Copulation and male calling in the swift moth, *Endoclita excrescens* (Butler) (Lepidoptera: Hepialidae)

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Abstract

Actual copulations of the swift moth, *Endoclita excrescens* (Butler), were recorded both in field observations at Takasaki in Kukizaki Town, Ibaraki Prefecture, Japan and in a large cage at the Forestry and Forest Products Research Institute during a research period from 1 September to 7 October 1999. Copulations took place during the dusk flight and the complete process of successful mating behavior in *E. excrescens* was observed. This demonstrates that the dusk flight of *E. excrescens* is the mating flight. In the beginning of the mating behavior, the female(s) flew straight toward the male(s) showing a swinglike pendulum flight around a branch of a particular tree in a particular spot. This result shows female attraction by the males (male “calling”). Preliminary experiments suggest that females are attracted to the flying males by visual cues from a long distance and the male scent from a short distance in the final encounter. The male flight pattern including the swinglike pendulum flight may have a “calling” function in long-range female attraction.

Key words: Hepialidae, *Endoclita excrescens*, copulation, male calling, mating behavior

INTRODUCTION

Hepialidae belongs to the primitive Lepidoptera and unique mating behaviors of many hepialid species in the world have been reported (Barrett, 1886; Deegener, 1902; Winn, 1909; McDunnough, 1911; Cockayne, 1912; Bethune-Baker, 1913; Cockayne and Jackson, 1913; Blair, 1918; Forbes, 1923; Madge, 1954; Edwards, 1964; Barton Browne et al., 1969; van Gerwen et al., 1972; Reynolds, 1973; Hardy, 1974; Turner, 1976; Mallet, 1984; Wagner and Rosovsky, 1991). Mallet (1984) has reviewed the mating behaviors of the Hepialidae. However, the details of mating behavior in Hepialidae are not well known except for some species such as *Hepialus hecta*, *H. humuli* (Turner, 1976; Mallet, 1984), *Oncopera alboguttata*, *O. tindalei* and *O. rufobrunnea* (Barton Browne et al., 1969), and *Korscheltellus gracilis* (Wagner and Rosovsky, 1991). Female attraction by male(s) (male “calling”) has been observed in the mating behavior of *H. hecta*, *H. humuli* and

Sthenopsis spp. (Barrett, 1886; Deegener, 1902; Cockayne, 1912; Cockayne and Jackson, 1913; Forbes, 1923; Turner, 1976; Mallet, 1984). On the contrary, male attraction by female(s) (female “calling”) as reported in many moths has been observed in *H. pyrenaicus* (Bethune-Baker, 1913), *H. sylvinus* (Blair, 1918) and *K. gracilis* (Wagner and Rosovsky, 1991).

The mechanisms by which females are attracted to males has been studied in *H. hecta* and *H. humuli* (Michael, 1949; Edwards, 1964; Reynolds, 1973; Schulz et al., 1990). Males of many hepialid species possess conspicuous tibial scent organs on their hind-legs. The tibial scent organs and their pineapple-like odor have been known in *H. hecta* (Barrett, 1882; Turner, 1976). The volatile components of male *H. hecta* emitted from the tibial scent organs have been studied by Schulz et al. (1990). They suggest that the volatile components of the male *H. hecta* function as a male sex pheromone, an “attractant” for conspecific females. In *H. humuli*, the mechanism by which females are at-

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tracted to males is controversial. *H. humuli* moths are sexually dimorphic in color. The male wings are white above and black below, while the females have a brown, mottled wing-pattern. Reynolds (1973) experimentally showed female attraction to a white paper model of male *H. humuli* by visual cues. On the other hand, Michael (1949) and Edwards (1964) suggest female attraction to a male *H. humuli* by olfactory cues, volatile components which are emitted from the tibial scent organs on the hind-legs of the males.

Endoclita excrescens (Butler) is a common hepialid species in Japan. Males of this species possess conspicuous tibial scent organs on their hind-legs like many other hepialids and emit a scent from the organs during flight. Fragmentary reports on the mating behavior of *E. excrescens* have been published by Matsuzawa et al. (1963) and Takahashi (1963) but little is known about this behavior. In the present study, the successful mating behavior in *E. excrescens* was observed and described both in the field and in a cage. The role of males in the mating behavior of *E. excrescens* is also discussed.

MATERIALS AND METHODS

Field observations. Field observations were made from 16:30 to 18:30 every day, except on rainy days, during the research period from 1 September to 3 October 1999 in the forest at Takasaki in Kukizaki Town (35°59'N; 140°7'E), Inashiki District, Ibaraki Prefecture, Japan. The field observation site was located about 1 km west of the Forestry and Forest Products Research Institute (FFPRI) at Matsunosato in Kukizaki Town. Wild adults of *E. excrescens* are found in Kukizaki Town from early September to mid-October every year, their mating behavior during the dusk flight was observed and recorded using an 8 mm video camera, Video Hi 8 Handycam CCD-TRV 92 (Sony, made in Japan), for further behavioral analyses. The period from the time the first male began to fly until the time the last male stopped flying was recorded as the flight period of males on each night. The flight period was recorded in the same way for the females as in the males. Light intensities at the time of the beginning and end of the dusk flight of both sexes were measured using a digital illuminometer, Digital Lux Meter (Model LX-1332, Custom Corporation, made in Japan).

The light intensity at 1 m above the ground in the center of the observation spot was measured.

In-cage observations. Male and female adults of *E. excrescens* used for the cage observations were reared from the larval stage at the FFPRI laboratory. Fourth to sixth instar wild larvae of *E. excrescens* were collected from shoots of willows (*Salix* spp.) and stems of Japanese knotweed (*Reynoutria japonica* Houtt.), mugwort (*Artemisia princeps* Pampan.), reed (*Phragmites australis* (Cav.) Trin. ex Steud), tall goldenrod (*Solidago altissima* L.) and daisy fleabane (*Erigeron annuus* (L.) Pers.) at Toyoda in Ishige Town, Yûki District, Ibaraki Prefecture on 2 June 1999. The field-collected larvae were kept individually and fed on artificial diet, INSECTA-LF(S) (Nihon-Nôsan Kôgyô, Inc., Japan), under constant conditions of $25 \pm 1^\circ\text{C}$ and 16L:8D from fourth to eighth larval instars and 10L:14D from ninth larval instar to pupa. After pupation, pupae were sexed to obtain unmated moths. The adult eclosion occurred during late September to mid-October 1999. The moths, too, were kept under constant conditions of $25 \pm 1^\circ\text{C}$ and 10L:14D until the beginning of the observation.

Observations on the mating behavior of the laboratory-reared adults of *E. excrescens* during their dusk flight were made in a wire-net field cage (7.7×9.6 m, 4.5 m in height; a net with a 1-cm mesh) located at FFPRI from 17:00 to 18:30 every day during a period from 4 to 7 October 1999. Two boughs (about 4 m in length) of a willow, *Salix* sp., were set in a water-filled bucket placed in the center of the cage. Five pairs of 1 to 4-d-old virgin females and males were transferred to the field cage about 3 h before the beginning of the observations and allowed to hang randomly on the willow twigs by their fore- and mid-legs in the resting position. Records of the flight periods for both sexes were taken in the same way as in the field observations. Light intensities at the beginning and end of the dusk flight of both sexes in the cage were measured in the same way as in the field observations. The mating behavior during the dusk flight of *E. excrescens* in the cage was observed and recorded also in the same way as in the field observations.

Fig. 1. Sunset times, flight periods of the wild *Endoclitia excrescens* males and females and their beginning copulation times in the field observation area at Takasaki in Kukizaki Town, Inashiki District, Ibaraki Prefecture, Japan, from 1 September to 3 October 1999. The period from the time at which the first individual began to fly (○) until the time at which the last individual stopped flying (●) was recorded as the flight period of the males (a) or the females (b) on each night. Figures above the markers indicate the total number of males (a) or females (b) observed flying on each night.

RESULTS

Field observations

Flight period, sunset time and light intensity

The wild males of *E. excrescens* were observed in the field on a total of 18 nights. The wild males were first observed on 6 September but their flight period could not be recorded on that night. Mean total number of males observed per night was 2.8 ± 0.3 SE (range 1–5; $N=18$) (Fig. 1a). The male(s) flew around a branch of a particular tree of *Quercus myrsinaefolia* Blume, *Q. serrata* Thunb. ex Murray or *Cryptomeria japonica* (Linn. fil.) D. Don which was located in a particular spot, that is, a spot on the border of an open area in the field. Two to 3 males were often observed to fly together

in the same spot but more than 3 males in a group were not observed. When a total of 4 to 5 males were observed in the observation area on a night, they flew in 2 to 3 different spots about 20 to 30 m from each other. The male flight periods and the sunset times are shown in Fig. 1a. The males began to fly 4 to 15 min after sunset (9.8 ± 0.7 min; mean $\pm$ SE, $N=17$) and stopped flying 15 to 26 min after sunset (21.7 ± 0.9 min; mean $\pm$ SE, $N=15$). Mean male flight period per night was 11.7 ± 0.9 SE min (range 5–18 min; $N=15$). The mean light intensities at the beginning and end of the male flight were 41.9 ± 6.4 SE lx (range 10.8–98.0 lx; $N=13$) and 2.0 ± 0.5 SE lx (range 0.5–7.0 lx; $N=12$), respectively.

On the other hand, the wild females of *E. ex-*

excrecens were observed in the field on a total of 5 nights (20, 22, 23 and 26 September and 1 October). A wild female was first observed on 20 September but her flight period could not be recorded on that night. Mean total number of females observed per night during these 5 observation nights was 1.2 ± 0.2 SE (range 1–2; $N=5$) (Fig. 1b). The female flight periods and the sunset times are shown in Fig. 1b. The females began to fly 12 to 17 min after sunset (14.2 ± 1.1 min; mean $\pm$ SE, $N=4$) and stopped flying 13 to 18 min after sunset (15.5 ± 1.4 min; mean $\pm$ SE, $N=4$). The onset of the female flight lagged 6.3 ± 1.5 min (mean $\pm$ SE, $N=3$) behind that of the male flight. The females stopped their flying 1.3 ± 1.3 min (mean $\pm$ SE, $N=3$) prior to the end of the male flight. Mean female flight period per night was 1.3 ± 0.3 SE min (range 1–2; $N=4$). The light intensity at the beginning of the female flight was 13.0 lx, recorded on 1 October. The light intensity at the end of the female flight could not be recorded.

Mating behavior

The mating behavior of wild *E. excrecens* was observed in the field on a total of 5 nights as mentioned above. Actual copulation was confirmed in 1 pair. This copulation took place at 17:54 on 20 September, 14 min after sunset and 8 min after the beginning of the male flight (Fig. 1). The whole process of the successful mating behavior in the 1 pair of *E. excrecens* is described as follows: a male from somewhere in the forest began to fly soon after sunset and flew to an open area in the forest. In the beginning of the mating behavior, the male showed a swinglike pendulum flight at about 4 to 5 m above the ground around a branch of a tree, *Q. serrata*, in a spot on the border of an open area in the forest. A female, which began her flying several minutes behind the onset of the male flight, flew straight toward the flying male from some distance (about 10 m) away. The female approached and hovered within about 0.8 m of the flying male, and then the male approached the hovering female. They touched each other in flight repeatedly. The female flew toward the forest and the male followed her immediately. The male approached the female and touched each other repeatedly. After which, the female flew into a Japanese cypress (*Chamaecyparis obtusa* (Sieb. et Zucc.) Endlicher) in the forest to hang from a twig by her fore- and mid-legs. The male alighted next to the female,

gripped her thorax with his fore- and mid-legs, and then bent his abdomen apparently to copulate with her. After the male copulated with the female in a ventral-ventral position, he relaxed his leg grip and hung vertically down from the female with only his claspers. The copulating pair was hanging from the twig of the tree about 3 m above the ground. After achieving copulation, the copulating pair was transferred from the branch to an insect cage (25×25 cm, 35 cm in height) and kept in the FFPRI laboratory at room temperature under semi-natural conditions. The pair remained copulating for more than 24 h and separated at dusk of the next day. Although actual copulations could not be confirmed in other pairs on the other 4 nights, the process of mating behavior observed on these nights was essentially the same as on 20 September. A male group consisting of 2 to 3 males was observed on 22 and 26 September and 1 October. As mentioned above, the males in a group flew together around a branch of a particular tree on the border of the open areas in the forest. In the beginning of the mating behavior, the female flew straight toward one of the males in a group. Disturbance by other males was not observed.

In-cage observations

Flight period, sunset time and light intensity

The flight periods of the laboratory-reared males and females of *E. excrecens* in the field cage were recorded every night from 4 to 7 October. All of the males and females flew every observation night and the flying males formed 2 small groups about 3 to 4 m from each other. Each male group consisted of 2 to 3 males. The male flight periods and the sunset times are shown in Fig. 2a. The males began to fly 9 to 17 min after sunset (13.3 ± 1.7 min; mean $\pm$ SE, $N=4$) and stopped flying 23 to 34 min after sunset (29.8 ± 2.4 min; mean $\pm$ SE, $N=4$). Mean male flight period per night was 16.5 ± 2.8 SE min (range 10–22 min; $N=4$). Mean light intensities at the beginning and end of the male flight were $24.7 = 11.9$ SE lx (range 10.3–48.2 lx; $N=3$) and 0.3 ± 0.2 SE lx (range 0.0–0.8 lx; $N=3$), respectively.

As shown in Fig. 2b, females began to fly 15 to 19 min after sunset (16.5 ± 0.9 min; mean $\pm$ SE, $N=4$) and stopped flying 24 to 30 min after sunset (27.8 ± 1.3 min; mean $\pm$ SE, $N=4$). The onset of the female flight lagged 3.3 ± 1.7 min (mean $\pm$ SE, $N=$

Fig. 2. Sunset times, flight periods of the laboratory-reared *Endocrita excrescens* males and females and their beginning copulation times in the cage at the Forestry and Forest Products Research Institute at Matsunosato in Kukizaki Town, Inashiki District, Ibaraki Prefecture, Japan, from 4 to 7 October 1999. The period from the time at which the first individual began to fly (○) until the time at which the last individual stopped flying (●) was recorded as the flight period of the males (a) or the females (b) on each night. Figures above the markers indicate the total number of males (a) or females (b) observed flying on each night.

4) behind that of the male flight, but the females finished their flight 2.0 ± 1.1 min (mean $\pm$ SE, $N=4$) prior to the end of the male flight. Mean female flight period per night was 11.3 ± 0.9 SE min (range 9–13 min; $N=4$). Mean light intensity at the beginning of the female flight was 7.7 lx (range 2.0–13.3 lx; $N=2$). The light intensity at the end of the female flight was 0.0 lx, recorded on 7 October.

Mating behavior

The mating behavior of the laboratory-reared adults of *E. excrescens* in the field cage was observed every night during the research period and a total of 2 actual copulations were recorded (Fig. 2). First copulation took place at 17:40 on 4 October, 21 min after sunset (8 min after the beginning of

the male flight and 4 min after the beginning of the female flight). Second copulation took place at 17:36 on 7 October, 21 min after sunset (7 min after the beginning of the male flight and 2 min after the beginning of the female flight). Each copulating pair was found on the wire-netting at a side of the cage, about 4 m above the ground. The sequence of the successful mating behavior of *E. excrescens* in the cage was essentially the same as in the field. After achieving copulation, each copulating pair was transferred from the field cage to a small insect cage and kept in the FFPRI laboratory at room temperature under semi-natural conditions. Each pair remained copulating for more than 24 h and separated at dusk of the next day.

DISCUSSION

Actual copulations of *E. excrescens* were observed both in the field and in the cage. The actual copulations took place during the dusk flight (Figs. 1 and 2). This proves the dusk flight of *E. excrescens* to be the mating flight.

The results from the present study revealed the entire process of successful mating behavior in *E. excrescens*. Compared to the usual or standard mating behavior of many other moths, the mating behavior of *E. excrescens* is quite unique.

As mentioned above, the wild males of *E. excrescens* flew around a branch of a particular tree of *Q. myrsinaefolia*, *Q. serrata* or *C. japonica* in the forest. In addition, the wild males were also observed to fly around other trees of various species such as *Castanea crenata* Sieb. et Zucc., *Diospyros kaki* Thunb. ex Murray and *Salix* sp. in a forest in Chiyoda Town, Ibaraki Prefecture and in the nursery of FFPRI in 1997 and 1998 (Kan and Kitajima, unpublished observations). Furthermore, these trees of different tree species with different shapes and different heights were always located on the border of an open area in the forest. From these results it is suggested that the particular tree in the particular spot represents some landmark in many dipteran swarms.

Sometimes 2 to 3 males of *E. excrescens* formed a group both in the field and in the cage. Although the number of males in a group has not been recorded, similar male group formation has been reported in some hepialid species, such as *Sthenopsis thule* (Winn, 1909), *Sthenopsis* spp. (Forbes, 1923), *O. fasciculata* (Madge, 1954), and *H. hecta* and *H. humuli* (Turner, 1976; Mallet, 1984). In *H. hecta* and *H. humuli*, copulations take place in the male group (Mallet, 1984). In *E. excrescens*, however, the copulations did not take place in the male group. Why and how the males of *E. excrescens* form the male group is still unknown.

In the present study, the female *E. excrescens* flew straight toward the flying males at the beginning of the mating behavior. This indicates female attraction by the males or male "calling" in *E. excrescens* as reported in other hepialids, such as *H. hecta* (Barrett, 1886; Deegener, 1902; Cockayne, 1912; Cockayne and Jackson, 1913; Turner, 1976), *H. humuli* (Mallet, 1984) and *Sthenopsis* spp.

(Forbes, 1923). From the present observations, it appears that females of *E. excrescens* are attracted to the flying males by visual cues from a long distance and olfactory cues (male scent) from a short distance. To confirm this experimentally, preliminary experiments were carried out in the field cage: 5 pairs of laboratory-reared males and females of *E. excrescens* were used. In the presence of flying males with their hind-legs intact, all females flew straight toward the males from about 4 to 5 m, approached, hovered within about 0.6 to 0.8 m of the males and repeatedly touched each other in flight. Mean flight period per night was 11.3 ± 0.9 SE min ($N=4$) in the females and 16.5 ± 2.8 SE min ($N=4$) in the intact males. On the contrary, in the presence of flying males with their hind-legs excised, all females flew straight toward the males from about 4 to 5 m, but passed them over without hovering and touching. Soon, the females stopped flying and settled on the wire-netting. Mean flight period of the females per night was 4.0 min ($N=2$). While, mean flight period of the treated males per night was 16.5 min ($N=2$), almost equal to the intact males (Kan, unpublished data). Although the experiments were only preliminary, they suggest the process of female attraction by flying males of *E. excrescens* consists of 2 stages: (Stage 1) females are attracted to flying males by visual cues from a long distance, and (Stage 2) females are attracted by male scent from a short distance (probably, within about 1 m) in the final encounter of both sexes. The male flight pattern in *E. excrescens* including the swinglike pendulum flight probably has a "calling" function in long-range female attraction and the male scent probably functions as an "attractant" at close range.

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