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Mating Behavior of the Black Chafer, *Holotrichia loochooana loochooana* (Coleoptera: Scarabaeidae)

Futoshi KAWAMURA, Norio ARAKAKI, Mitsunobu KISHITA and Yasutsune SADOYAMA

Okinawa Prefectural Agricultural Experiment Station, 4–222 Sakiyama, Naha, Okinawa, 903–0814 Japan

Abstract. Mating behavior of the black chafer, *Holotrichia loochooana loochooana* (Sawada) (Coleoptera: Scarabaeidae), was observed in Miyako Island, southwestern Japan. Females settled on colonies of the beach naupaka, *Scaevola sericea* Vahal after evening flight. They showed clumped distribution within the colonies. Soon after they settled on the leaves, they commenced feeding on the leaf margin and extruded the abdominal gland (calling behavior). Attracted males landed on or near the calling females, and immediately mounted them. All the females accepted the males upon contact. After the genitalia was successfully jointed, the male raised his legs and suspended by his genitalia. Mean duration of copulation was 60.0±12.1 min (mean±SD, N=27). Of 17 calling females collected in the field, six females laid fertilized eggs. This indicates that females are polyandrous. In the laboratory, females and males stayed in a vermiculite layer in boxes during the day and came to the surface in the evening. Mating activity was observed only in the evening. Females and males continued to feed in the night, and went into the vermiculite layer at dawn.

Key words: *Holotrichia loochooana loochooana*, black chafer, scarab beetle, Scarabaeidae, sex pheromone, mating behavior.

Introduction

Azuma & Oshiro (1967) and Nagamine (1980a, b) recorded 10 or more species of Scarabaeidae as the sugarcane pests in the Ryukyu Iss. of Japan, including the black chafer *Holotrichia loochooana* (Sawada). *H. loochooana* was divided into two subspecies; *H. loochooana loochooana* (Sawada) that occurs in Miyako, Irabu, Ishigaki and Iriomote Iss., and *H. loochooana okinawana* (Sawada) in Okinawa Is. (Takahashi, 1997). Larvae of both subspecies feed on the roots of sugarcane and consequently cause wilting or deterioration of stalks.

For the control of *Anomala albopilosa sakishimana* Nomura, the most important pest of sugarcane, a total of about 1,000 light traps were set up on Miyako and Irabu Iss. The light trap is a very effective control method for species with a strong phototaxis. However, this is not effective for *H. l. loochooana* and *H. l. okinawana* since both show a weak phototaxis (unpublished data and Ogimi & Nozato, 1963, respectively). Application of a synthetic sex pheromone is potential for monitoring and/or control but it requires greater knowledge of *H. l. loochooana* mating behavior. Although some studies have been conducted on the mating behavior of the large black chafer, *Holotrichia parrella* (=*Lachnosterna morosa*) (Yoshioka & Yamasaki, 1984; Leal *et al.*, 1993), published information on the mating behavior of *H. l. loochooana* is scant. In this paper, we report the female settlement pattern on hosts prior to mating and the subsequent mating behavior.

Materials and Methods

Settlement on the plants by the female

Field observations were conducted on coastal plant communities growing along the pavement at Nishi-Henna-Zaki, the northern cape of the Miyako Is., on June 29–30, 1999. There were four main plant species in the colonies: the beach naupaka *Scaevola sericea* Vahl, the beach apple *Pandanus odoratissimus* L.f., *Wikstroemia retusa* A. Gray, and the Japanese pampas grass *Miscanthus sinensis* Anderss. We observed settlement patterns by *H. l. loochooana* females on *S. sericea* plants along the pavement (ca. 250 m), at about 30-min intervals from 16 : 00 to 20 : 00. Only one side of plants (ca. 0.8 m in half width) facing the pavement was studied. When females appeared to settle on the plants, we gently marked the nearby leaf with a numbered adhesive tape to avoid any disturbance by direct individual marking. To analyze a

spatial distribution, 30 cm height × 100 cm length of block size was treated as a unit, but at the edges of plants, when plants occupied only a part of block, this was treated as half-unit. The spatial distribution pattern of females on the host plants was analyzed by m–m^* linear regression analysis (Iwao, 1968).

Mating behavior of H. l. loochooana *in the field*

Mating behavior of *H. l. loochooana* was observed on colonies of *S. sericea* at Nishi-Henna-Zaki, on June 27, 2000. When females settled on the plants, we marked the nearby leaf with a numbered adhesive tape. To confirm the virginity of calling females, 17 calling females were collected at Nishi-Henna-Zaki, in June 1999, and were reared individually in plastic cups (300 m*l*) with soil and *S. sericea* leaf as food. Eggs laid by the females were checked for hatching.

Daily activity of H. l. loochooana *in the laboratory*

Females of *H. l. loochooana* that took the calling posture on the leaves, and males, were captured at Nishi-Henna-Zaki on May 25, 2000. Females and males were kept separately in two different plastic boxes (35 × 20 × 21 cm) with *S. sericea* leaves at 25°C and under natural light condition in the laboratory of the Okinawa Prefectural Agricultural Experiment Station at Naha, Okinawa Is. Ten females and ten males were confined individually the day before experiments in transparent plastic boxes (21 × 13.5 × 13.5 cm) that contained vermiculite (1.5 cm depth) and *S. sericea* leaves. The behavior of the insects was observed at 10-min intervals for 24 h on June 10–11, 2000. Light intensity was measured at the upper surface of the plastic box.

In addition to this observation, it was checked for further five days whether they came out the surface every evening.

Results

Settlement on the plants by females

All *H. l. loochooana* females (N = 54) were found only on *S. sericea* leaves in the evening (Table 1, Fig. 1A). Females settled on seven of ten colonies of *S. sericea*. There was no relationship between the number of female beetles and size of plant colonies (Fig. 2). Female beetles were spatially clumped in distribution ($P < 0.01$, ANOVA, Table 2) in three colonies where relatively large numbers of females settled. Mean plant height of *S. sericea* growing at the surveyed area was 156.4 ± 20.2 cm (mean ± SD, N = 30). Mean height of the plant leaves that females settled on was 54.7 ± 35.6 cm (mean ± SD, N = 45). Twenty-five females (55.6%) settled on leaves at a 30–60 cm height (Fig. 3).

Mating behavior of H. l. loochooana *in the field*

In the evening, the first female alighted on *S. sericea* leaves at 17 : 08 when light intensity was 2210 lx (Fig. 4). Mean appearance times of females and males were 18 : 16 ± 33 (mean ± SD, N = 34, 1480 lx) and 18 : 34

Table 1. Number of *H. l. loochooana* females counted on the plants along the pavement at Nishi-Henna-Zaki on the Miyako Is. on June 29–30, 1999.

Host species	Total length of plant colonies along the pavement (m)	No. of plant colonies with beetles/plant colonies	No. of beetles
Scaevola sericea	150.5	7/10	54
Pandanus odoratissimus	35.0	0/ 4	0
Wikstroemia retusa	26.4	0/ 1	0
Miscanthus sinensis	18.9	0/ 2	0

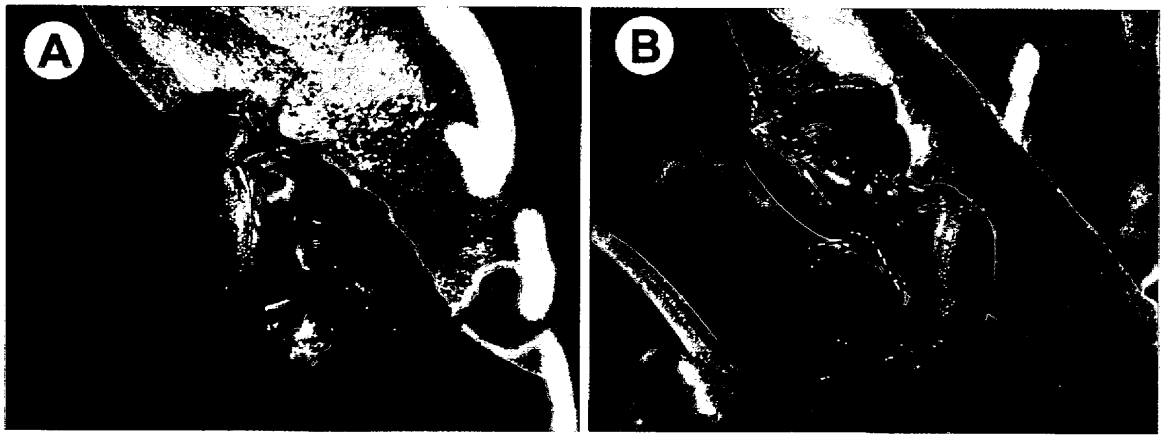

Fig. 1. A: Female *H. l. loochooana* exposing abdominal gland to release sex pheromone, B: Female (left) and male (right) in copulation.

±35 (N=34, 1240 lx), respectively. They were significantly different at $P<0.05$ by Mann–Whitney U-test. The last male appeared at 19:36 when light intensity was 41 lx. Females started to feed on the leaf margin and extruded their abdominal glands within a minute after females settling on the leaves (calling behavior, Fig. 1A). During the calling behavior, most females lifted their hind legs from the substrate, but in windy conditions, they held the substrate with all legs when calling. They continued to feed while they took the calling posture. Females stopped calling by 20 : 00 when light intensity was below 0.1 lx.

The male landed on or near the calling female, and immediately mounted her. Mating started just after male appearance (Fig. 4). All the females seemed receptive when males contacted them. The female was motionless when a male mounted her. The male moved backward with his abdomen bending and inserted his genitalia into the female abdomen. When the genitalia were successfully jointed, the male raised

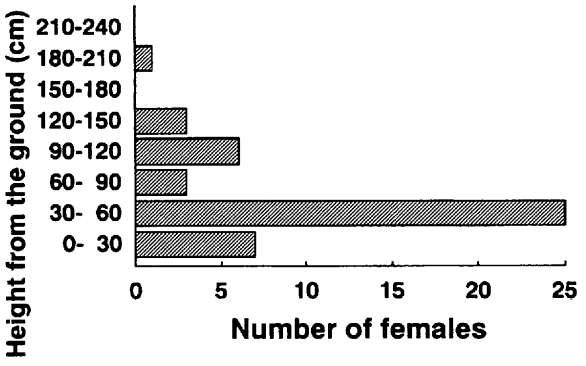

Fig. 3. Settling height on *S. sericea* by *H. l. loochooana* females.

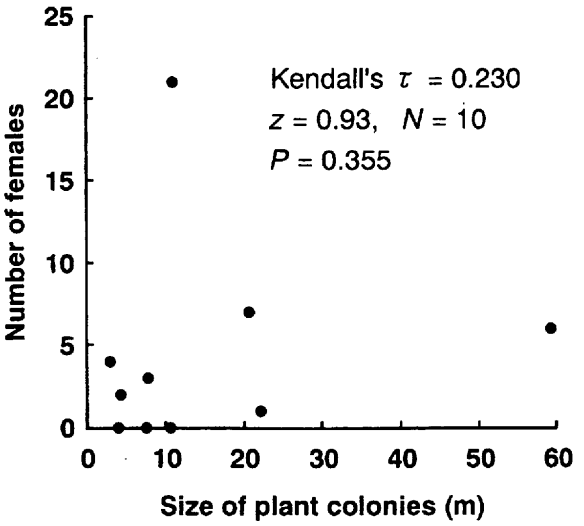

Fig. 2. Relationship between the colony size (expressed as the length of plant colony along the pavement) and the number of females found on the colony.

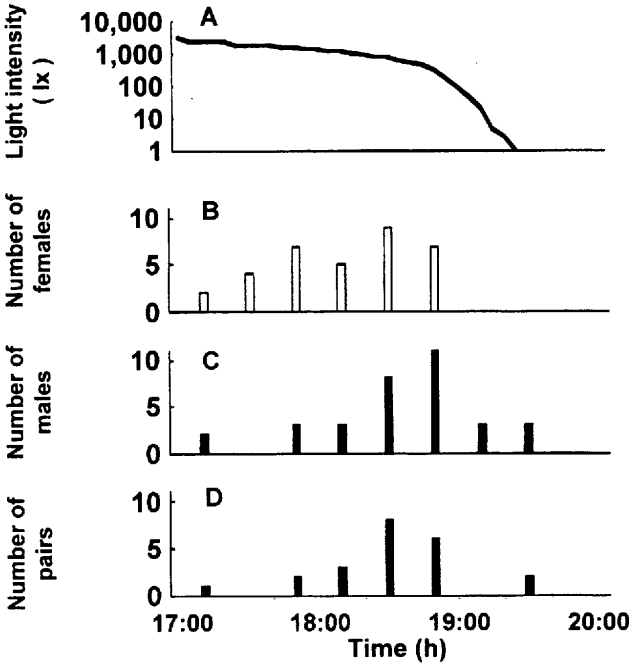

Fig. 4. Numbers of females (B) and males (C) that appeared and settled on *S. sericea* leaves, and number of pairs that started to mate (D) between 17 : 00 and 20 : 00, June 27, 2000 at Nishi-Henna-Zaki in Miyako Island. (A): Light intensity

Table 2. Spatial distribution of *H. l. loochooana* females on colonies of *Scaevola sericea*.
x is the mean no. of females per unit, x^* the mean crowding of females, and I_δ Morisita's index for females.

Colony	Length of colony (m)	No of units[1)]	No of females	x	x^*	x^*/x	I_δ	F	P
#1	11.0	55.0	21	0.38	1.71	4.49	4.7	2.4	<0.01
#2	20.7	103.5	7	0.07	0.86	12.67	14.8	1.8	<0.01
#3	59.2	298.0	6	0.02	1.33	65.76	80.2	2.3	<0.01
#4	3.0	15.0	4	0.27	0.50	1.88	2.5	1.3	NS
#5	7.8	39.0	3	0.08	0	0	0	0.9	NS
#6	4.3	21.5	2	0.09	1.00	10.75	7.2	1.3	NS
#7	22.2	111.0	1	—	—	—	—	—	—

[1)] Block size, 30 cm height × 100 cm length, was treated as a unit of measurement.

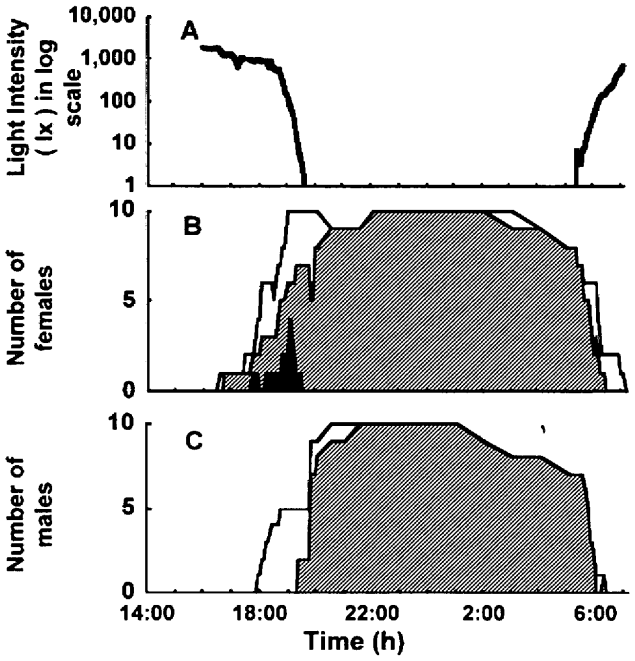

Fig. 5. Number of females (B) and males (C) that stayed on the vermiculite surface (white), fed (shaded) and called (black), between 14 : 00 and 07 : 05 in the laboratory. Ten females and ten males were confined individually in boxes with a vermiculite layer and provided with *S. sericea*. (A): Light intensity

all of his legs and remained hanging by his genitalia (Fig. 1B). Mean copulation duration was 60.0±12.1 min (mean±SD, N=27). During the copulation, the female continued to feed, but the male did not. After mating, both sexes remained on the leaf and fed on it.

Of 17 calling females collected at Nishi-Henna-Zaki, 14 females laid eggs, but three females did not. Six females laid fertilized eggs, and their mean number of eggs oviposited was 14.2±5.0 (±SD). But eight females laid unfertilized eggs, and their mean number of eggs oviposited was 5.6±4.4.

Daily activity of H. l. loochooana *in the laboratory*

Female and male adults remained under the vermiculite layer for most of the day, and came to the surface in the evening (Fig. 5). Females started calling at 17 : 40 when light intensity was 926 lx. The peak of calling occurred at 127–85 lx, and ceased at 3.4 lx. Females and males continued feeding during the night, and went into the vermiculite layer at dawn. In five-day continuous observations, adults came to the surface every evening in the laboratory.

Discussion

We found that *H. l. loochooana* adults assembled on the coastal colonies of *S. sericea* with evening flight. Although this coastal area is dominated by four plant species, *H. l. loochooana* adults settled and fed only on *S. sericea* leaves (Table 1). In the large black chafer, *H. parallela*, adults are known to feed on leaves of plants such as cherry, peach, fig, chestnut, willow, and arrowroot (Yoshioka & Yamasaki, 1984). However, *S. sericea* is the only known host plant for *H. l. loochooana.*

H. l. loochooana females settled on seven of ten colonies of *S. sericea* in the evening. There was no relationship between the number of females and size of plant colonies (Fig. 2). Females showed clumped distribution patterns (Table 2) within three colonies. Therefore, *H. l. loochooana* females have the tendency to aggregate at specific colonies. But factors that cause these aggregations are still unknown.

H. l. loochooana females have the tendency to settle on leaves at 30–60 cm height (Fig. 3). This reason would be that females and males of *H. l. loochooana* were frequently observed flying at around the knee-height before the settlement on colonies of *S. sericea* in the evening (unpublished data).

During the evening, *H. l. loochooana* females alighted on *S. sericea* leaves. They soon commenced feeding on leaf margins, and displayed a calling behavior by extruding an abdominal gland (Fig. 1A). In *H. parallela*, similar calling behavior was observed in the field (Yoshioka & Yamasaki, 1984; Leal *et al.*, 1993). *H. parallela* females settled on leaves of host plants, and extruded abdominal glands in the evening. In *H. parallela*, the pheromone substance was extracted from this abdominal gland (Leal *et al.*, 1993). The abdominal gland of *H. l. loochooana* should also be related to pheromone release.

Of 17 calling females collected from Nishi-Henna-Zaki, six laid fertilized eggs. In the laboratory, several females conducted calling on the day after copulation (unpublished data). Therefore, *H. l. loochooana* females appear to be polyandrous; namely, they mate more than once during their life.

Copulation duration in *H. l. loochooana* was much longer (60.0 min, mean) compared with that of a congenerous species, *H. parallela* whose copulation duration was only 3–6 min (Yoshioka & Yamasaki, 1984). For butterflies, prolonged copulations are observed and have been explained as a male's in-copula guarding strategy; his body acts as a nuptial plug, and impedes additional copulations of the females (e.g.,

Svard & Eiklund, 1988). Evaluation of the significance of the possible prolonged copulation duration in *H. l. loochooana* should be a topic for further research.

In the case of the large black chafer, *H. parallela*, adults appeared on the surface every other evening soon after sunset. This species has 48-h periodicity in the activity rhythm (Yoshioka & Yamasaki, 1983, 1984). In *H. l. loochooana*, however, adults came to the surface every evening in the laboratory observation. Therefore, *H. l. loochooana* should have an ordinary 24-h (circadian) periodicity.

In the daily activity rhythm of adults in the laboratory, the adults remained under the vermiculite layer during the day and emerged on the surface in the evening. They conducted calling and mating only in the evening. Females and males continued feeding during the night. At dawn, they returned to the vermiculite layer. These facts suggest that adults in the field burrow under the ground near colonies of *S. sericea.*

Migration of the lineate chafer, *Mimela testaceipes* from sward to forest was observed (Torikura, 1991). Forests are important sites for feeding, mating and egg-developing for newly emerged adults. Gravid females then returned to sward for oviposition. Similar migration was observed in the cupreous chafer, *Anomala cuprea* between the peanuts fields to the chestnut fields (Inou & Takai, 1984).

So far, migration of *H. l. loochooana* between sugarcane fields and costal area has not been reported. Colonies of *S. sericea* grow only in rocky coastal areas with shallow upper soils. Such areas are not habitats of *H. l. loochooana* larvae. This species spends the larval and pupal stages in sugarcane fields (Nagamine, 1980a, b). Emerged adults may then move to the coastal area, and spend several days near colonies of *S. sericea*. During this coastal phase, females feed voraciously on *S. sericea* and mate repeatedly. After several days, gravid females may return to the sugar cane fields to oviposit. To clarify migration of *H. l. loochooana* between sugarcane fields and costal area, further studies are need.

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