Reproductive Biology of the Yellow-Spotted Longicorn Beetle,
Psacotheta hilaris (PASCOE) (Coleoptera: Cerambycidae)
IV. Effects of Shape and Size of Female Models
on Male Mating Behaviors

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The effects of color, shape and size of female models on the mating behavior of male *Psacotheta hilaris* (PASCOE) were examined. Various forms treated with female extract were presented to mature males. Male mating behaviors were strongly dependent on the shape and the size of the models but not on the color. With some models, such as an oval, a sphere and a regular prism, males exhibited mating behavior just as they did with intact females, although they showed unusual behavior with other models, such as a hemisphere, a triangular prism, and an isosceles triangular prism. Normal mounting and copulatory attempts apparently required footholds which permitted both fore- and midlegs to be positioned parallel and symmetrical to one another. Males appeared to search for a protrusion using parameral contact in order to decide on the appropriate point for copulation. Among the three dimensions of the size of the model, height was considered to be the most important factor for eliciting mating behavior because the male showed mating responses only toward models taller than 2 mm. The optimal model size for eliciting a copulatory response was nearly the same as the average size of a wild female.

Key words: *Psacotheta hilaris*, cerambycid, mating behavior, body size, configurational factor

INTRODUCTION

Adult males and females of the yellow-spotted longicorn beetle, *Psacotheta hilaris* (PASCOE) (Coleoptera: Cerambycidae) feed on mulberry trees (*Morus bombycis*) and exhibit promiscuous mating behavior on the host tree (IBA, 1993; YOKOI, 1989, 1990). Males displayed mating behavior toward artificial models treated with female pheromone extract in almost the same manner as they did toward intact females (FUKAYA and HONDA, 1992).

In some insects, the importance of color, color-pattern, shape and size of the subjects (mates or their models) has been demonstrated in courtship behavior (HIDAKA, 1972; HIDAKA and YAMASHITA, 1975; SPIETH, 1974; WALL, 1989; SHOREY and GASTON, 1970). Nevertheless, few studies on non-chemical factors have been undertaken in cerambycid species.

In the present study, we examined the effect of color, shape and size on *P. hilaris* male behavior using artificial female models in order to analyze the regulation system of male mating behavior.

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Fig. 1. Models used in the experiment. A: transparent capsule, diameter (d) = 8, length (l) = 24; B: sphere, d = 12; C: hemisphere, d = 12, height (h) = 6; D: column, d = 15, h = 8; E: square prism, side length (sl) = 15, h = 8; F: triangular prism, side lengths (sls) = 17, 17, 17, h = 8; G: isosceles triangular prism, sls = 21, 21, 12, h = 8; H: rectangular prism, l = 20, width (w) = 8, h = 8; I: triangular prism, l = 20, w = 8, h = 6; J: house-like prism, l = 20, w = 8, h = 10; K: hexagonal prism, l = 30, sl = 18, w = 8, h = 8. Unit of values: mm.

MATERIALS AND METHODS

Insects. Beetles were the progeny of wild populations collected in Saitama and Kanagawa Prefectures in 1988. The insects used were 14 to 60 d post-emergence. They were reared individually on mulberry leaves or an artificial diet for young silkworm (Nippon Chlorella Co. Ltd.) at $25 \pm 2^\circ\text{C}$ under a 15L : 9D photoregime (FUKAYA and HONDA, 1992). Mature but virgin males (20.4–21.4 mm in length) used for the bioassay were selected by checking their response to intact females before the assay. Six to 32 males were examined for each model but each male was tested only once per day.

Female extracts. Females were killed by freezing and soaked in ether (8 ml/insect) for 12 h at room temperature. After desiccation with anhydrous sodium sulfate, extracts were concentrated under reduced pressure and stored at -20°C until use.

Female models. To examine the effect of model color on male mating behavior, transparent gelatin capsules (Japanese Pharmacopoeia, No. 00) lined with various colored paper were used. The colors of the models were specified by CIE1976 $L^*a^*b^*$ ($C/2^\circ$) color space (see ROBERTSON, 1977; HUNTER and HAROLD, 1987) with an NR-3000A colorimeter (Nippon Denshoku, Tokyo, Japan) (Table 1).

To examine the effect of configurational factor on male mating behavior, the following 12 types of models were used (Fig. 1): a capsule (Japanese Pharmacopoeia, No. 00) (A); a sphere (B); a hemisphere (C); a column (D); a square prism (E); a triangular prism (F); an isosceles triangular prism (G); a rectangular prism (H); a triangular prism (I); a house-like prism (J); and a hexagonal prism (K). Model A was an empty transparent gelatin capsule (Japanese Pharmacopoeia, No. 00), models B and C were made of glass and the others were rubber blocks covered with aluminum foil. In preliminary experiments, males showed the same response to the same-sized capsule models made of the three different materials.

For determining the effect of model size on male behavior, rectangular prism models of

different sizes were presented to the males. These models were rectangular prisms (20 mm × 8 mm × 8 mm) and one of the three dimensions (width, length or height) was varied in each model.

Bioassay. Each model was fixed on a filter paper disc (9 cm dia., Toyo No. 2) and treated with the female extract. The upper surface of the capsule and rounded models (A–C) was coated with 0.5 female equivalent (FE)/model and the top surface of the other models (D–K) was coated at 0.3 FE/cm² of hexane solution using a microsyringe. The amount of extract was determined from the optimum dose which we reported previously (FUKAYA and HONDA, 1995).

The bioassay was carried out according to the method of FUKAYA and HONDA (1992). A model was presented 4 cm in front of a walking male and the male was able to contact the model with his antennae. Male behavior was observed for 5 min after first making palpal and/or tarsal contact with the model. Behavioral responses recorded were holding (HD), mounting (MT), and abdominal bending (AB) behaviors which were previously determined by FUKAYA and HONDA (1992): when a male contacts a female model with the antennae, tarsi or palpi, he immediately tries to grasp the model and holds it with the forelegs (HD), adjusts his body axis parallel to the model axis and consequently mounts it with his fore- and midlegs (MT), bends the abdomen downward (AB) and protrudes the paramere toward the tip of the model.

RESULTS

Effect of model color on male behavior

Each of the color models treated with female extract elicited AB response from more than 70% of the males (Table 1). No male showed AB response to the models treated without pheromone extract, although one male showed HD response to a black model. The reason for this was not clear.

Effect of shapes

The behavior sequences on the various shaped models were principally the same as those which had been observed on the intact females (FUKAYA and HONDA, 1992). However, males often failed to continue mating behaviors on some models. They occasionally showed abnormal behaviors such as changing their footing, repeated circling on the models or mounting on the lateral surfaces of the models.

Holding (HD). Males tried to hold all the models (Fig. 1) with or without dash behavior (see FUKAYA and HONDA, 1992) when they contacted the models with their palpi and/or tarsi (Table 2: HD attempt). On models C (hemisphere) and I (triangular prism), males tried to grasp the model but failed to get a foothold on them.

Mounting (MT). When a male got a foothold on the model with the fore- and midlegs, 100% of them showed MT toward models A and H, and 40 to 90% did so toward the other models. On models E, F, G and K, however, 10–30% of males repeatedly circled the top of the model (Table 3, circling before MT) until they got a preferable foothold or left the model. On models E, F, G and K, males often clung to the sides (Table 3, MT on lateral side).

Abdominal bending (AB). Models A and H induced AB response from 100% of males and models B, D, E, J and K did so from 40–70% of males. Males bent their abdomens to make parameral contact with the model at a point just below the body axis on models A, B,

Table 1. Mating responses of *P. hilaris* males to various colored gelatin capsules used as female models

Colors ^a	Male response (%)			
	With female extract ^b		Without female extract ^c	
	HD	AB	HD	AB
Black	100	100	10	0
White	100	90	0	0
Red	100	80	0	0
Yellow	100	90	0	0
Green	100	90	0	0
Blue	80	80	0	0
Violet	90	90	0	0
Silver	100	100	0	0
Transparent	80	80	0	0

^a Color scale presented by the CIE 1976 L*a*b* (C/2°) color space: black, 18.7/0.6/0.7; white, 89.8/0.5/3.5; red, 38.0/50.5/26.2; yellow, 72.9/−8.5/77.2; green, 43.1/−46.4/11.1; blue, 45.5/2.9/−51.3; violet, 56.7/20.9/−26.8; silver, 24.1/26.5/13.8. ^b 0.5 FE (female equivalent)/capsule. ^c Treated with solvent only.

HD: holding, AB: abdominal bending (see text for details). Ten insects were tested on each model.

Table 2. Mating responses of *P. hilaris* males to various shapes of female models treated with female extract

Model ^b	Male response (%) ^a			
	HD attempt	HD	MT	AB
A	100	100	100	100
B	90	90	90	50
C	40	0	0	0
D	80	80	70	70
E	60	60	60	60
F	80	70	60	60
G	88	88	65	59
H	100	100	100	100
I	80	0	0	0
J	83	67	67	67
K	80	60	40	40

^a HD: holding, MT: mounting, AB: abdominal bending (see text for details).

^b See Fig. 1. Dose of female extract: 0.5 FE (female equivalent)/model.

Number of insects: A, B, C, D, E, F, H, I: 10; G: 32; J: 6; K: 15.

D and K (ex. Fig. 2K). On the contrary, they moved their abdomen toward a vertical edge on models F, G, H and J, which have no edge just below the body axis (ex. Fig. 2G, H and J).

Some males (28–50%) continued to circle on the top of models E, F and G until the paramere contacted the model edge. On model E, males made parameral contact with the model edge when they could successfully mount along the diagonal line of the model (Fig. 2E), but they circled when they failed to mount. On models F and G, ca. 30% of males changed their foothold and/or circled on the top surface of the models even after making

Table 3. Male circling behaviors and clinging to a lateral surface of a model observed on various models treated with female extract^a

Model ^c	Male response (%) ^b				
	Circling before MT	MT on lateral surface	Circling before parameral contact	AB on lateral surface	Circling after parameral contact
E	20	20	40	0	0
F	20	20	30	40	30
G	13	13	28	0	28
K	30	30	0	0	0

^a Dose of female extract and number of insects: see footnote for Table 2. ^b See text. ^c See Fig. 1.

Fig. 2. Positions of male legs and paramere on various shapes of artificial female models.

parameral contact. Especially on model G, males repeatedly changed the parameral contact point and consequently, the mounting position, between two different edges (Fig. 2G).

Effects of model size on mating response

Length. When the length of the rectangular prism models was varied from 60 to 100 mm (Fig. 3a), all the models elicited an HD response from more than 70% of the males. The AB response rate increased as the model length increased, peaked at 22 and 24 mm, and then decreased. On models longer than 30 mm, males showed abdominal bending posture only when they successfully mounted an end of the model (Fig. 2L).

Width. When the width was varied from 2 mm to 20 mm (Fig. 3b), an HD response was observed in 70% or more males. AB response was induced in 50% of males to a 4 mm wide model, over 50% to 6–16 mm models, and below 50% to models over 16 mm. To

Fig. 3. Male mating response to artificial female models of different lengths (a), widths (b) and heights (c). □: holding, ●: abdominal bending, △: circling behavior (males which showed circling behavior before MT, before AB and/or after parameral contact).

models 10 mm or wider, more than 50% of males showed circling behavior, as observed on models D, E and F.

Height. The height was varied from 0 to 14 mm (Fig. 3c). Models with heights of 0 mm (8 mm × 20 mm sheet of aluminum foil) and 2 mm induced no response even when a male contacted the model surface with his antennae, tarsi and/or palpi. Maximum HD and AB responses were observed when the model width was 6 and 8 mm. On models higher than 10 mm, AB response decreased, where male hindlegs could no longer reach the base when the males tried to take a mounting posture.

DISCUSSION

Effects of color and shape of models on male mating sequence

Males were observed to attempt to hold models of different colors and shapes when the models were treated with female extract (Tables 1 and 2), which reconfirmed that a chemical factor is essential in induction of mating behavior. Moreover, shape and size were also considered to be essential since no male responded to very flat models even when they were treated with female extract (Fig. 3c). Male mating behavior was modified by the shape and size of models, although model color did not affect male mating behavior (Table 1).

Male footholds were considered to be important for the mating sequence to proceed normally. When a model had a shape which served unstable footholds on sloped sides or ventrally curved shapes (models C and I), they slipped off of them and left without holding (Table 2). Moreover, it appeared that males could maintain the mounting posture when their fore- and midlegs were symmetrically placed in parallel on the models. The male foothold for MT was parallel and symmetric even on an isosceles triangular prism (Fig. 2G) and a square prism (Fig. 2E). Males often circled on the top side of models when they tried to mount (Table 3). Some males mounted a lateral surface on models E, F, G and K (Table 3). It is considered that males were searching for stable footholds through such behaviors.

When a male bent his abdomen toward a model, he appeared to use his paramere to search for a protrusion on the model, since the movement was terminated on the vertical edge of models (Fig. 2). On models with curvatures (models A, B, and D), the male abdomi-

nal tip moved downward along the model surface to the base. These paramere movements could be attributed to the male's decision pertaining to the parameral contact point for copulation on the female abdomen.

Male circling behavior was observed before parameral contact on models E, F, and G (Table 3). This was interpreted as follows: when a male tried to point his paramere toward the vertical edges of such models, the abdominal tip was compelled to move far from his body axis and consequently the footholds became unstable, so he was obliged to circle on the model. Males also circled after parameral contact on models E and F. After the male paramere reached and stopped on an edge, the male tried to replace his leg to stably grasp the model. When he could not find a stable foothold at that position, he again began to circle on the model.

Males would adjust their posture on a female according to the female body shape and then mount and bend their abdomens toward the correct direction and copulate with the female.

It was demonstrated that in *P. hilaris* males, the shape of the object was an important factor for mate recognition as well as female sex pheromone, although further experiments are necessary for a detailed understanding of this. The female pheromone must be present on any object which will allow a male to take preferable foothold for grasping the object with his fore- and midlegs and possesses a protruding point below the male body axis for making parameral contact.

Male behavior and size of mating subject

It has been reported in *Trichoplusia ni* and *Glossina morsitans morsitans* that males show the highest response rate to models resembling living females in size (SHOREY and GASTON, 1970; HUTTON et al., 1980). In *P. hilaris*, height of the models appeared to be an essential cue for initiation of male mating behavior, because the males showed no holding attempt when models were 2 mm or shorter (Fig. 3c). This indicates that males cannot recognize the short models as 'female' even in the presence of female pheromone. The optimum range of model size for inducing more than 50% AB response but 10% or less circling behavior was 20–30 mm in length, 6–8 mm in width and 6–8 mm in height (Fig. 3a, b, c). For comparison, wild beetles were collected in Ibaraki in 1989 and the body size was measured: 22.0 ± 2.1 mm in length, 6.8 ± 0.79 mm in width (mean $\pm$ S.D., $N=56$). The mean height of the prothorax was 5.7 ± 0.6 mm and the actual body height was estimated to be 6.2–7.7 mm because the legs add 0.5 to 2 mm to the height. These values were included in the optimum range of model size.

When the size of the model was beyond the optimum range, mating behavior after HD did not progress normally even when HD response was induced. It appeared to be difficult for males to hold and mount short models, because of insufficient footholds for the male's fore- and midlegs. Long models over 30 mm were often grasped near the center of the long axis but males failed to mount. AB response was, however, only observed when males mounted the end of the model (Fig. 2L). On models wider than 8 mm, AB response decreased and circling behaviors were observed more frequently (Fig. 3b). These behaviors were considered to have been due to difficulties in obtaining footholds and/or parameral contact points on the wider models, as mentioned above for models E, F and G. The lower rate of AB response on higher models (Fig. 3c) could be attributed to the unstable footholds for successful MT behavior.

In *P. hilaris*, there is considerable variation in size (females 18.3–27.4 mm, males 13.6–

28.5 mm in length: Ibaraki population 1989). It is considered that males probably chose mates through recognition of the relative size of females. Thus far variation in male body size has been reported to be related to female choice and male-male competition in other coleopteran insects, including a cerambycid (GOLDSMITH, 1987; GOLDSMITH and ALCOCK, 1993; JOHNSON, 1982; MASON, 1980; McLACHLAN and NEEMES, 1989). The effects of male size and relative behavior are of interest for clarifying the reproductive strategies of *P. hilaris*.

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