Mating behavior of the scarab beetle *Dasylepida ishigakiensis* (Coleoptera: Scarabaeidae)

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(Received 5 December 2003: Accepted 22 July 2004)

**Abstract**
Mating behavior of the scarab beetle *Dasylepida ishigakiensis* was observed in a sugar cane field in Miyako Is., Okinawa, Japan. In field observations of tethered females on 6 February 2002, calling behaviors were observed only within 30 min of sunset time (18:25–18:55, JST), when light intensity decreased from ca. 500 lx to 1 lx. Mating was strongly affected by temperature: adults appeared and subsequent mating occurred when the temperature at 18:00 was higher than 18°C. Females appeared from the soil, flew to settle on sugar cane leaves and commenced rhythmical abdominal expansion and contraction. Males were attracted to the calling females from leeward, landed on or near the calling female, and immediately mounted. After genital connection, the male raised his legs and suspended himself with his genitalia. Mating lasted for ca 2 h. Most mated *D. ishigakiensis* females neither appeared from the soil nor attracted males until the end of March, so are considered monogamous. In contrast, males appeared from the soil after mating on evenings warmer than 18°C and probably repeat mating if females are available.

**Key words:** White grub; *Dasylepida ishigakiensis*; Scarabaeidae; mating behavior; sugar cane

**INTRODUCTION**

The white grub *Dasylepida ishigakiensis* (Niijima et Kinoshita) (Coleoptera: Scarabaeidae) was first recognized in 1997 as an important pest species that infests the roots of sugar cane in Miyako and Irabu Isls., Okinawa, Japan (Sadoyama et al., 2001). We previously documented the two-year life cycle of this species (Oyafuso et al., 2002), which synchronizes well with the cultivation cycle of sugar cane in Miyako Is. Adults fly from early February to mid-March, when host plants are still young (ca. six months after planting). Females lay eggs in the soil, probably in March and April. Larvae hatch in April and May and grow as they feed on the underground parts of sugar cane. Young third instar larvae feed voraciously and cause severe damage in September and October, just before the harvest season (January–March). After harvest, the grown larvae continue moderate feeding on the residual roots and stems that remain in the fields until June or July. Then they move into the deep soil to survive the summer season, as the upper soil layer is turned for planting in September. They form tunnel cavities and pupate from late October to early November. Adults emerge between mid- and late November and remain in the cavities until the following February (Oyafuso et al., 2002).

When sugar canes are heavily infested, the leaves droop and turn yellow, as in the early symptoms of drought. In the autumn of 1998, sugar cane damage in Miyako Is. (more than 200 ha) was mistakenly attributed to the black chafer, *Horotrichia loochooanan loochooana* Sawada. After the damage was recognized to be due to *D. ishigakiensis* in 1997 (Sadoyama et al., 2001; Oyafuso et al., 2002), rapid development of pest control methods become

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DOI: 10.1303/aez.2004.669
necessary. Generally, control of soil dwelling pests of sugar cane through insecticide application is problematic, because of the absence of direct contact of insects with the chemicals. Chemical applications to soil may create a water pollution risk, especially in Miyako, where groundwater is the source of drinking water.

Synthetic sex pheromone has potential for monitoring and control but greater knowledge of *D. ishigakiensis* mating behavior is required. We found that adults appear from the ground in the sugar cane fields to mate in the evening. We conducted field observations of the mating behavior of wild and tethered females in sugar cane fields in the evening and report our findings in this paper.

**MATERIALS AND METHODS**

**Insects.** Virgin females used for these experiments were obtained from about 250 third-instar larvae, collected from the sugar cane fields in Hirara, Miyako Is. in February 2001. They were kept in two concrete frames (185×185 cm) in the field cage of the Okinawa Agricultural Experiment Station at Naha, Okinawa Is. for ca. 10 months. Young sugar canes were planted in the frames as food for the larvae. Pupae and adults were carefully excavated in late December 2001. Pupae were kept under laboratory conditions until adult emergence, and adults were sexed according to the difference in size and shape of the antennal clubs. Adults were individually kept in plastic cups (6.2 cm dia.×3.5 cm height) that contained moistened vermiculite and natural photoperiod conditions in the laboratory at 25°C.

**Daily fluctuations in the number of mating pairs.** Daily fluctuations in the number of mating pairs of *D. ishigakiensis* was surveyed at two sugarcane fields (Field A: 60 m×40 m, B: 59 m×57 m) in Hirara, Miyako Is. from February to March 2002. In these fields, *D. ishigakiensis* larvae had seriously infested sugar cane in the autumn of 2000. Since they take ca. 1 y for emergence after infestation (Oyafuso et al., 2002), the adults were expected to emerge in October and November 2001 and to appear from the soil in February 2002. The sugar cane was planted again in September 2001 and the plants were ca. 1.0 m tall (stalk length was about 50 cm). We walked in the fields with flashlights and visually identified and collected mating pairs from 18:00 to 20:00 (Japan Standard Time).

Field surveys were conducted every evening from 7 January to 13 March 2002 in field “A” and from 7 February to 23 February 2002 in field “B”. Daily air temperatures during the survey periods at Miyako Is. were obtained from the Okinawa Meteorological Observatory.

**Calling and male attraction by tethered virgin females.** Virgin females (*N*=15) were tied with ca. 20 cm of a waxed cotton thread (No. 80, Japan Industrial Standard) at the thorax (see Fig. 2). They were individually tethered on the stems or leaves of sugar cane at ca. 20 cm height and at 1 m intervals. The tethered females were set at around 17:30 on 6 February 2002 in field “B”. All males that approached within 30 cm of the females were caught with 15×25 cm net. Temperature and wind velocity were measured at 1.5 m above the ground every 5 min from 18:00 to 19:00. The mean temperature was 18.4°C and mean wind velocity was 1.1 m/s. Light intensity was measured at 1 m above the ground every 5 min from 18:00 to 19:00. The sunset time on that day at Hirara Miyako Is. was 18:24.

**Mating behavior and copulation duration of adults in the field.** Mating behavior and copulation duration of *D. ishigakiensis* was observed in field “B” on 21 February 2002. From 18:00 to 21:17, we visually surveyed pairs in copula at 15-min intervals with a flashlight. When pairs in copula were found, the sites were immediately marked by placement of a 2-m pole ca. 30 cm away from the pair to prevent disturbance. The times of start and ending of copulation were recorded.

**Attraction of males by virgin and mated females in the field.** To clarify whether *D. ishigakiensis* females mate only once or more than once, attractiveness was compared between virgin and mated females. A funnel trap with crossed vanes was used (15 cm dia.×38.5 cm ht., Trécé Inc., Salinas, Calif., U.S.A.). Two traps were baited with two virgin females and two mated females, respectively, in which females were confined in the wire net cage (5.5 cm dia.×7 cm ht.). The mated females were collected from the field while in copula on 6 and 21 February and used as bait for field trap tests conducted on 7 and 22 February (1 d after mating). The females collected on 6 February were also used on 18 and 22 February (12–16 d after mating).
The traps were set at an interval of ca. 10 m on the ground, with wire and a stick (0.8 cm dia. × 90 cm) that was inserted into the soil at ca. 10 cm depth. Traps were placed in the field between the cane rows at approximately 18:00 and recovered at 20:00–20:30. Each trap test had a total of eight replications, which were conducted on 7, 18, 21 and 22 February 2002.

**Multiple copulation.** To clarify whether multiple copulations occur in females, a total of 847 copulated pairs were collected from field “A” from 4 to 22 February 2002. Females were individually numbered on their elytra with an oily dye using felt-tip pens (Mitsubishi Paint Co., Ltd.) and soon released into a field cage [6 m × 18 m × 2.4 m (ht.)] at the Miyako Branch of the Okinawa Agricultural Experiment Station at Hirara. Males were also released into the same cage without numbering to save time and labor. All the beetles were confirmed to enter the soil by the following morning.

**RESULTS**

**Daily fluctuations in numbers of mating pairs**

Mating pairs in the field were observed from 4 to 24 February, while sporadic and small numbers of male flights were observed until early March (Fig. 1). Daily air temperature at 18:00 ranged from 14.3°C to 21.7°C (mean: 18.7°C) during February. Mating pairs in the fields were only observed for 11 days in field “A”, when the air temperature was above 18°C.

**Calling and male attraction by tethered virgin females**

In the field observations of tethered females on 6 February, we observed that the tethered females commenced rhythmical abdominal expansion and contraction at around 18:25 when light intensity decreased to 470 lx (Fig. 2). We considered this to be calling behavior. The frequency of this behavior in the tethered females increased thereafter reaching a maximum at 18:40 (60 lx) but soon ceasing by 19:00 (0.2 lx).

Males were observed to approach the calling females by flying from the leeward just before calling was first observed at 18:20 (760 lx). The number of males attracted to tethered females peaked at 18:40 (60 lx) and terminated by 18:55 (0.8 lx).
Mating behavior and copulation duration in the field

Male flights were observed to start at around 18:30. Males repeated zigzag flights to search for mates while buzzing between the rows of sugar cane at a height of approximately 10–30 cm. Females appeared from the soil and settled on the sugar cane leaves or stalks at heights of about 20–50 cm and took calling postures (Fig. 3A). Males landed on or near the calling females and immediately mounted. All the females seemed receptive because no mate refusal was observed. The female retracted her elongating abdomen to stop calling when a male mounted her. The male moved backward, curved his abdomen to feel the female abdominal tip and inserted his genitalia into the female abdomen. When the genitalia successfully jointed, the male raised all of his legs and turned his body backward. He was then hanging by the genitalia (Fig. 3B). During the copulation, neither the male nor female consumed any food. All twelve of the copulations (onset of copulations) observed were limited to a 22 min interval between 18:47 and 19:09.

The first termination of copulation took place at 20:40 and the last at 21:17. Mean copulation duration was 116.5 ± 13.2 min (mean ± SD, N=12). Females and males were observed to enter into the soil soon after copulation. Most unmated males also entered into the soil immediately after an active flight period at dusk but a small number of males were observed to remain on the plants for several hours after this period.

Attractiveness of virgin and mated females in the field

Large numbers of males were captured with the traps baited with virgin females, but few males were captured with females that had mated 1 d or 12–15 d previously (Table 1). These differences were statistically significant (p<0.001, t-test).

Multiple copulation

Among 847 copulated pairs, 98.6% of the females never appeared from the soil (Table 2). Only 12 and 6 females (1.4% and 0.7%) appeared and copulated again (until late March). Most of the mated males appeared repeatedly from the soil when air temperature was 18°C or higher.
DISCUSSION

Many Melolonthinae species are known to appear from the soil and copulate only in the evening, e.g. the black chafer *H. l. loochooana* (Kawamura et al., 2001) and the yellowish elongate chafer *Heptophylla picea* Motschulsky (Yamamoto, 1989). Mating behavior of *D. ishigakiensis* was observed only for 30 min on warm evenings around sunset and at 18°C or warmer (Fig. 1). Appearance from the ground was also strongly restricted by low temperature. Neither appearance nor mating occurred temperatures at lower than 18°C. Females were observed to appear and fly from the soil around 18:30–19:00. Light intensity reduction to ca. 300 lx or lower probably induced females to take off (Fig. 2). However, further studies are need to clarify the relationship between the appearance of females and the light intensity. Females flew a short distance for a few minutes within the field and then perched on sugar cane leaves. They immediately elongated and rhythmically expanded and contracted their abdomens (Fig. 3A). Males were soon observed to fly toward the females. Since all the males approached females from the leeward with zigzag flights, the females were considered to emit an airborne pheromone and the fe-

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**Table 1.** Capture of male *D. ishigakiensis* by traps baited with two mated or virgin females in the field (Hirara, Miyako Is., 2002)

<table>
<thead>
<tr>
<th>Days after mating of mated females</th>
<th>Mean no. of males captured&lt;sup&gt;a&lt;/sup&gt; (mean±SE)</th>
<th>Mated females</th>
<th>Control (virgin)</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5±0.3</td>
<td>94±12</td>
<td>p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>12–16</td>
<td>0.4±0.4</td>
<td>38±8</td>
<td>p&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>See text for details on females.

<sup>b</sup>Trap data (x) were transformed to log(x+0.5) and submitted to t-test. Values presented are the means and SE for eight traps for two days, consequently 16 replications.

**Table 2.** Appearance and mating of mated *D. ishigakiensis* females collected as mating pairs from sugar cane fields, marked and released together with unmarked males in a field cage (Miyako Is., Okinawa, 2002)

<table>
<thead>
<tr>
<th>Dates&lt;sup&gt;a&lt;/sup&gt; of release</th>
<th>No. of females released&lt;sup&gt;b&lt;/sup&gt;</th>
<th>No. of females&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Appeared</th>
<th>Mated</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>76</td>
<td>1 (6)</td>
<td>1 (6)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>116</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>46</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>301</td>
<td>5 (14, 14, 21, 26, 27)</td>
<td>1 (14)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>16</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>103</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>26</td>
<td>3 (22, 27, 28)</td>
<td>2 (22, 27)</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>28</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>77</td>
<td>1 (22)</td>
<td>1 (22)</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>51</td>
<td>2 (25, 25)</td>
<td>1 (25)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>All the dates indicated are in February 2002. The field survey was from 7 January to 13 March, but zero data are not presented.

<sup>b</sup>Numbers in parentheses represent dates of appearance or mating (February).
male’s behavior was defined as calling. Mating lasted for about 2 h. This duration was longer than those for other scarab species: ca. 20 min in the green chafer, *Anomala albopilosa sakishimana* (Arakaki et al., 2004) and the oriental beetle, *Exomala orientalis* (Waterhouse) (Facundo et al., 1999), ca. 60 min in *H. l. loochooana* (Kawamura et al., 2001) and only 3–6 min in *H. parallela* (Yoshioka and Yamasaki, 1984). The reasons for the longer mating times are not clear and are a subject for future studies.

There are different patterns of mating receptivity of females in insects, ranging from those that mate only a single time to those that are always sexually accessible and have a steady succession of copulations (Thornhill and Alcock, 1983). Many scarab beetles are polyandrous, as are other pest species of sugar cane, *H. l. loochooana* (Kawamura et al., 2001) and *A. a. sakishimana* (Arakaki et al., 2004). In contrast, mated females of *D. ishigakiensis* seldom appeared from the soil (Table 2) and attracted only a few males even 12–15 d after mating (Fig. 4). Therefore, *D. ishigakiensis* females appear to be monogamous. Furthermore, these results imply that mated females may not migrate but stay in the soil to lay eggs.

Many herbivorous scarab beetles are known to aggregate on certain plants for feeding and mating, such as *H. l. loochooana* (Kawamura et al., 2001) and *A. a. sakishimana* (Arakaki et al., 2004). These two scarab beetles migrate from the sugar cane fields to coastal plant colonies, voraciously feed on *S. sericea* and repeatedly mate there. However, *D. ishigakiensis* adults have not been observed to feed on any plants, probably due to having degenerated mouthparts. Therefore, females and males of this species are considered to appear from the ground only for mating. This interpretation indicates sex pheromone may be effective for control of this severe pest of sugar cane. Our present findings concerning *D. ishigakiensis* mating behavior will be useful to establish techniques for isolating the sex pheromone.

**ACKNOWLEDGEMENTS**

We thank Masayuki Sunagawa, Katsumi Uechi, Tadashi Miyaguni, Kazufumi Yoya, Hiroaki Sunagawa and Tsuneo Kinjo of the Okinawa Prefectural Agricultural Experiment Station, Miyako Branch for their assistance with the field survey. The manuscript was improved by Serge Glushkoff.

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