbered the clusters as follows: Cluster 1 composed from only one 5×5 cm^2 paper, Cluster 2 from four 2.5×2.5 cm^2 papers, Cluster 3 from 16 1.25×1.25 cm^2 papers and Cluster 4 from 64 0.625×0.625 cm^2 papers. Thus, the total blue areas of square pieces of the four clusters were equal (Fig. 3).

Series 4

Butterflies may also perceive the odor of flowers. I prepared two sets of experimental apparatus to test this hypothesis. Each set had two plastic cylinders: one contained flowers in water while another contained water only (control). The flowers of *Speraea chamaedrifolia* were used because of their strong odor. The tops of the cylinders were covered with 5×5 cm^2 papers perforated with many small cuts. One set was covered with vivid blue papers, while another was covered with pale blue papers. Both sets apparatus were placed by the net window to control for wind direction (Fig. 4).

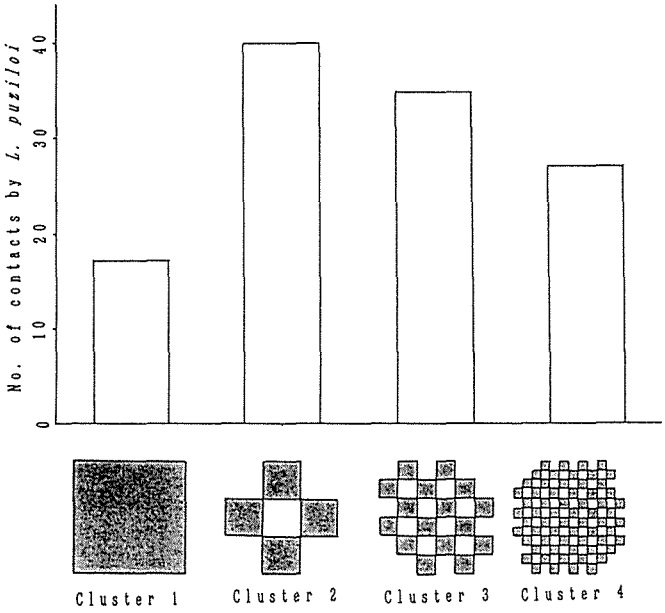

Fig. 3. Results of experiment Series 3.

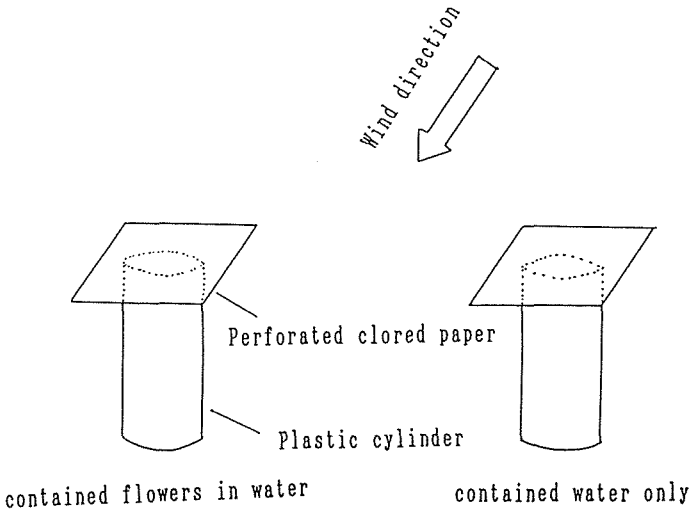

Fig. 4. Experimental apparatus used in Series 4.

Results

Series 1

When blue squares of various sizes were presented, the butterflies of both species visited the larger papers more frequently (Fig. 1). Because the successively smaller paper was one quarter the area of the previous paper, the number of contacts of the successively smaller paper should be one quarter of the previous paper if the butterflies responded merely to the blue area. However, the results did not agree with this hypothesis. Instead, the number of contacts on the successively smaller paper was about one half. This suggests the butterflies may have responded to the lengths of the papers (e.g. marginal lengths) rather than the area.

Series 2

When 5×5 cm^2 blue papers with the differnt-sized white squares were presented, the butterflies visited the paper with larger white squares more frequently (Fig. 2). These data reject the hypothesis stating that butterflies visit flowers in response to the area of blue. The butterflies alighted on the paper and elongated their proboscises indicating they were being attracted to blue. They did not respond to the white square in the paper. The blue papers presented in this experiment were equal in the length of outer margin, but varied in the length of inner margin. The numbers of contacts were proportional neither with the lengths of the outer margin nor the inner margin. Instead, the number of contacts increased as the total length of both outer and inner margins increased (Fig. 2).

Series 3

Among the four masses of square blue papers, the butterflies visited the cluster consisting of four 2.5 × 2.5 cm^2 papers most frequently, while the 5 × 5 cm^2 paper attracted the least number of butterflies (Fig. 3). As the masses of paper(s) had the same blue area, it appears that the butterflies did not respond only to the blue area. The total margin length of Cluster N was twice of that of Cluster N+1: 20 cm for Cluster 1, 40 cm for Cluster 2, 80 cm for Cluster 3 and 160 cm for Cluster 4. The butterflies did not merely respond to the total margin lengths of blue papers.

Series 4

In the case of the set of cylinders covered with vivid blue papers, the butterflies contacted equally the papers with odor and without odor ($P>0.05$, Chi-square test). The butterflies in this case seem to have visited papers merely in response to the color of papers.

In contrast, the butterflies visited the paper with odor more frequently than that without odor when covered with pale blue papers ($P<0.01$). This would indicate that the odor attracted more butterflies in this case (Fig. 5).

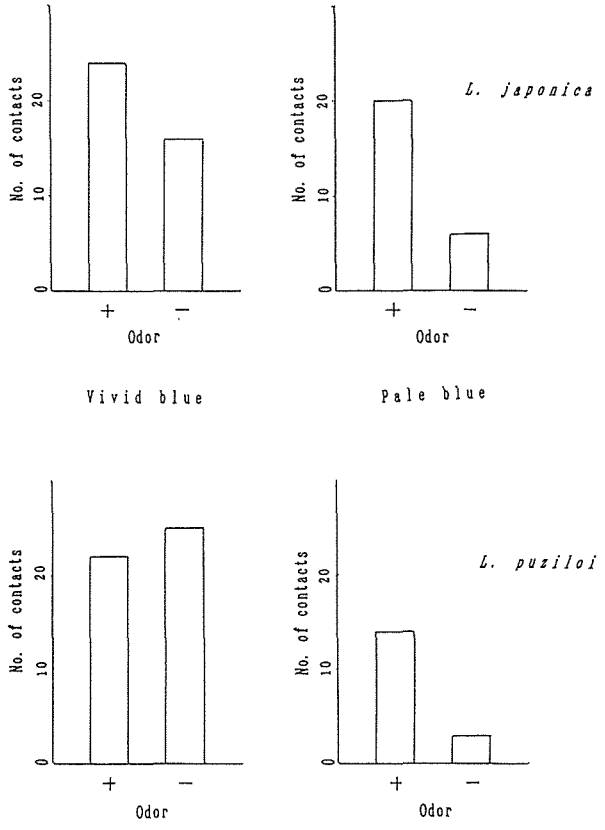

Fig. 5. Results of experiment Series 4. Above: *L. japonica*, Below: *L. puziloi*, Left: covered with vivid blue papers, and Right: covered with pale blue papers.

Discussion

Luehdorfia butterflies are able to locate nectar sources innately by approaching and then alighting on the blue-violet objects. They visit purplish flowers most often in their natural habitat because those flowers have a peak reflection of blue-violet around 450 nm in wavelength (Fujii 1999). However, they do not visit purplish flowers equally: they seem to have favorite flower species (e.g. Fukuda *et al.* 1982; Fujii 1999).

The results of this study have shown that *Luehdorfia* butterflies distinguish several patterns of blue-violet objects, and that they also sense the odor of flowers.

In Series 1, the butterflies visited larger papers more frequently, but the numbers of visits were not in proportion to the blue area. Instead, it seems to have been proportional to the total margin lengths of blue paper. Because the size of flowers usually visited in their natural habitat is around 10-50 mm in length, the 10x10 cm^2 paper seemed too large and the 0.625x0.625 cm^2 paper seemed too small for *Luehdorfia* to visit. *Luehdorfia* butterflies might have a preference for size of flowers, but they still visit flowers of extraordinary sizes. This preference seems adaptive in nature because they would be able to visit larger and/or smaller flowers if they existed in their habitat.

It is interesting that a contradictory result was obtained in Series 2: the butterflies visited the papers with smaller blue area more frequently than those with larger blue areas. However, this might be explained by the butterflies responding to the total lengths of both inner and outer margins of the blue paper. Because *Luehdorfia* butterflies usually alight on the edge of larger papers or larger flowers like *Erythronium*, and then elongate their proboscises, the marginal areas seem more attractive than the total blue area. This might also be as a result of the ability of compound eyes to distinguish edges as has been shown with honey bees (Frisch 1914; Hertz 1929, 1930, 1931).

The result of Series 3 suggests that the butterflies do not respond to each paper or each flower, instead they respond to the papers or flowers as a cluster. Though larger flowers (e.g. *Erythronium*) attract more butterflies than smaller flowers (e.g. *Viola*) do when blooming as single flowers, a mass of smaller flowers should attract more butterflies than single large flowers.

The results of Series 4 could be explained as visual cue is of primary importance while olfactory cue is of minor importance. As shown in the previous study (Fujii 1999), vivid blue is the most attractive color for *Luehdorfia* while pale blue is less attractive. Therefore, it seems that the butterflies visit blue-violet objects regardless of flower odor. However, they will visit pale blue objects frequently if they excrete the appropriate odor. *Luehdorfia* butterflies might also use olfactory cues when visiting yellowish or whitish flowers in their habitat.

Acknowledgements

The author would like to express his appreciation to Mr. Y. Kikuchi who kindly reared materials and permitted me to use his outdoor cage for experiments. This work was supported in part by a Grant-in-aid (No. 58121003) for Special Project Research on Biological Aspects of Optimal Strategy and Social Structure from the Japan Ministry of Education, Science and Culture.

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