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Role of Male Scent in the Mating Behavior of *Pieris melete* Ménétriès (Lepidoptera: Pieridae)*

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Abstract — A *Pieris melete* male emits species-specific scent from the wings, while a virgin female of *P.melete* is frequently observed to take the so-called "mate-refusal posture" in response to courting males. The role of the male scent in the mating behavior of *P.melete* was investigated experimentally by using scented and scentless male models. It is suggested that the male wing scent can function primarily as a sex pheromone to "seduce" the virgin females that assume the mate-refusal posture, and can therefore induce a successful copulation.

Introduction

Pieris melete Ménétriès is sympatric with *P. rapae crucivora* Boisduval in Japan. *P. rapae crucivora* males rely on visual cues for conspecific mate recognition. *P. rapae crucivora* has a sexual dimorphism of an ultraviolet reflection pattern which serves for female recognition (Obara & Hidaka 1968; Obara 1970). The use of sexual dimorphism in ultraviolet reflectance for mate recognition has also been found in several other pierid butterflies such as *P. protodice* (Rutowski 1981), *Colias eurytheme* and *C. philodice* (Silberglied & Taylor 1978) and *Eurema lisa* (Ghiradella et al. 1972; Rutowski 1977a & 1978). *P. melete* does not have such clearly distinguishable sexual dimorphism in the ultraviolet reflectance (Hidaka & Kan unpublished), and it is believed that it does not rely on visual cues for mate recognition.

P. melete males have scent scales (androconia) which are responsible for their wing scent (Abe et al. 1986; Yata et al. 1986). There have been several studies on the chemistry of components of these scales (Hayashi et al. 1978; Kuwahara 1979; Abe et al. 1986; Yata et al. 1986), but the biological functions of the scale scent have not been experimented with. While, *P. melete* females, both mated and virgin, are often observed in a "mate-refusal posture" (Obara 1964; Suzuki et al. 1977) in response to male approach. The female spreads and depresses her wings and raises her abdomen upright, while the male keeps hovering above and behind her. In this study, the role of the male wing scent was investigated experimentally by using scented and scentless male models.

Materials and Methods

Butterflies

Male and female *Pieris melete* Ménétriès adults used in this study were reared from eggs in our laboratory at 25℃, 12 L: 12 D. Larvae hatching from the eggs were fed on their normal host plant, *Rorippa indica*. Pupae obtained from the larvae were kept and allowed to emerge in separate transparent plastic containers (10 cm diameter at base and 13 cm at top; 6 cm ht). After the emergence, adults were kept in the separate rearing containers for 2 to 4

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days until use in the study. Adults were fed on 5% sucrose solution soaked in a small cotton wads that were changed everyday.

Male models

Scentless and scented male models were used in the model presentation experiments.

Wings of 3 - to 4 - day-old virgin males were used for the male models because these males were considered to be fully sexually mature and with a vigorous scent. Soon after emergence, male butterflies (in their rearing containers) were kept in a cardboard box (110×90×90 cm) placed in the laboratory. The box was covered with a piece of black curtain to keep the interior dark. The butterflies stayed motionless in the dark, without damaging their wings, until they were killed (scent was emitted from their wings in the dark, too). These males were fed on 5% sucrose solution 3 times a day, under a lamp.

(a) Scentless male model

Wings were removed from a body of a freshly killed male butterfly by pressing his thorax. The fresh wings were deodorized with 2 immersions (each 1 h) in diethyl ether at 10℃. Ten ml of diethyl ether was used per 2 pairs of wings. The deodorized wings were then put on a filter paper and air-dried at room temperature for 1 day. In the preliminary experiments, there was no significant difference in the ultraviolet reflectance of ether-treated and control wings.

After the treatments, 2 pairs of the deodorized wings (2 fore-wings and 2 hind-wings) were glued on a piece of transparent acrylic plate (1×2×0.5 cm) fixed at the tip of a transparent acrylic stick (0.3×0.3×50 cm). A transparent glue (CEMEDINE C, Cemedine Co., Ltd., Japan) was used. The wings were glued with their dorsal surfaces up in a position identical to that of living *P. melete*, with the wings open.

(b) Scented male model

Theoretically, the best way to make the scented male model may be to deodorize male wings and then impregnate them with scent extracts. However, this ideal model rapidly lost its scent, and had uncontrollable volatility during the experiment. Fresh male wings were therefore used as scented male models. These models could emit male scent at an almost constant rate for at least 1 h. Thirty min before the beginning of the trials, the wings were removed from a body of a freshly killed male and glued to the acrylic plate (as described in (a)).

Observations and experiments

Observations on the mating behavior in *P. melete* and male model presentation experiments were carried out between 8 : 30 and 13 : 30 from May 26 to June 29 in 1990, in a net cage (2×2×2 m) on the grounds of Kyoto University in Kyoto.

Two-day-old virgin females and 3 - to 4 - day-old virgin males were used. Soon after releasing 4 females, 4 males were released into the net cage. Their mating behavior was observed and recorded for 2 h by a VHS video camera (AG 350, National Co., Ltd., Japan).

In the male model presentation experiments, 2-day-old virgin females were used. In each experiment, a virgin female was released into the net cage and then carefully placed on a leaf of vegetation to give her a rest for at least 15 min. After a stationary posture with closed wings was confirmed, the experiment was started. In every experiment, presentations of the scentless male model was carried out first, and then followed with the scented male model after 10 min.

The scentless or scented male model was presented to the female, where the male model on the tip of the acrylic stick, held at the same level as the female, was rotated by hand at a distance of about 10 cm from the female. Presentations were alternated between right and left sides at 1-min intervals. The trial was repeated 10 times in each series.

The following response patterns of the virgin females were observed in the preliminary experiments.

Mate-refusal posture (MR) : See above. Despite the doubt posed by Wiklund and Forsberg (1985), we use the popular term of "mate-refusal posture."

Leaning (L) : The female with her wings closed leaned slightly toward the model (Fig. 1).

Fig. 1. "Leaning" response to the presented scented male model by female *Pieris melete*: (left) female, (right) scented male model.

Stationary posture (S): The resting female assumed a stationary posture with her wings closed.

Head shaking (H): Sometimes the resting female shook her head.

Flying (F): The female that had assumed the mate-refusal posture abruptly closed her wings and flew away.

It was often observed that after several presentations of the scentless male model, the female in the stationary or mate-refusal posture shook her head. Once started, this behavior continued whether or not the model was presented.

Results

I. Mating Behavior

The mating behavior of *P. melete* varied considerably from pair to pair, especially among females: a few virgin females copulated with males without ever taking the mate-refusal posture, but most of them took this posture before they accepted copulation. Their mating sequence was long-lasting, and not always leading to successful copulation.

1. Successful mating sequences

The male found a resting female with her wings closed and approached her. The resting female took the mate-refusal posture (MR).

The male alighted on the female without stopping the motion of his wings. The female either continued MR or fluttered her wings and synchronously twitched her abdomen up and down. Keeping his balance, the male tapped her on her wings, and bent his abdomen laterally or ventrally. Usually, the male remained on her for about 1 min, and then he took off and began to "hover." This consisted of staying at a fixed position in the air, above and behind the female, at a distance of about 40 cm. Sometimes the hovering lasted for more than 1 min but was highly variable in duration. While the male continued hovering, the female maintained MR or took the stationary posture with her wings closed and often leaned slightly toward the male hovering at her side. Sooner or later, the male stopped hovering, alighted beside the female, and then attempted copulation by curving his abdomen laterally. Usually, the copulation did not occur instantly at this stage. The female either maintained MR or flew away to escape from the male. In the former case, the male alternated the hovering and the

copulatory attempt until the female took the stationary posture. In the latter case, the male pursued the escaping female. After the female alighted on a plant or the cage net, the male approached her and again alternated hovering and copulatory attempts. At the moment that the female discontinued these mating-avoiding behaviors to take the stationary posture, the male caught her by the front margin of her fore-wings with his fore- and mid-legs. Then the male curved his abdomen laterally, and inserted it between her hind-wings to take hold of the tip of her abdomen with his genitalia.

Soon after copulation had started, the male took off for a short distance flight, dragging the female upside-down with her wings closed. Usually, the couple alighted on the nearby plants or the net. Then the sexes faced opposite directions and continued copulation there.

2. Unsuccessful mating sequences

These sequences were also initiated by the male approaching a resting female. The courting male sometimes tried to alight on her, shifted to hovering, or attempted to copulate, but the female continued to maintain MR endlessly. In 3 cases, the female kept her rigid MR for 2 h, and copulation was not achieved.

Sometimes the female flew away after she took MR in response to the courting male and then alternated this escaping flight with MR until the end of the observation. In this case also, no successful copulation occurred.

II. Male Model Presentation Experiments

Thirteen virgin *P. melete* females were tested in the male model presentation experiments. The results are shown in Fig. 2 and Tables 1-a and b, and 2.

The "leaning (L)" response of the virgin females was observed only in the presentation of the scented male model (Tables 1-b and 2). The MR was taken by the females not only during the presentation of the scented male model but also in response to the scentless male model (Table 1-a). However, all females eventually dropped MR to the scented male models and showed the L response, while most of them persisted in MR toward the scentless male models throughout the experiments.

As shown in Table 1-a, all females took MR towards the scentless male model on the 1st trial, spreading and depressing their wings and raising the abdomen at angles of 30° to 60°. Two out of 13 virgin females (Nos. 1 and 2 in Table 1-a) took MR on every trial until the final (10th) one. Nine out of 13 females (Nos. 3, 5, 7, 8, 9, 10, 11, 12 and 13) took MR in combination with the head shaking (H) at later stage of the experiments. The rest of the females (Nos. 4 and 6) ceased MR, and then

Table 1-a. *Pieris melete* female responses to the scentless male model on each presentation trial.

Individual No. of females	Trial No.									
	1	2	3	4	5	6	7	8	9	10
1	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR
2	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR
3	MR	MR	MR	MR	MR	MR	MR	MR	MR/H	–
4	MR	MR	MR	MR	MR	MR	MR	MR	MR→S/H	–
5	MR	MR	MR	MR	MR	MR	MR	MR/H	–	–
6	MR	MR	MR	MR	S/H	–	–	–	–	–
7	MR	MR	MR	MR	MR	MR/H	–	–	–	–
8	MR	MR	MR	MR	MR	MR	MR/H	–	–	–
9	MR	MR	MR	MR	MR	MR/H	–	–	–	–
10	MR	MR	MR	MR	MR	MR/H	–	–	–	–
11	MR	MR	MR	MR	MR	MR/H	–	–	–	–
12	MR	MR	MR	MR	MR	MR	MR/H	–	–	–
13	MR	MR	MR	MR	MR	MR/H	–	–	–	–

MR : Mate-refusal posture ; S : Stationary posture ; and H : Head shaking.

Table 1-b. *Pieris melete* female responses to the scented male model on each presentation trial.

Individual No. of females	Trial No.									
	1	2	3	4	5	6	7	8	9	10
1	MR	MR	MR	MR	MR	MR	F→L	–	–	–
2	MR	MR	MR⇄F	F→L	–	–	–	–	–	–
3	L	–	–	–	–	–	–	–	–	–
4	MR	MR	L	–	–	–	–	–	–	–
5	MR	MR	MR	MR	MR⇄F	MR⇄F	L	–	–	–
6	MR	MR	L	–	–	–	–	–	–	–
7	MR	MR	F→L	–	–	–	–	–	–	–
8	MR→F	F→MR	F→L	–	–	–	–	–	–	–
9	MR	MR	MR	MR	MR	MR	MR	F→L	–	–
10	MR	F→L	–	–	–	–	–	–	–	–
11	MR	MR	MR	F→L	–	–	–	–	–	–
12	MR	MR	MR	L	–	–	–	–	–	–
13	MR⇄F	MR⇄F	MR→F→L	–	–	–	–	–	–	–

MR : Mate-refusal posture ; L : Leaning ; and F : Flying.

Table 2. "Leaning" response to presented models.

Model types	No. of females "Leaning" response		Total
	(+)	(−)	
Scentless male model	0	13	13
Scented male model	13	0	13

they took the stationary posture (S) in combination with H, on the 8th and 5th trials respectively. With the scented male model, 12 females out of 13 took MR on the 1st trial (Table 1-b). Nine of them (Nos. 1, 2, 5, 7, 8, 9, 10, 11. and 13), however, then took off, flew (F) for some distance, alighted on vegetation or net, and resumed MR. After this take-off, the females were followed by the model. They alternated flying up and landing with successive MR, until they showed the L response. Three others (Nos. 4, 6 and 12) showed L on the 3rd or the 4th trial without further take-off. One female (No. 3) showed L as early as on the 1st trial without taking MR.

The number of presentations of the model needed for the L response is shown in Fig. 2.

The scented male model thus induced the L response of the female, while the scentless male model did not.

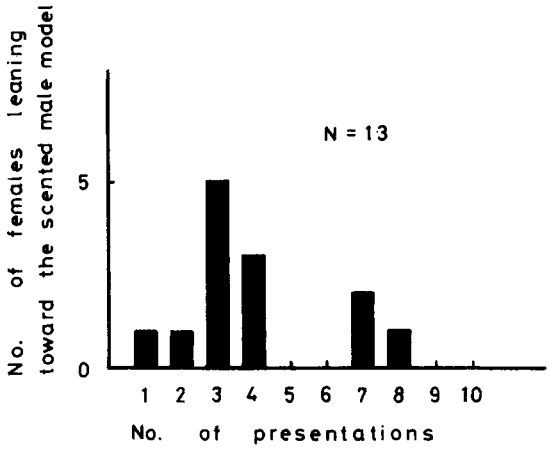

Fig. 2. No. of model presentations needed for induction of "leaning" response in *Pieris melete*.

Discussion

The results of this study show that *P. melete* virgin females usually took the mate-refusal posture in response to the scented male model at the initial stage of the experiments but eventually showed the leaning toward the model (Tables 1-b and 2). In contrast, the females showed different responses to the scentless male model. They also took the mate-refusal posture at the initial presentations, but maintained them throughout the experiments and did not lean toward the model (Tables 1-a and 2). Although the typical leaning response was not so frequently observed in the wild, females that ceased the mate-refusal posture leaned slightly toward the courting male that emitted the wing scent. Soon after the leaning, the female allowed the male to copulate. Therefore, our results indicate that *P. melete* females rely on olfactory cues for conspecific mate recognition. The male wing scent of *P. melete* may act as a sex pheromone "seducing" the conspecific female for the cancellation of her mate-refusal posture and for the induction of the leaning response leading to successful copulation. Ohguchi & Hidaka (1988) suggest a possibility that *P. melete* female may recognize her conspecific male by the male scent when the male hovers and/or makes contact with her during the period of her mate-refusal posture. Our present results seem to support this hypothesis. The variety in the timing of the leaning response may be due to the difference in the physiological state of the females, but it may also be related to the problem of the female choice.

The importance of male wing scent in the conspecific recognition and its probable connection with scent-scale secretions has been reported: *C. eurytheme* and *C. philodice* (Taylor 1973; Silberglied & Taylor 1978; Grula et al. 1980; Rutowski 1980), *E. lisa* (Rutowski 1977b), *Lycaeides argyrognomon* (Lundgren & Bergström 1975) and *Zizeeria maha argia* (Wago 1978). Our results show that it applies to *P. melete*.

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