

## **Female Abdomen Position Signals Receptivity During Courtship in the Japanese Sulfur Butterfly, *Colias erate* (Pieridae)**

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Source: The Journal of the Lepidopterists' Society, 65(4) : 259-263

Published By: The Lepidopterists' Society

URL: <https://doi.org/10.18473/lepi.v65i4.a6>

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FEMALE ABDOMEN POSITION SIGNALS RECEPTIVITY DURING COURTSHIP IN THE JAPANESE SULFUR BUTTERFLY, *COLIAS ERATE* (PIERIDAE)

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**Abstract.** Females of the Japanese sulfur butterfly, *Colias erate*, eclose in the grassland vegetation in early morning and copulate immediately after their wings fully expand. Once mated, females lose their interest in mating with males that approach them. Receptive females exposed their abdomens toward courting males, whereas unreceptive females hide their abdomen. Field observation of males' courtship behavior to females was carried out by focusing on the abdomen posture of females. We glued the wings of females together, forced their abdomen to be either exposed from their wings or hidden in their wings, and presented them to males. When males found the females, most males hovered around the female. Males alighted near the females when their abdomens were exposed. However, when their abdomens were hidden, males stopped courting and left the females. Females with exposed abdomens, irrespective of mating experience, copulated with the male courting. Thus, the female posture of hiding her abdomen was a signal of unreceptivity, as well as the rejection of the courting males.

**Additional key words:** Exposed abdomen, mate refusal, mating experience, receptivity

Although sexual communication in butterflies involves the use of multiple signals at different phases of mate recognition and choice, constituting a complex scenario (Klein & Araujo 2010), initial attraction usually takes place by visual channels (Scott 1972). The information contained in the male visual signals alters or controls the female's behavior, and vice versa. Alfalfa fields in the USA and Canada are a favorable habitat for sulfur butterflies, *Colias* spp., providing abundant food for the larvae and adults (Hovanitz 1944), showing mating behavior within the field in which they emerged (Stern & Smith 1960). In Japan, there are few single alfalfa fields, while a lot of ski slopes are abundantly covered with clovers such as *Trifolium repens* and *T. pratense* in the cool temperature zone (Watanabe & Nakanishi 1996). In this region, the Japanese sulfur butterfly, *C. erate*, is abundant and can be seen flying and courting in the middle of the day during summer.

Flight is essential to butterflies in looking for mates, food and new habitats. Silberglied & Taylor (1978) clarified that males of *C. eurytheme* and *C. philodice* search for females visually, using UV-reflectance, and that some pheromone is vital to the females. In *C. erate*, Watanabe et al. (1997) showed that males can easily encounter females when they fly around the habitats, probably using visual cues. Irie & Watanabe (2009) also reported that newly-emerged females are, in general, easily found by mate-searching males on the wing in the early morning. Such virgin females simply accept

courting males and then copulate. On the other hand, mated females become unreceptive to male courtship advances (Hasegawa & Watanabe 2008). Consequently, almost every flying female in the daytime seems to be unavailable for searching males. Mated females showed a pierid-specific mate refusal posture at the perching site (wings-spread, abdomen nearly vertically upturned) or do a so-called ascending flight followed by the courting males (Hasegawa & Watanabe 2008). However, the dissection of females captured in the fields indicates that females mate a couple of times during their life span (Nakanishi et al. 1996), indicating that mated females do not always refuse males. Therefore, males have a chance to mate with experienced females as well as with virgin females.

In the daytime, males actively patrol on the wing, searching for females in grasslands (Watanabe & Imoto 2003). When they find a female perching or flying, they visit the female to start courtship behavior. Males hover around the perching female, or near the flying female inhibiting her flight course. When the female accepts the courtship of the male, she alights on the vegetation, remains motionless on the perch site, and extends the abdomen toward the male from her hindwings for copulation, as reported in other *Colias* butterflies (Rutowski 1980). The male follows her, alights next to the female, and bends his abdomen to the tip of the female's abdomen for copulation. However, when the female shows mate refusal behavior due to a recent copulation,

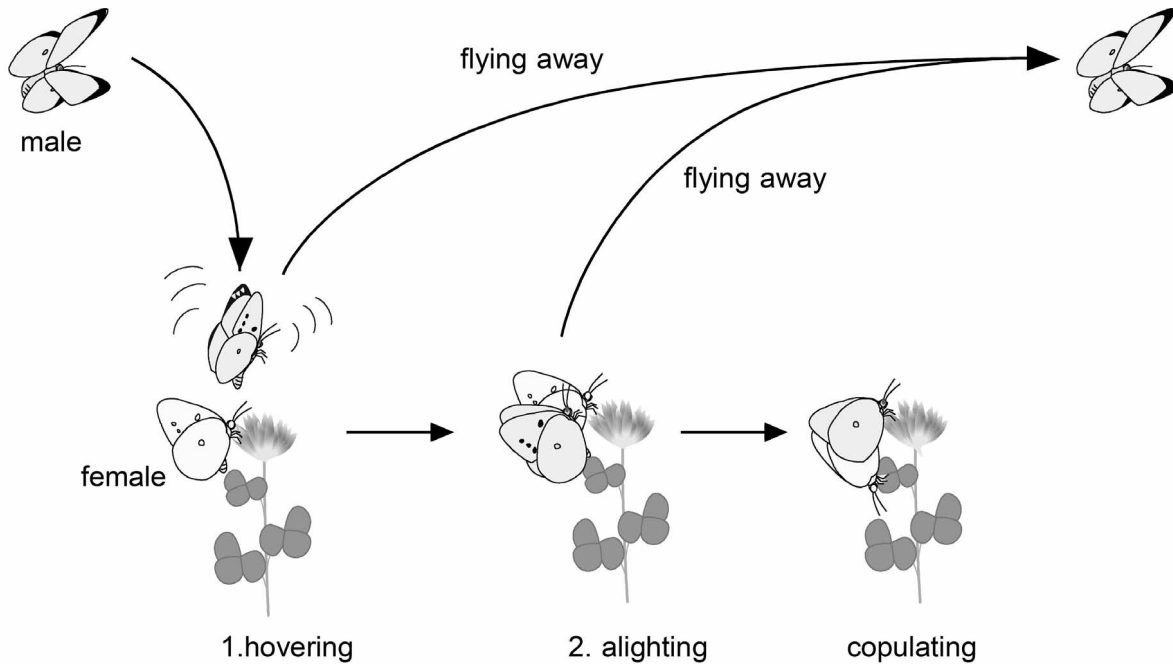


FIG. 1. A schematic representation of the courtship behavior of the *Colias erate* male to the presented female (c.f., Hasegawa & Watanabe 2008). **1.** A flying male visits the presented female and starts to hover in front of the female. **2.** The male alights on the edge of female's wings, walks down, and bends the abdomen towards the female, then the male and the female copulate. In each stage, when the males abandon copulation, they fly away from the female.

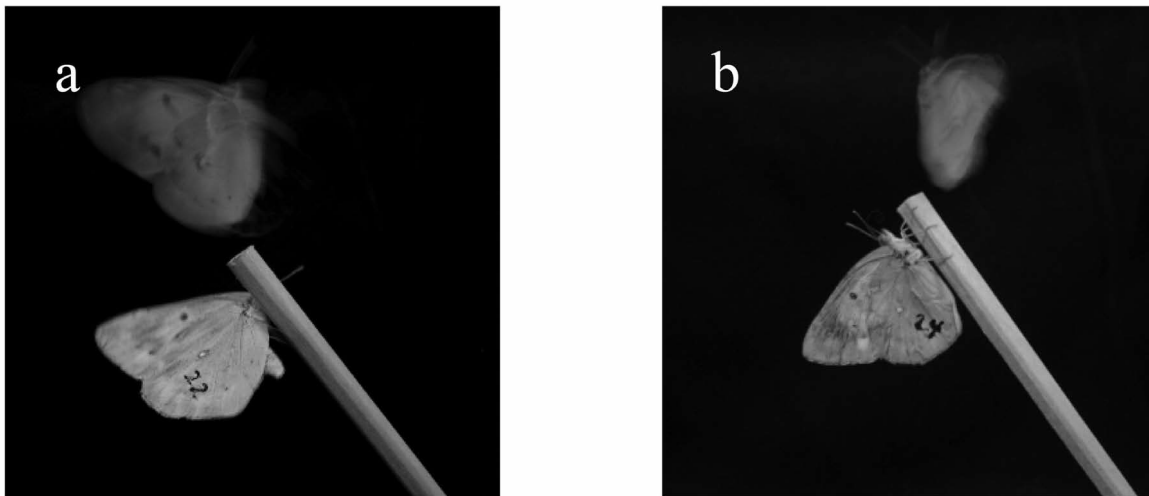


FIG. 2. The female response to the fluttering male. **a.** the virgin female showed the exposed abdomen to the male. **b.** the unreceptive female did not expose the abdomen.

males stop their courtship behavior and fly away from her (Hasegawa & Watanabe 2008). In order to identify the counter behavioral signal of the mated females against the male courtship behavior, inhibition of such behaviors was useful in the field experiment (Irie & Watanabe 2009). In the present study, in order to understand the adaptive significance of female refusal, we quantitatively investigated the courtship behavior of males to females whose abdominal movements were manipulated.

#### MATERIALS AND METHODS

**Female response to male courtship.** The abdominal responses of females to courtship behavior of males were investigated using virgin and non-virgin females in August 2008. Virgin females were the offspring of wild *C. erate* females captured in July 2008 in Tsukuba, Ibaraki Prefecture, Japan (36°7'N, 140°30'E, alt. 30 m). Immediately after emergence, they were kept in a refrigerator (10°C) until the experiment. Non-virgin females, young ones judging by the degree of wing wear (Watanabe & Nakanishi 1996) to exclude any age effect on the male behavior, were captured in the fields, Tsukuba, Ibaraki Prefecture in June 2008. In addition, we used young laboratory-reared females that had mated on the day prior to the experiment. They were also kept in a refrigerator (10°C) until the experiment. After the experiment, each female was dissected to confirm the number of spermatophores in the bursa copulatrix, because the number of spermatophores indicates the number of copulations in the females (Nakanishi et al. 1996).

Young males were the offspring of wild females captured in Tsukuba, in June and July 2008. They were tethered with a fine black cotton thread tied to the neck. A thread of 20 cm did not seem to inhibit courtship behavior of the male because they fluttered freely within the reach of the thread. Forewings of each female were glued together in order to inhibit mate refusal posture and flying away. Then, the females were put on the tip of a 10-cm wooden stick to perch and were presented to tethered males fluttering in the laboratory. The distance between male and female was about 5 cm. Each trial lasted at least 30 seconds, which is longer than the duration of hovering in wild males (Irie & Watanabe 2009). Abdominal responses of the females were observed.

**Male courtship behavior in relation to abdominal posture of females.** Courtship behavior of males to perching females with different abdominal postures was observed for the summer generations in the grasslands of ski slopes in Sugadaira, Nagano Prefecture (36°31'N, 138°20'E, alt. 1320 m) in late July 2008. The grasslands were flat, and were covered with dense *T. repens* and *T.*

*pratense*, which are the host and nectar plants for the larvae and adults of *C. erate*, respectively. In the middle of the day, most males flew above the grass searching for a mate while females were resting, nectaring, and being courted.

On the day before the experiment, resting or nectaring young females were captured with a net in the grassland. Virgin females reared in the laboratory were also used for the experiment. Both forewings and hindwings of each female were glued together to become a closed wing posture, and their abdomen was forced to be either exposed, out from their wings, or hidden in their wings. The females were not able to move their abdomen. Thus, four groups of females were prepared (virgin/non-virgin, and abdomen hidden/ exposed). From 1200 to 1500 on sunny days, females were placed on a *T. pratense* flower in the grassland. When males visited the perching female their courtship behavior was observed.

The courtship behavior of males was divided into hovering and alighting (Hasegawa & Watanabe 2008). In the present study, as shown in Figure 1, when males noticed and visited the female perching, they began to hover around her. Next, the male alighted on the edge of the female's wings, walked down and bent his abdomen toward the female in order to copulate. The duration of hovering and alighting was measured using a stopwatch (accuracy 0.01 s) and a voice recorder. Each trial was carried out until a total of 10 males alighted; by that time, the scales of the female were detached by the males. Females that experienced fewer than 10 instances of male alightings were withdrawn 90 minutes after the start of the experiment. This was because of an increase in their body temperature due to direct sunlight and non-feeding. After the experiment, each female was dissected to confirm the number of spermatophores in the bursa copulatrix.

#### RESULTS

**Female response to male courtship.** Each female whose forewings were fixed remained motionless on the tip of a stick soon after being placed there. At this time, their abdomen was hidden in their hindwings. Immediately after the male appeared with their wings fluttering, the virgin female pushed her abdomen out of the hindwings toward the male (Fig. 2a). In contrast, when the male stopped fluttering, the female put her abdomen back into her closed wings. When the male resumed the fluttering, the female again exposed her abdomen toward the male from the hindwings. Table 1 shows that all virgin females exposed their abdomen in response to male fluttering. On the other hand, mated females that were reared in the laboratory did not expose the abdomen when a fluttering male was presented (Fig.

TABLE 1. The number of females that extended or hid their abdomen when presented with fluttering males.

Mating experience of the females	Extended Abdomen	Hid Abdomen	Total
Virgin	11	0	11
Mated (laboratory reared)	0	4	4
Mated (captured in the field)	0	7	7

2b). They remained perched in place without showing any behavior to the fluttering males. Mated females captured in the fields also perched and did not display their abdomen.

**Male courtship behavior in relation to abdominal posture of females.** When females were placed on flowers in the grassland, searching males visited them. Out of 13 visits to virgin females whose abdomen was hidden in hind wings, 10 males stopped hovering and left the female, while 3 males attempted copulation. When the female's abdomen was exposed, more of the visiting males attempted copulation (16 out of 22 males). Before leaving, the males hovered for  $2.8 \pm 0.4$  s ( $\pm$ SE,  $n=10$ ) for virgin females with their abdomen hidden, and for  $1.7 \pm 0.3$  s ( $\pm$ SE,  $n=7$ ) for virgin females with their abdomen exposed.

When mated females were presented the same results were obtained. Out of 18 visiting males, 14 stopped hovering after  $1.9 \pm 0.2$  s ( $\pm$ SE,  $n=10$ ) when the female's abdomen was hidden. Out of 35 male visits to females with exposed abdomens, 19 males attempted copulation. The duration of hovering was  $7.8 \pm 2.7$  s ( $\pm$ SE,  $n=11$ ). Hovering duration was different between the groups, though the statistical test indicated only marginal significance (log-transformation to normalize the data distribution, abdomen hidden:  $Z=-1.784$ ,  $p=0.07$ ; abdomen exposed:  $Z=-1.859$ ,  $p=0.06$ ). When the female's abdomen was hidden in the wings, about one fourth of the males that visited the females alighted on them after hovering, irrespective of the female's mating experience. Alternately, when males hovered around a female whose abdomen was exposed, more than half of them alighted on the females. Thus, the proportion of males that alighted was not affected by the female mating experience, but by the female abdomen posture (Log-Linear Model, female mating history:  $Z=-0.911$ ,  $p=0.15$ ; female posture:  $Z=1.428$ ,  $p<0.01$ ; interaction:  $Z=-0.911$ ,  $p=0.36$ ).

It was physically impossible for males to copulate with females whose abdomen was hidden. Males that alighted on a virgin female with the abdomen hidden continued to attempt copulation for  $19.0 \pm 11.4$  s ( $\pm$ SE,  $n=3$ ), which

was not significantly different from the time spent by males that alighted on a female with the abdomen exposed ( $8.3 \pm 2.4$  s,  $\pm$ SE,  $n=9$ ) ( $U=10$ , n.s.). After that time, they gave up attempting to copulate and flew away from the female. Attempts to copulate with non-virgin females with the abdomen hidden lasted for  $21.1 \pm 10.8$  s ( $\pm$ SE,  $n=3$ ), which was not significantly different from the time spent by males that alighted on virgin females with the abdomen hidden ( $19.0 \pm 11.4$  s,  $\pm$ SE,  $n=3$ ) ( $U=3$ , n.s.). The males flew away after copulation ended.

Some females with the abdomen exposed mated with the males. Out of 8 virgin and 12 non-virgin females with their abdomen exposed, 5 and 5 females copulated, respectively. The proportion of remated females in the non-virgin females was not significantly different from that in the virgin females ( $\chi^2=0.29$ , n.s.).

#### DISCUSSION

In the Lepidoptera, there are many reports on the sequence of nuptial communication between males and females before copulation, including the female's response to the male's courtship behavior (e.g. Scott 1972; Rutowski 1984; Wiklund 2003). The female posture of exposing her abdomen towards the male during the courtship behavior has often been reported as a signal of female receptivity in butterflies such as *Precis coenia* (Scott 1975), *Leptidea sinapis* (Wiklund 1977), *Eurema lisa* (Rutowski 1978), *C. eurytheme* and *C. philodice* (Grula et al. 1980). In the present study, receptive virgin females of *C. erate* also exposed their abdomen for males approaching them.

In *C. erate*, females show ascending flight or mate refusal posture when they do not want to copulate (Nakanishi et al. 1996). Interference due to males courtship attempts drives ovipositing females off of the flight areas (e.g. Shapiro 1970). In addition, because copulation of *C. erate* lasts for about an hour, females suffer from restricted oviposition time (Watanabe et al. 1997). Thus, females gain benefits by avoiding harassment by males and excessive matings. Mate refusal posture or ascending flight in females often leads to interruption of the male courtship behavior (Hasegawa & Watanabe 2008), functioning as signals of unreceptivity. However, even when the female shows the mate refusal posture, some males continue courtship behavior, achieving copulation. Female abdominal position must play a role in altering or controlling the male's behavior.

In the present study, ascending flight and mate refusal posture in females were artificially restricted by fixing their wings, such that males could alight near the female and attempt to copulate. The proportion of the non-virgin females that copulated is clearly higher than the proportion of females in the field, compared with the

mating frequency of young females reported by Nakanishi et al. (1996). Thus, the abdomen extension of females is vital behavior for copulation. Alternatively, females can refuse copulation by hiding their abdomen, suggesting that females can choose males. While wing fluttering at the perching site functions as refusal of males courting in *Lycaena phlaeas* (Watanabe & Nishimura 2001), the posture of hiding the abdomen is the male avoidance behavior in *C. erate*. It is physically impossible for the male to put his genitalia onto the tip of female's genitalia in the hindwings for copulation. Suzuki (1981) reported that unreceptive *Pieris rapae* females can avoid copulation by lifting their abdomen into their wings.

Watanabe et al. (1997) suggested that females could identify the age of males copulating, and that females may choose males based on the sex pheromones and scales on the wings of males. Males can gauge the receptivity of a female by abdominal posture, and they will not attempt to copulate while females hide their abdomen. Although polyandrous *Colias* females accept remating depending on the intensity of the male's courtship (Rutowski 1985), prolonged courtship behavior costs males by reduced searching time. Thus, interrupting courtship behavior to an unreceptive female is adaptive for males. Courtship proceeded along the behavioral sequence in which male and female recognize each other as a suitable mate. Successful copulation occurs only after the typical courtship behavior is achieved (Silberglied & Taylor 1978). More specifically, male and female behaviors in *C. erate* mutually release the next behavior. In order to evaluate female receptivity during courtship behavior, *C. erate* males exploit the positive female behavior. The present study clarified that the abdominal extension towards the male is an indication of female receptivity, and that it may be a releaser of male alighting. Birch et al. (1989) demonstrated that when the females move off against the male courtship behavior in *Mamestra brassicae*, the males fly away from the female without any attempt for copulation. Therefore, these studies suggest that butterfly females readily repel male's attempts so that antagonistic coevolution between males and females does not escalate in butterflies.

#### ACKNOWLEDGEMENTS

Thanks are due to Brett Setmoore, Arizona State University, who greatly improved the original text with his comments.

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Received for publication 16 April 2010; revised and accepted 15 August 2010.