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AUTHOR(S):

Fujii, Hisashi

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Ethological Studies on the Flower-visiting Behavior of *Luehdorfia* Butterflies (Lepidoptera; Papilionidae) III. Learning Flower Color

HISASHI FUJII

Department of Zoology, Faculty of Science, Kyoto University,
Sakyo, Kyoto, 606-8502 Japan
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Abstract *Luehdorfia japonica* butterflies were trained to initiate feeding behavior by white (or bee-white), red and yellow (or bee-purple) for more than 7 days in the laboratory. The butterflies were then tested to ascertain whether they had memorized each color. The results of experiments indicated that they could learn to distinguish yellow but neither white nor red. Similarly, *L. puziloi* were conditioned for yellow (or bee- purple) flowers for only 2 hours in an outdoor cage. Before conditioning, the butterflies visited bluish papers more frequently than yellowish ones, but they began to visit yellowish papers during the conditioning. After conditioning, the butterflies changed their preference to visit yellowish papers and bluish papers equally.

Key words Learning, Flower-visiting Behavior, Memory, *Luehdorfia*, Butterfly

Introduction

Most diurnal insects should be able to discriminate several colors. Some butterfly species are known to use particular color(s) or color patterns in feeding and/or mating behaviors (Ilse 1928, 1932; Hidaka & Obara 1968; S. L. Swihart 1969, 1970, 1972; Swihart & Swihart 1970; C. A. Swihart 1971; Hidaka & Yamashita 1976; Miyakawa 1976; Lutowski 1977; Shilberglies & Taylor 1978; Fujii 1999a, b). Though some students believe that insects are largely at the mercy of their instincts (e.g. Shields 1967), several insects are able to memorize or learn color, shape, location and so on (e.g. Matthews & Matthews 1978). As far as butterflies are concerned, several species are believed to learn the color of flowers (Ilse 1928; S. L. Swihart 1970, 1972; Swihart & Swihart 1970; C. A. Swihart 1971), the location of food flowers and larval host plants (Gilbert 1975; Matthews & Matthews 1978), the leaf shape of host plants (Rausher 1978; Papaj 1986) and how to extract nectar from flowers (Lewis 1986, 1989).

My previous studies have shown that *Luehdorfia* butterflies visit flowers primarily in response to blue-violet reflection from flowers so that they should visit purplish flowers most often in their natural habitat. Adults of *L. puziloi* have a minor color preference for yellow, whereas those of *L. japonica* do not have a preference for either yellow or white.

The odor of flowers attracts *Luehdorfia* butterflies when the flower color is less attractive (Fujii 1999b). Thus, *Luehdorfia* butterflies might visit yellowish or whitish flowers mainly in response to odor, at least before they have experienced a number of visits to those flowers.

Butterflies of both *Luehdorfia* species began to visit yellow or white flowers in a cage soon after I had presented such flowers, and kept visiting the same flowers repeatedly (Fujii unpublished). The butterflies are likely to have learned some traits, particularly flower color. The objective of this study is to examine whether *Luehdorfia* butterflies could memorize or learn the color(s) of flowers.

Materials and Methods

Experiments 1:

Female *Luehdorfia japonica* were collected in Kyoto in April 1984. Eggs were obtained from them in the laboratory. Larvae were reared on the fresh leaves of *Heterotropa aspera*. Pupae were kept in a refrigerator at 4°C from February 20 until March 23 or 28, 1985. Afterwards, they were restored to room temperature for emergence.

Naive adults (30 males and 30 females) were prepared for the experiments. They were divided into three groups, one trained for yellow (Series 1), one for white (Series 2) and one for red (Series 3), respectively. A simple apparatus (Fig. 1-a) was used to allow them learn a given color. Sugar solution was supplied every night until the insects fin-

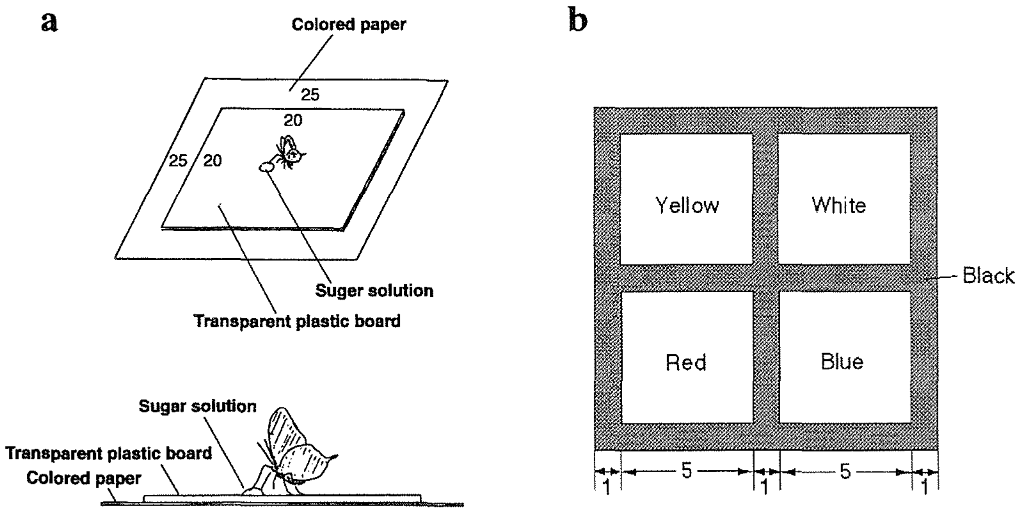

Fig. 1. a) Experimental apparatus for color conditioning, and b) experimental board for the color preference test in Experiment 1.

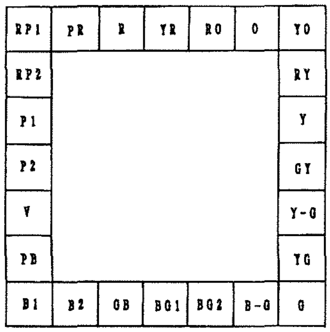

Fig. 2. Arrangement of color papers in Experiment 2. Abbreviations, see Fig. 3.

ished feeding. This training continued for over a week until the experiment was completed. Ten individuals (5 males and 5 females) were used in each experiment, and the remaining ten were reserved. When a trained butterfly died, a reserved individual of the same sex was added to the cage, so that the number of butterflies in the experiments was always ten. The experiments were carried out in a laboratory cage from the 8th through 10th days after emergence (for details of the cage, see Fujii 1999a). The duration of the experiments was 6 h a day (0900-1500), with a total of 18 h. The experimental board (Fig. 1-b) was used in every experiment. The behavior of butterflies was recorded with a video camera (National MacLord). The number of butterflies that touched and/or alighted on each colored paper was counted from the video records.

Experiment 2:

Eggs of *L. puziloi* were collected in Hiraka, Aomori in May 1987. Larvae were reared on the leaves of *Asiasarum Shieboldii*. Pupae were kept in a refrigerator at 4°C till early April 1988, and then moved to an outdoor cage, in Hiraka (for details see Fujii 1999a) for emergence. The experiment was carried out in the cage from 1 to 2 May 1988. Early in the morning, a number of purplish flowers such as *Erythronium* and *Viola* were placed on the ground of the cage. Naive butterflies were then released into the cage. They freely visited these flowers for about 4 hours. I then conditioned the butterflies for yellow for two hours by replacing the flowers with many yellow dandelions (*Taraxacum officinale*). The butterflies soon began to visit the dandelions. The flowers were changed again when two hour conditioning period had finished. I tested for color preferences before conditioning, during conditioning and one day after conditioning.

Twenty-four square colored papers (5×5 cm^2 in size) were used for the color preference test. The papers were arranged in circle-square so that the colors changed gradually, and were fixed on the ground of the cage (Fig. 2). The reflection spectra of each color paper, measured with a spectrophotometer (Shimadzu UV-visible Recording Spectrophotometer UV-240, Graphicord), are shown in Fig. 4. The behaviors of the butterflies was recorded throughout the experiment with a 8mm video camera (SONY V-900). Afterwards, the number of contacts for each color paper was tabulated.

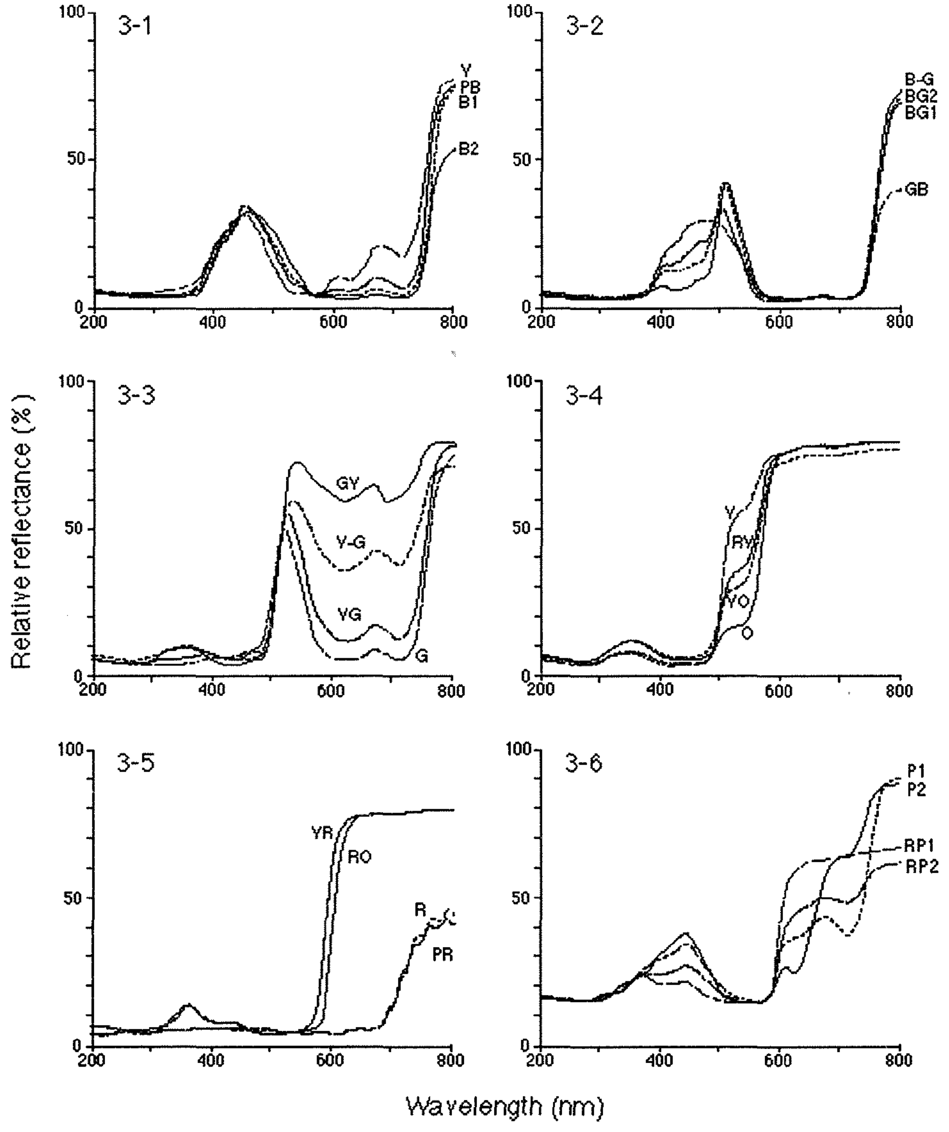

Fig. 3. Reflection spectra of color papers used in Experiment 2. PR: vivid purplish red, R: vivid red, YR: vivid yellowish red, RO: vivid reddish orange, O: vivid orange, YO: vivid yellowish orange, RY: vivid reddish yellow, Y: vivid yellow, GY: vivid greenish yellow, Y-G: vivid yellow green, YG: vivid yellowish green, G: vivid green, B-G: vivid blue green, BG2: vivid bluish green 1, BG1: vivid bluish green 2, GB: vivid greenish blue, B2: vivid blue 2, B1: vivid blue 1, PB: vivid purplish blue, V: vivid violet, P2: vivid purple 2, P1: vivid purple 1, RP2: vivid reddish purple 2, RP1:vivid reddish purple 1.

Results

Experiment 1:

Series 1

When trained for yellow, the butterflies visited yellow more frequently than others (P<0.001, Chi-square test; Fig. 4). Their color preference was significantly different from those in Series 2 and 3 (P<0.01). The butterflies appeared to have memorized or learned yellow as the color of nectar sources.

Series 2

When trained for white, the butterflies visited blue more frequently than others (P<0.001; Fig. 4).

Series 3

When trained for red, the butterflies visited blue more frequently than others (P<0.001; Fig. 4). Their color preference was no difference from that in Series 2 (P>0.05).

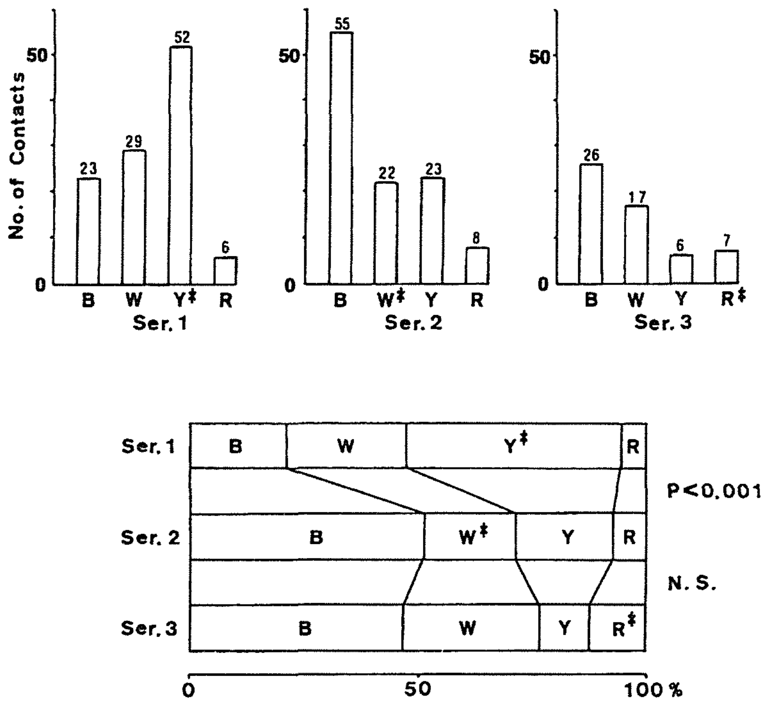

Fig. 4. Results of Experiment 1. *: the color conditioned.

Experiment 2:

Before conditioning, the butterflies clearly preferred blue to other colors. This did not change during the 1st one hour of conditioning. However, their preference shifted from blue to orange-yellow during the second hour of conditioning. The butterflies still visited orange-yellow frequently even one day after the conditioning. This therefore suggests that *Luehdorfia puziloi* can memorize the color yellow as nectar sources in a matter of 2 h (Fig. 5).

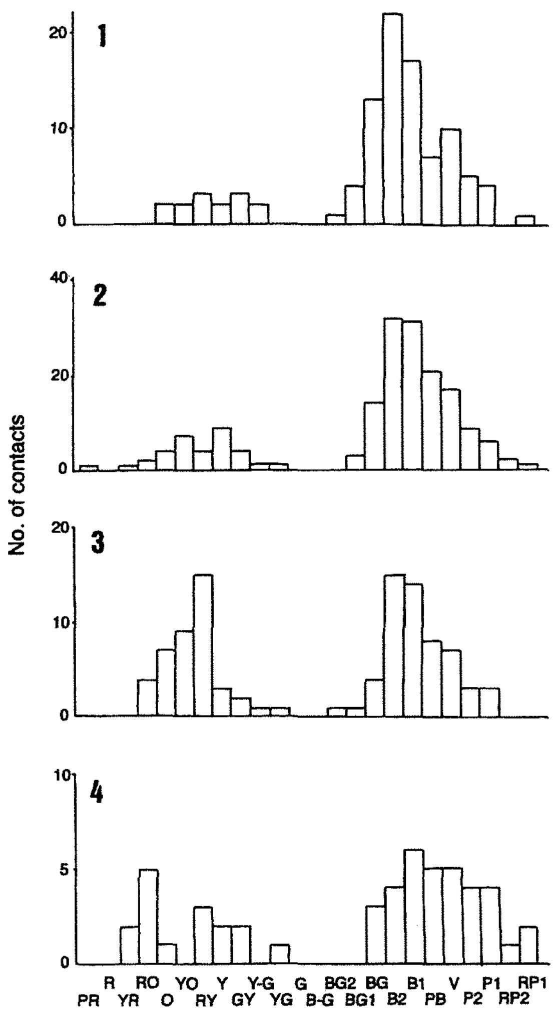

Fig. 5. Changes in color preference. 1: before conditioning. 2: 1st 1 h during conditioning. 3: 2nd 1 h during conditioning. 4: 1 day after conditioning.

Discussion

As Ilse (1928) noted, the color preference of feeding butterflies may have two attributes: (1) innate preference, and (2) acquired preference. The previous studies have already proved the first attribute in *Luehdorfia* (Fujii 1999a, b). The results of this study justified the second attribute in *Luehdorfia*.

Innately, adult *L. japonica* prefer blue-violet to yellow, red and white (Fujii 1999a). The results of Experiment 1 suggest that adult *L. japonica* can learn to recognize yellow (or bee-purple) but cannot learn to recognize white nor red as the color of nectar sources. It should be noted that, in both Series 2 and 3, the butterflies visited vivid blue most frequently regardless of the trained color. This means adult *L. japonica* have innate color preference for blue-violet (or bee-blue) as my previous studies have already suggested (Fujii 1999a, b). Nevertheless, the proportions of contacts on white and yellow papers are larger than those in the previous study (Fujii 1999a). Because the training lasted for more than 7 days, the butterflies might have slightly changed innate color preference as a result of my training.

Adult *L. japonica* are unlikely to have innate color preference for yellow (Fujii 1999a; this study). This is a probable explanation for their rare visits to yellowish flowers in nature (e.g. Fukuda *et al.* 1982). However, as a result of learning, an adult *L. japonica* may begin to visit yellowish flowers in response to yellow reflection if many yellowish flowers are available in its habitat.

As for *L. puziloi*, they are likely to have not only innate color preference for blue-violet but also minor preference for yellow (Fujii 1999a; this study). This should explain why adult *L. puziloi* visit not only purplish flowers but also yellowish flowers occasionally (in most habitats) or frequently (in several habitats in Hokkaido) in the natural habitat (e.g. Fukuda *et al.* 1982; Watanabe 1985; Kanda 1987). The result of Experiment 2 suggests that *L. puziloi* can change their color preferences fairly easily from innate preference for blue-violet to acquired preference for yellow (or orange-yellow) if yellowish flowers are more abundant than purplish flowers. Thus, acquired preference for yellow should constitute another reason for occasional or frequent visits on the yellowish flowers.

As pointed out in the previous paper (Fujii 1999b), the odor of yellowish flowers may also attract *Luehdorfia* butterflies. After conditioning, however, the butterflies began to visit yellow or yellowish papers that lacked odor. Therefore, the odor of flowers seems important only before the butterflies learn the color of flowers. This would constitute adaptive nature, because recognition time would be shorter if the insect recoginizes food visually than when it recognizes it through its olfactory sensors (e.g. Krebs 1978).

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