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Mate Recognition in Two Sympatric Species of Butterflies, *Pieris rapae* and *P. melete*

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Abstract – Female butterflies should distinguish conspecific males from males of different species. The aim of the present study was to evaluate the female role in avoiding allospecific mating between 2 sympatric pierids, *Pieris rapae crucivora* (*P. rapae* hereafter) and *P. melete*. Since it is relatively rare to observe responses of a wild female to a courting male of different species, we substituted the wings of a female with those of a female of the other species. Such a female attracted allospecific males. Female *P. melete* took "mate-refusal posture" to courting males, regardless whether the male belonged to different species or not. Female *P. melete* discontinued the posture only when the male was conspecific. Such a behavioural difference indicates that the female discriminated its conspecific male. On the other hand, when only the mate-refusal posture was taken into account, a female *P. rapae* did not differently respond to courtship of males of both *P. melete* and *P. rapae*. Having once approached, the male *P. melete* attempted more frequently to copulate with its conspecific females than with allospecific ones, while the male *P. rapae* indiscriminately attempted to copulate with both allospecific and conspecific females. The male *P. melete* tended to persist in its courtship to females of both *P. rapae* and *P. melete* who took the mate-refusal posture. The mate recognition system by male and female in these 2 species is discussed in connexion with the difference in mating behaviour pattern of the species.

Individual butterflies identify a conspecific mate from others by using visual, olfactory and/or mechanical cues provided by their partner (reviews by Scott 1973; Silberglied 1977), after which they can make a decision to choose a proper mate. Since the mechanism of recognizing the proper mate can be used in not only male (or female) recognition but also species recognition, it should function as one of the isolating mechanisms between closely related and quasi-sympatric species (Taylor 1973; Silberglied & Taylor 1973, 1978).

It has been reported that males of many butterfly species may chase and sometimes court females of different species: *Eumenis semele* L. (Tinbergen et al. 1942); *Pieris bryoniae, P. napi* and *P. rapae* (Petersen et al. 1952; Petersen & Tenow 1954); *Colias eurytheme* and *C. philodice* (Taylor 1973), suggesting that the males have a relatively poor discrimination ability (Silberglied 1977). Therefore, females should also discriminate and choose conspecific males to avoid allospecific mating, and to save both time and energy. However, little is known about how female butterflies behave when they are courted by allospecific males freely flying in the field.

Artificial "chimeras" of females of 2 quasi-sympatric pierids, *Pieris melete* and *P. rapae*, were prepared to induce such allospecific courting in the field. The chimeras had their own head and body but wings of the other species, and attracted, "despite her intention", males of the different species.

Materials and Methods

Butterflies

Virgin females of both *Pieris rapae crucivora* Boisduval (*P. rapae* hereafter) and *P. melete* Ménétriès were used after they had emerged in our laboratory.

Both pierids are common in the Toky region, but *P. rapae* prefer open and sunny areas like a cabbage field, while *P. melete* often fly around in residential quarters and in areas adjacent to forests where many shaded habitants are presents. Males of the both are patrol-type strategists, and detection of the female is made visually (Obara & Hidaka 1968; Obara 1970; Suzuki et al. 1977).

Wing Operation

Since visual signals of female wings play an essential role for males of both species in recognizing their conspecific females (Obara 1970; Suzuki et al. 1977), wings of a female of either *P. rapae* or *P. melete* were exchanged with those of the other species so that the female was enabled to attract allospecific males. By using this method, we could easily observe responses of a female that was courted by males of a different species (see also Silberglied & Taylor 1978).

Two days after emergence, the wings of a recipient female were cut apart and changed with the wings of a donor female (Fig. 1). The wings of the donor were glued on the stumps of the recipient's wings by using Aron-α (produced by Tôagôseikagaku Ltd.). After the wing operation the recipient could move both body and wings. The follwoing 4 types of females were tested.

mFr: female *P. melete* with wings of female *P. rapae*
mFm: female *P. melete* with wings of another female *P. melete*
rFm: female *P. rapae* with wings of a female *P. melete*
rFr: female *P. rapae* with wings of another female *P. rapae*

Experiments were carried out between 11 : 00 and 16:00 from May to August 1975, in Fuchû and Takao regions in Tokyo. Each female in every test was settled on a leaf of a plant.

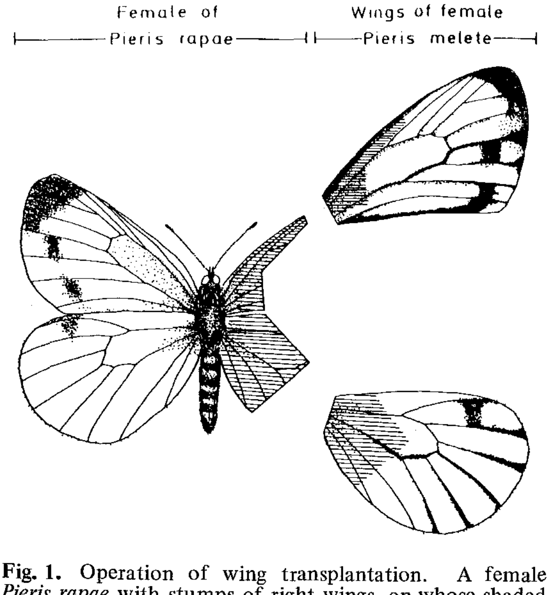

Fig. 1. Operation of wing transplantation. A female *Pieris rapae* with stumps of right wings, on whose shaded parts right wings of a female *P. melete* are adhered.

In order to maximize the chance of allospecific courting, female chimeras with wings of female *P. melete* (rFm) (and also mFm) were placed in a residential quarter where mainly male *P. melete* were seen flying. Female chimeras having wings of female *P. rapae* were placed in an open and sunny area like a cabbage field where male *P. rapae* were abundant. We did not design the experiments to investigate the response of mFr to male *P. melete*, or that of rFm to male *P. rapae*.

Each observation was carried out for 30 min, but terminated when mFm and rFr copulated with their conspecific males.

Behaviour Patterns Recorded

The following patterns of behaviour were observed and recorded.

Approach: When a male flew to within ca. 1 m of a female, decreasing its flight speed and often changing both direction and a wavelike mode of flight to a straight approach toward the female, this was recorded as an "approach". When a single male which had once flown away approached again, the second approach was acounted as another approach.

Contact: When an approaching male touched a female, it was recorded as a contact. When a male which had once flown away approached and touched the female again, the second contact was counted as another contact.

Copulatory attempt: When a contacting male tried to copulate with a female, bending its abdomen toward the genitalia of the female, this was recorded as a copulatory attempt. Each bend of the abdomen was counted as a copulatory attempt.

Mate-refusal posture: Females spread and depressed their wings and raised the abdomen upright. Since this posture is characteristic of mated females of both species which are refusing the coutship of conspecific males (Obara 1964; Suzuki et al. 1977), it has been called conventionally "the mate-refusal posture". Although Wiklund and Forsberg (1986) pointed out that this term specifically implies the function of this behaviour when it should not, we used the popular term "mate-refusal posture" and counted each use of this posture in response to an approach as one occurrence.

Copulation: A courting male can copulate with a female which does not show any refusal posture, and with a female which discontinues the mate-refusal posture. Copulation occurrs only once for each female.

Results

In the observation periods, males approached only the females whose wings belonged to their conspecific female. Males of both *P. melete* and *P. rapae* are often observed chasing an allospecific female which is flying some distance ahead and sometimes court the female (Obara personal communication). However, a perching female is rather seldom courted by an allospecific male. The results obtained here were therefore plausible.

Since frequency of approaches to individual females varied considerably, relative numbers of contacts to approaches (a), copulatory attempts to approaches (b) and mate-refusal postures to approaches (c) were calculated for each female and averaged over the group (Fig. 2). Those results were statistically analysed by non-parametric methods: Mann-Whitney U-test and Fisher exact probability test.

Female Recognition by Male P. rapae

Male *P. rapae* approached females with transplanted wings of a female *P. rapae*, regardless of whether the females were *P. rapae* (rFr: mean frequency of approaches observed during each observation period ± SD = 8.7 ± 6.9) or *P. melete* (mFr: 9.0 ± 3.6). Having once approached, the male *P. rapae* contacted the female *P. melete* (mFr) as frequently as the female *P. rapae* (rPr) (Fig. 2a). After the contact the male *P. rapae* attempted to copulate indiscriminately with mFr and rFr (Fig. 2b).

Female Recognition by Male P. melete

Male *P. melete* approached the female with transplanted wings of a female *P. melete*, regardless of whether the female was *P. rapae* (rFm: mean ± SD = 10.2 ± 15.0) or *P. melete* (mFm: 8.7 ± 12.7). Having once approached, the male *P. melete* indiscriminately contacted mFm and rFm (Fig. 2a).

However, after the contact the male *P. melete* exhibited more copulatory attempts with conspecific females (mFm) than with the allospecific ones (rFm) (Fig. 2b, P < 0.01, U-test, 2-tailed).

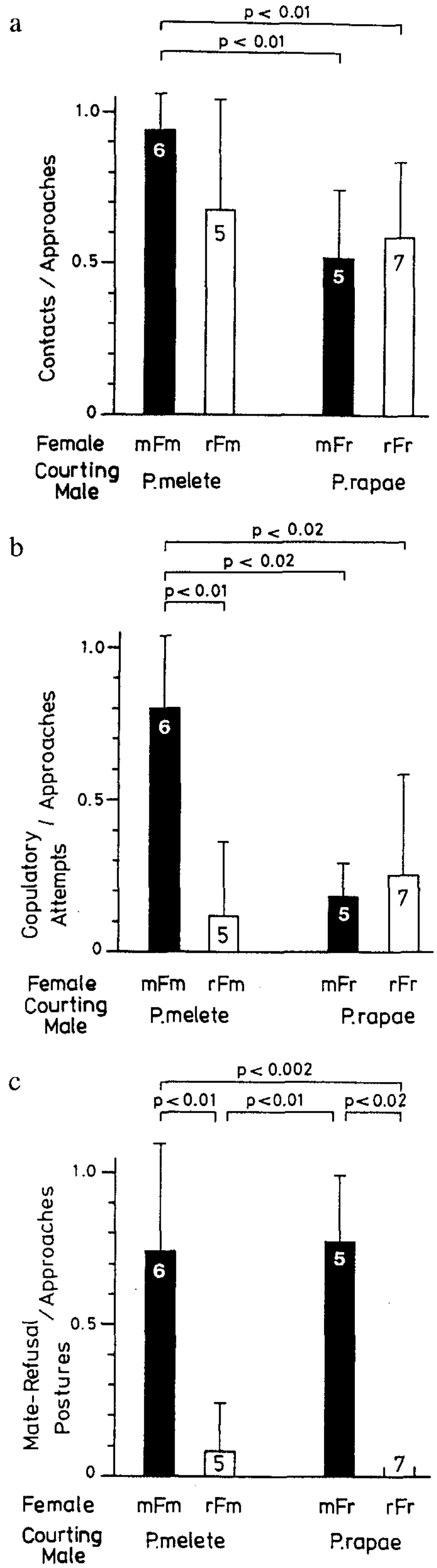

Fig. 2. Response of males when they courted both conspecific and allospecific females: relative numbers of contacts to approaches (a), and copulatory attempts to approaches (b) in 4 different types of females. Response of females when they were courted either by conspecific or allospecific males: relative numbers of mate-refusal postures to approaches (c) in the 4 types of females. The numbers in columns show the numbers of females tested. Solid columns: female *P. melete*, open columns: female *P. rapae*. vertical bars: S.D. Statistics: Mann-Whitney U-test, 2-tailed. Responses are statistically nonsignificant in pairs not connected at the top of each figure. For further explanations see text.

Five out of 6 female *P. melete* (mFm) took the mate-refusal posture during the contact and all of them experienced copulatory attempts by male *P. melete*. Male *P. melete* did not attempt to copulate with 3 female *P. rapae* (rFm) which did not take the mate-refusal posture, while males frequently showed couplatory attempts with a female *P. rapae* (rFm) which displayed the mate-refusal posture.

Responses of Female P. rapae

Three out of 7 female *P. rapae* (rFr) copulated with conspecific males, while allospecific copulation between 5 female *P. rapae* with wings of female *P. melete* (rFm) and male *P. melete* did not occur.

All 5 female *P. rapae* (rFm) did not show any obvious rejecting behaviour when they were approached by the male *P. melete*. After the approach contact occurred in 4 of these 5. One of the females exhibited the mate-refusal posture against the contact of a male *P. melete* (Fig. 2c), although a virgin *P. rapae* usually does not take this posture.

Responses of Female P. melete

Virgin female *P. melete* took the mate-refusal posture more often than virgin female *P. rapae* did, when they were approached or contacted by conspecific males (Fig. 2c, $P < 0.05$, U-test, 2-tailed) as well as by allospecific males ($P < 0.05$, U-test, 2-tailed).

Four out of 6 female *P. melete* (mFm) discontinued the mate-refusal posture against the courtship of male *P. melete*, and all of them copulated with the males. All 5 female (mFr) continued the mate-refusal posture when they were courted by male *P. rapae* and allospecific copulation did not occur. The difference of the female response to the male courtship was statistically significant ($p < 0.05$, Fisher exact probability test, 1-tailed).

Discussion

In the present study, we attempted to observe the mating behaviour of individual female butterflies during allospecific coutship by males. We did not conduct experiments to analyze the stimuli which release various responses of the mating behaviour: the study was concerned with the mating behaviour as a whole, but not analytically with its components.

Mate Recognition in Pieris rapae

At the level of contact and copulatory attempts in the sequence of mating behaviour, the male *P. rapae* did not seem to discriminate the allospecific female with *P. rapae* wings. Wings of female *P. rapae* reflect near-ultraviolet light (380–400 nm), being different from male wings. Such sexual dimorphism of an ultraviolet reflection pattern serves as the basis for precise female recognition by the male (Obara & Hidaka 1968; Obara 1970; Rutowski 1977a; Silberglied 1977; Silberglied & Taylor 1978). The visual cue used for recognition of the female before approach by the male should play an important role in avoiding allospecific courtship. On the other hand such obvious sexual dimorphism in *P. rapae* may reduce the importance of further cues for recognition of the female by the male.

Silberglied & Taylor (1978) showed by elaborate experiments that females of both *Colias eurytheme* and *C. philodice* actively avoided allospecific copulation. In the present study most virgin *P. rapae* did not show any obvious rejecting activity, such as the mate-refusal posture (Obara 1964; Suzuki et al. 1977) against approaching or contacting male *P. melete*. The female seemed to play only a passive role in avoiding allospecific copulation. However, it is still unclear whether the female *P. rapae* was not discriminating conspecific from allospecific males.

Mate Recognition in Pieris melete

The present study showed that a male *P. melete* distinguished its conspecific female after the contact with her, while male *P. rapae* did not. However, the male *P. melete* seemed not to discriminiate the female *P. rapae* with the wings of female *P. melete* when she took the mate-refusal posture. The mate-refusal posture may be one of the behavioural cues with which the male *P. melete* distinguishes its conspecific females.

Male cannot copulate with a virgin female *P. melete*, as long as she persists in taking the mate-refusal posture (Suzuki et al. 1977). To avoid wasting time spent in taking the mate-refusal posture before an adequate copulation, she must distinguish a conspecific male for herself and cancel the mate-refusal posture when she finds a proper partner. After the contact with a male, a female *P. melete* cancelled the mate-refusal posture when the male was conspecific, but not when the male belonged to a different species.

Cancellation of the mate-refusal posture might result from male manipulation rather than from the decision of the female; the male *P. melete* made the female close her wings and depress her abdomen by pressing against her body. However, the female *P. melete* was often observed cancelling the posture while the courting males were flying around her. Obviously the female response must be attributed to some decision on her part. Of course, this conclusion does not deny the role of male *P. melete* in mate recognition, because the individual male itself should choose its proper mate, optimally investing its time and energy.

Rutowski (1981) showed that the female checkered white (*Pieris protodice*) visually discriminates conspecific males and females at close range on the basis of differences between the sexes in ultraviolet reflectance rather than on the basis of differences in movement patterns or chemical signals. The wings of the male and female *P. melete* are not clearly distinguishable as regards the ultraviolet reflection (Hidaka unpublished). On wings of the male *P. melete*, however, a number of scent scales occur. The male *P. melete* hovers above and behind the female *P. melete* which is taking the mate-refusal posture, presumably promoting her perception of the odour emitted from the scent scales (Hidaka unpublished; see also Tinbergen et al. 1942; Scott 1973; Rutowski 1977b). The female *P. melete* may discriminate its conspecific males by the male odour, when the male hovers and/or makes contact with her during the period of the mate-refusal posture. This possibility should be tested in analytical experiments.

The results of our present analysis indicate that female *P. melete* play an important role in avoiding allospecific copulation. Another species of pierid, *P. napi*, is widely distributed in Japan and occurs in some areas together with *P. melete*. *P. napi* has a similar appearance to *P. melete* and its virgin females also exhibit the mate-refusal posture against courtship of conspecific males (Suzuki et al. 1977). Studies on allospecific mating behaviour between *P. melete* and *P. napi* should provide further knowledge on the female role in avoidance of allospecific mating.

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