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Initial Mate Recognition in the British Cabbage Butterfly, *Pieris rapae rapae*

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ABSTRACT—Visual features of the wing color, with special reference to the UV (ultraviolet) color, of the British subspecies of the cabbage butterfly, *Pieris rapae rapae* and its mating behavior were investigated. Both sexes of the British subspecies were found to lack UV color and differed only slightly in color in the visible color range, with female wings more yellowish. It follows that they show only slight sexual dimorphism in wing color. It was shown that the initial mate recognition was mediated visually, based on the wing color. Males discriminated between the sexes visually, but only marginally and were occasionally observed to approach other males mistakenly. The resting males approached by female-searching males displayed a flutter response, deterring the approaching males from attempting to copulate with them; i.e. it functioned as “mechanical isolation mechanism” against maladaptive copulatory attempts between males. The results are discussed in terms of the comparative ethology of the mating behavior with that of the Japanese subspecies. It is suggested that the British subspecies is ancestral to the Japanese subspecies.

INTRODUCTION

In many species of butterfly, males take the initiative in mating behavior. They fly in search of females in appropriate areas, and when they encounter females, they approach them. This initial phase of mating behavior, or initial recognition of potential mates, is mediated visually, female wing color patterns playing an important role (Tinbergen *et al.*, 1942; Crane, 1955; Magnus, 1958; Obara and Hidaka, 1968; Obara, 1970; Wago and Unno, 1976; Rutowski, 1977a, 1978, 1981; Silberglid and Taylor, 1978). However, chemosensory stimuli are required for mating behavior to proceed beyond this initial stage. (Tinbergen *et al.*, 1942; Magnus, 1958; Brower *et al.*, 1965; Rutowski, 1977b; Wago, 1978a,b). It has been established that in many species of butterflies, final mate recognition of females by males is usually chemosensory.

Males of the Japanese cabbage butterfly, *Pieris rapae crucivora*, fly over cabbage fields searching for females (Obara and Hidaka, 1968). Female wing color plays an essential role in eliciting a mating response from males. In this subspecies UV colors are present on the wings of females, but not males, i.e. males and females are strikingly sexually dimorphic in the UV spectrum (Obara, 1970). Initial, and final mate recognition as well, by the male *P. rapae crucivora* is entirely dependent upon visual cues, the female wing color including UV color as an essential component (Obara, 1970). Males can discrimi-

nate females from males, even when these are sealed in an impermeable transparent film (Saran wrap), thereby preventing chemosensory stimuli (Obara, unpublished).

Preliminary observations of museum material suggested that the sexual dimorphism for UV wing color of *P. rapae crucivora* is not present in the British subspecies of the cabbage butterfly, *Pieris rapae rapae*. In this subspecies, females have no UV color. These observations raise some interesting questions. For instance, how do British males recognize mates? Is presence or absence of female UV wing color the ancestral state?

In the present study, we aimed first to determine whether the preliminary observations in respect of female UV color, mentioned above, are true. Second, we observed the mating behaviors of British subspecies and carried out experiments to determine how British males recognize mates.

MATERIALS AND METHODS

Butterflies

Butterflies used for UV color examination were derived from British (Cambridge area in UK) larvae raised to adulthood in the laboratory. Recently eclosed butterflies were killed by freezing and carefully set to minimize physical damage to the wings. Male butterflies for behavioral observation were derived from the Cambridge stocks, or were freshly collected around Cambridge.

Color examination

To compare the visual features of the wing color between the sexes, a female and a male were examined in two ways: photographic

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examination and reflectance spectrometric analysis.
For the visible color examinations of the wings, 4 male specimens with similar wing color appearance and 4 female specimens likewise with similar wing color appearance were photographed under normal sun light, using color print film (Fuji Super G Ace 400). For the ultra violet color examinations the same specimens were photo-

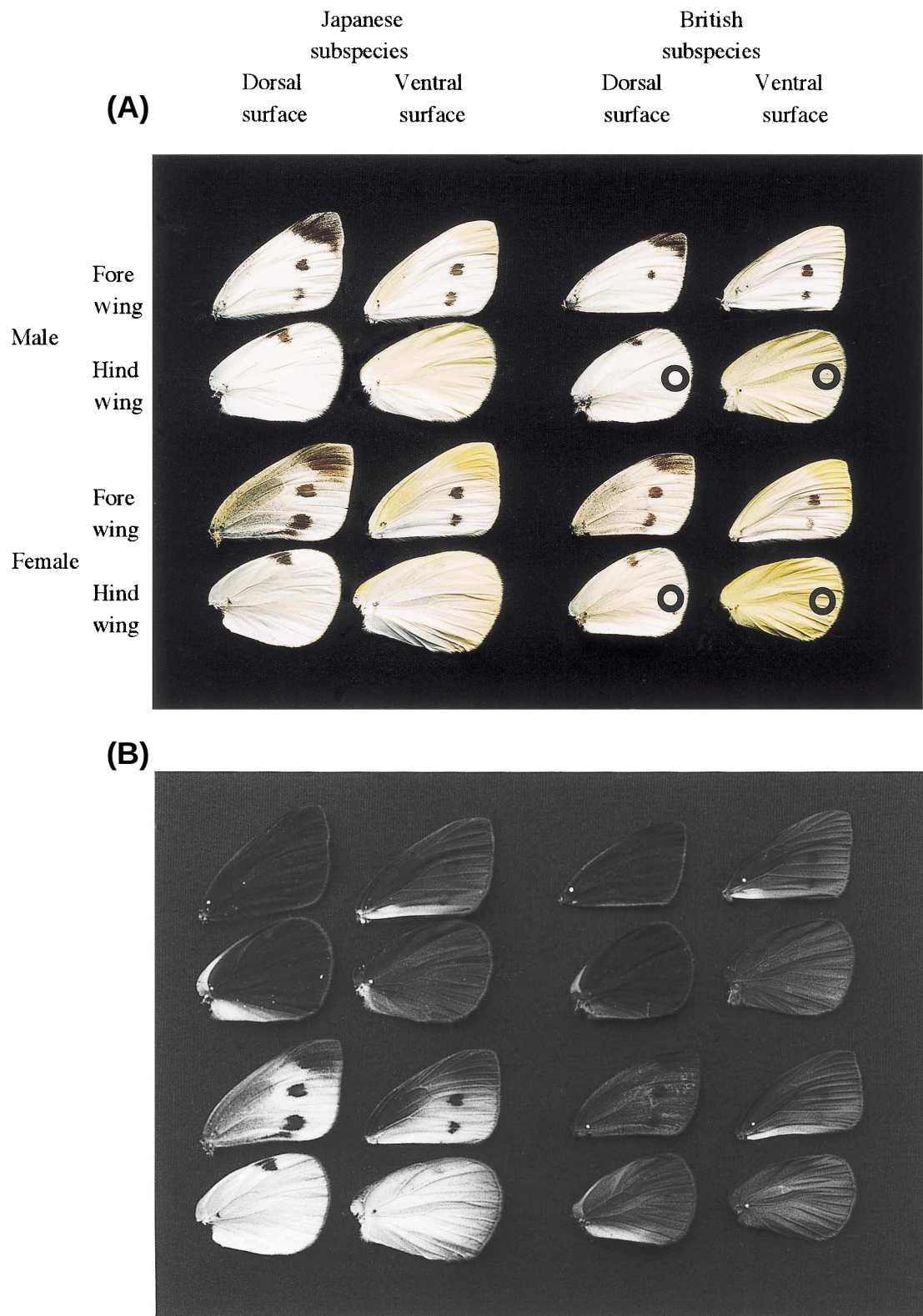


Fig. 1. Japanese and British subspecies of cabbage butterflies photographed normally (A) and through UV filter(B) under sun light. Circles on the hind wings of the British subspecies depict the portion subjected to the spectrophotometric assessment.

graphed using the monochromatic film (Fujifilm Neopan SS) through a UV filter (Hoya UV36) following procedures described by Obara and Hidaka (1968). This filter is transparent to light with wavelengths from ca 300 to 400 nm with a transparency peak at 360 nm. Thereafter, the UV reflectance characteristics of butterflies were assessed using a spectrophotometer (Shimadzu UV-260, Integral Sphere Attachment ISR-260). For this assessment, a 5mm x 5mm section from the middle, distal part of the hind wings was carefully excised (see circles in Fig.1) and placed in the cassette of the spectrophotometer, which assesses the percentage of light of specific wavelengths (at 4 nm increments) that is reflected from the specimen. Only the ventral surfaces, and the dorsal surfaces for comparison, of the hind wing samples were assessed as it is this surface that females expose to searching males and which, in the Japanese subspecies, functions as a visual releaser.

Behavioral observation

Initial observations were intended to provide a general view of the mating behavior of British subspecies. Attention was paid to determination of which sex initiates mating behavior, and to the detailed behaviors of males and females during courtship. Data from these observations were compared with similar, previously published details of the courtship and copulatory behavior of the Japanese subspecies (Obara, 1970).

As a result of the initial behavioral observations, we examined which body parts of the female attracts males and elicits male mating behavior. The wings of females were cut away from the body, and left and right fore- and hind-wings were stuck together basally, in the posture assumed by a resting butterfly (wing dummy). The head, thorax and abdomen from which the wings had been removed, were retained as an integral unit (naked dummy). The wing dummy was placed vertically on the 20cm x 25cm Stigrofoam board covered with a black cloth, 15cm distant from the naked dummy. Observations were made of whether males were attracted to the wing dummy or the naked dummy, or both.

To determine whether a male could distinguish between a female and a male and recognize the former as a mate, female and male wing dummies were presented together to searching males. In this experiment 6 male wing dummies with similar wing color appearance and 6 female wing dummies likewise with similar wing color appearance were used. A female and a male wing dummy were placed 15cm apart, in resting posture with the wings closed, on cabbage leaves (termed exposed specimens). The positions of the male and female wing dummies were reversed every 15 min to exclude possible local positional effects.

In a separate experiment designed to examine whether chemosensory stimuli have a role in the recognition of females by British male, single female and male wing dummies were placed 15cm apart on a 20cm x 25cm transparent vinyl chloride board, framed thinly with wood. The wing dummies were then sealed with a transparent kitchen wrap film (Saran wrap:Asahi-Chemical Industry Co., Ltd.) (termed sealed specimens). The wood frame was attached to prevent the film wrap from being distorted by the specimens, so as not to cause diffused reflection. Transparent rather than black opaque board was used because we found that the film wrap acted as a mirror when set against the black board, causing unexpected artefacts when approaching males saw their own reflections. The transparent board with sealed wing dummies was placed vertically amongst cabbage plants.

The experiments were carried out in a 3m x 3m x 2m net cage erected outside at the Department of Genetics Field Station of Cambridge University in Cambridge, U.K. More than 70 males were released into the cage.

In these experiments the numbers of males that approached, came into contact with, and attempted to mate with, dummies of various types were recorded.

RESULTS

1. Visual features of the wings

British male and female can be distinguished readily from the markings on the dorsal surface of the forewings, for while females have two black spots on this surface, males only have one (Fig. 1A). Males have two black spots on the ventral surface of the forewing, and the posterior of these, which corresponds in position to the second black spot of females, can be discerned in males on close examination. This is in contrast to Japanese subspecies, in which both sexes have two black spots (Fig.1A). In addition, British females generally have darker coloration on the proximal and anterior edge of the forewing than do males.

On the other hand, the ventral wing surfaces of the two sexes differed only slightly in color, with female wings more yellowish. This was true both for the hindwing and anterior edge of the forewing that are exposed by females when at rest and so have the potential to be seen and used as sensory cues by males seeking mating partners, if males use visual cues to identify females. Given only a slight sexual dimorphism of resting individuals of the British subspecies in the human-visible spectrum, visual discrimination of the sexes, by males, on the basis of human-visible wavelengths, seems difficult.

This same conclusion appears applicable for UV color. The wings of British females reflect little UV light (Fig. 1b), although close observation of the UV photograph suggests very weak UV reflectance by the dorsal surface of the forewing.

Spectrophotometric measurements are consistent with the photographic results. The dorsal surface of male and female hind wings have slightly different reflectances in the ca 440 - 530 nm wavelength range, reflectance from the male being slightly stronger, as suggested by the photographs in Fig. 1b (Fig.2A). The ventral surfaces of the hindwings of the two sexes have very similar reflectance characteristics (Fig.2B).

In summary, British subspecies appears to exhibit only slight visible sexual dimorphism in human-visible wavelength, but scarce in UV wavelength.

2. Mating behavior

The mating behavior of the British subspecies is essentially the same as that of the Japanese subspecies. Males initiate mating behavior, flying in search of females and approaching and attempting to copulate with those they find. Virgin females respond to male mating behavior by assuming a resting posture with the wings closed, while previously mated females reject males by assuming a mate refusal posture with wings open and depressed, and abdomen erect. Females maintain this posture as long as flying males remain in close proximity (Obara, 1964).

The "flutter response", previously described for the Japanese subspecies (Obara and Hidaka, 1964), was also observed in the British subspecies. This response involves rapid wing fluttering by resting males in response to approaches by

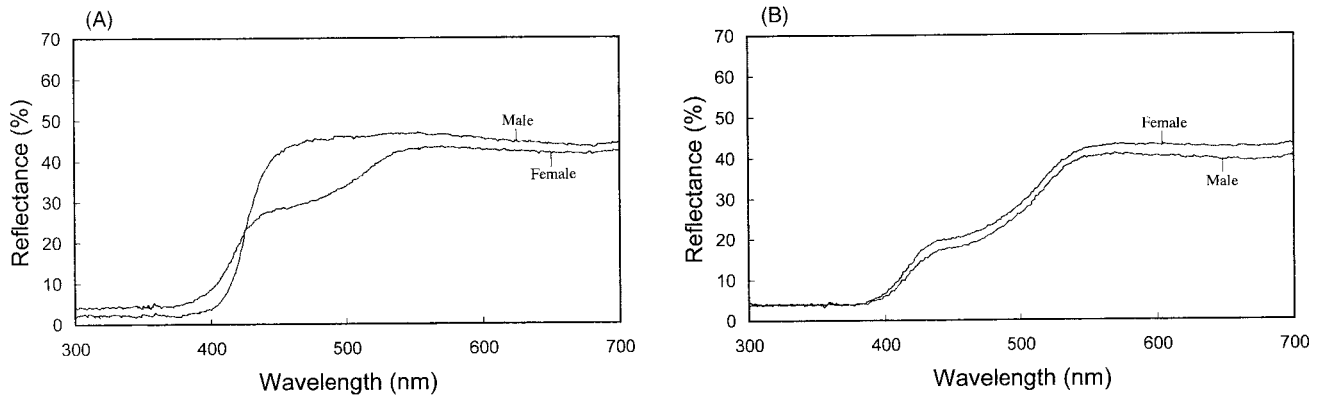


Fig. 2. Light reflectance of the ventral (A) and dorsal (B) surface of the hind wings of the British male and female cabbage butterfly.

flying males. The total of 36 approaches by the female-searching males to the resting males were all followed by the flutter responses by the latter. All of the female-searching males were then observed to fly away following brief behavioral interactions with the resting males, without copulatory attempt.

In the experiment with the naked dummies and wing dummies, 37 males were observed to approach the wing dummies and 31 of them to contact the wing dummies. Twenty-two of the latter displayed attempted copulation to the wing dummies. No males, on the other hand, were observed to approach the naked dummies.

In the experiment using the exposed specimen, 42 males approached the female specimen, whereas 28 males approached the male specimen (Fig. 3). Of the 42 males that approached the female specimen, 39 (93%) males made contact with the specimen. Similarly, 25 of the 28 (89%) males that approached the male specimen did so. The difference between the two sexes is only marginally significant for approach and contact with the p values of 0.12 and 0.10 (Two-tailed binominal test), respectively.

Twenty-two of 42 (52%) males that approached females proceeded to attempt copulation with the female specimen, while 12 of 28 (43%) males that approached males attempted copulation with the male specimen. Here again difference in the proportions for the two sexes is only marginally significant (Two-tailed binominal test, $p=0.12$). Once the approach has been made, a male is not significantly more likely to attempt to copulate with a female wing dummy than with a male wing dummy (χ^2 test, $p>0.25$).

Similar behaviors were observed for the sealed specimen (Fig. 4). Thirty-eight males approached the female specimen and 26 males approached the male specimen. Most males proceeded to make contact with the specimens they approached (for females, 37 (97%); for males, 25 (96%)). The difference between the two sexes is not significant with the p value, 0.17 for approach and 0.16 for contact (Two-tailed binominal test). Only five (13%) males that approached the female specimen attempted to copulate with it and none of the males that approached the male specimen attempted copulation with it. Some males appeared to try to alight on the film wrap covering the specimen. However, this seemed difficult

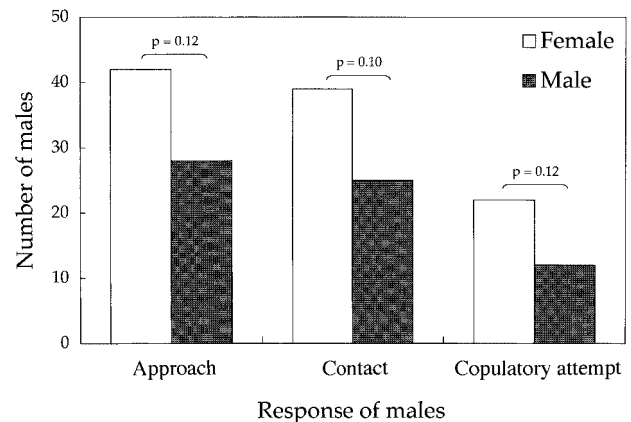


Fig. 3. Male behavioral response to the exposed specimen of the male and female wings.

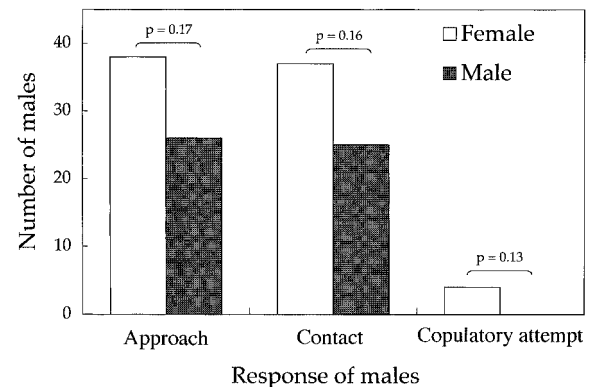


Fig. 4. Male behavioral response to the sealed specimen of the male and female wings.

as the smooth film, when positioned vertically, gives little hold for the tarsal claws.

DISCUSSION

1. Sexual dimorphism and mate recognition

The results of the experiments with the female wing and naked dummies revealed that in British subspecies, as in Japanese subspecies, it is the female wings, not the female body

itself, that attracts males and elicits their mating behavior. Furthermore, the results of the exposed and sealed wing dummy experiments showed that visual, rather than tactile nor chemosensory, cues from the female wings prompt male's mating behavior; i.e. the initial mate recognition is mediated visually (Fig. 3 and 4).

The results of the experiment with wing dummies demonstrated that the ventral surface of the female wings are sufficient in eliciting mating behavior from males. As in the Japanese subspecies, British female keeps their wings closed in a resting position when found by a conspecific male. In such a posture the exposed wing parts of the female are the ventral anterior edge plus the apex of the forewing (fw-VA) and the whole ventral surface of the hind wings (hw-WV). Thus the hw-WV comprises most of the wing area exposed to males and probably plays the critical role in their initial mate recognition.

The present study revealed only slight sexual dimorphism for the wing color in the fw-VA or the hw-WV, in human visible spectra, but little in UV spectra (Fig. 1). The similar appearance between the sexes was apparent both when specimens were viewed by eye, and when they were subjected to spectrophotometric analysis (Fig. 2). The very slight difference in the ventral surfaces of female wing compared to male wings in UV (Fig. 1), is in sharp contrast to the differences seen in Japanese subspecies, in which female, but not male, ventral wing surfaces reflect UV strongly (Fig. 1; Obara and Hidaka, 1968; Obara, 1970).

The difference in wing color sexual dimorphism between the two subspecies explains why males of the two subspecies have different mating behaviors. For British males using visual cues to recognize mates, sexual discrimination must be difficult due to only a slightly appreciable optical wing dimorphism and a proportion of males will be expected to mistakenly make copulatory approaches to conspecific males. This was shown to be the case in the present observation. On the other hand, in Japanese subspecies with distinct sexual dimorphism in the UV wing color, virtually no males (one out of 107 males) approached conspecific male wing dummies when single male and female dummies are juxtaposed 20 cm apart (Obara, 1970).

How, then, is final mate recognition achieved by the British males? In cases when a male approached conspecific resting males, no copulatory attempts occurred because the resting male displayed the flutter response. This effectively prevented the approaching male from landing beside the resting male, which is a prerequisite for a male to attempt copulation. It follows that the flutter response of resting males plays an important role in the mate recognition when female-searching males approach; i.e. the flutter response functions as a "mechanical isolation mechanism" against mating interaction between males.

2. Flutter response

Ohtani and Yamamoto (1980) suggested that the flutter response by a male *in copula* functions to dispell males who

approach and attempt to copulate with the female *in copula*. They presented, however, no explanation for the flutter response displayed by males not *in copula*. Obara and Hidaka (1964) reported that the flutter response serves no apparent purpose to the males who elicit it nor to the males who perform it. The flutter response thus seems functionless and overt benefit will accrue to neither of the males.

This discussion allows speculation on which of the two subspecies is evolutionarily ancestral to the other. It is improbable that the flutter response has evolved in the Japanese subspecies independently, due to the costs to both female-searching males and resting males, with no overt benefit accruing. On the other hand, for the British subspecies with little sexual dimorphism, the flutter response may have been critically necessary for mate recognition by the male. It may also have been beneficial to males by increasing the temporal and energetic efficiency of their mate-search. Once the UV color cue become available for mate recognition in the Japanese subspecies, however, the flutter response would have been superfluous in mate recognition.

Based on this discussion we conceived no necessary behavioral-ecological factors that would have promoted the evolution of the flutter response in the Japanese subspecies, whereas the flutter response should have been favoured by natural selection in the British subspecies. Consequently it may be possible that the flutter response probably evolved first in the British subspecies as a mate recognition or "mechanical isolation mechanism" between males and that it has remained in the Japanese subspecies as a rudimental behavior with no overt function. If so, this may be evidence for the hypothesis that the British subspecies, *P. r. rapae* is ancestral to Japanese subspecies, *P. r. crucivora* and consequently that the absence of female UV wing color is the ancestral state.

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