Mating Behavior of *Ascogaster reticulatus* WATANABE
(Hymenoptera: Braconidae), an Egg-Larval Parasitoid
of the Smaller Tea Tortrix, *Adoxophyes* sp.
(Lepidoptera: Tortricidae)

II. Behavioral Sequence and a Role of Sex Pheromone¹

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Mating behavior of the egg-larval parasitoid *Ascogaster reticulatus* WATANABE was observed in the laboratory. The sequence of mating behavior of the male parasitoid was summarized as follows: random walking, antennal searching, orientation, pursuit, approach, antennal contact, mounting, and copulation. A hexane extract from the female body, drawn across the bottom of a petri dish, elicited male antennal searching while walking along the line. Dose-response curve of the behavior had a peak between concentrations of 10^{-3} and 10^{-2} female equivalent per line. In another experiment, male parasitoids actively searched the tea leaves on which females had walked. These facts indicated that the male responded to a sex pheromone left by females. The sex pheromone deposited by the females seemed to activate male searching and increase the chances of mating behavior.

INTRODUCTION

Sex pheromones in parasitic Hymenoptera are summarized by ELLER et al. (1984) in which the evidence of sex pheromones was shown in seven families; Aphelinidae, Chalcididae, Cynipidae, Pteromalidae, Scelionidae, Braconidae and Ichneumonidae. However, there are few cases in which the sex pheromones of parasitic Hymenoptera were identified. Among them, the sex pheromone of an ichneumonid *Itopectis conquisitor* was identified as neral and geranial (ROBACKER and HENDRY, 1977), and that of another ichneumonid *Syndipnus rubiginosus* was identified as ethyl (Z)-9-hexadecenoate (ELLER et al., 1984). Other than the biological interest on the sex pheromones of parasitic insects, pheromone traps can be practically used to evaluate a parasitic population (LEWIS et al., 1971).

Ascogaster reticulatus WATANABE (Hymenoptera: Braconidae) is a solitary endo-

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parasitoid of the smaller tea tortrix, *Adoxophyes* sp. (Lepidoptera: Tortricidae). KAINOH (1986) examined the conditions for mating of this species and briefly described the mating behavior. He reported that male behavior changed from random walking to pursuit after encounter with females, but did not suggest the presence of a sex pheromone. In our later observation, we recognized that males of *A. reticulatus* often antennated onto the certain points of a container in which females had previously been confined. This suggested that a chemical trace left by females acts as a sex pheromone for males to search for a mate.

We report here more detailed observation of mating behavior and the presence of a female sex pheromone of this insect.

MATERIALS AND METHODS

Insects. The parasitoids used were from our stock culture based on the rearing method by KAINOH and TAMAKI (1982) and KAINOH (1986). About 10 adult parasitoids of each sex were kept in a transparent plastic container (15 cm dia. 12 cm ht.) until experiments. Honey drops were streaked on the walls and wet cotton was put on the bottom of the containers. All the insects used for observations and experiments were 3-day-old unmated adults. The rearing of insects and following experiments were conducted at $25 \pm 1^\circ\text{C}$ and $50 \pm 20\%$ RH with a 16L-8D photoperiod. Behavioral observations and experiments were conducted between 8 and 12 hr after start of L period, during which mating activity was frequent (KAINOH, 1986).

Observation of mating behavior. A male and a female *A. reticulatus* were introduced into a transparent plastic container (8 cm dia. 4 cm ht.) in which wet cotton had been placed. Their behavior was observed for five min after the introduction. The behavioral sequence was analysed based on observations with a total of 67 pairs.

Bioassay of female extract. Three-day-old virgin female parasitoids were dipped in hexane (100 μl /female) for 24 hr at 5°C . The extract was filtered through a filter paper, concentrated by a rotary evaporator, and then serially diluted with hexane. Five microliters of the diluted extract [3.2×10^{-5} – 10^{-2} female equivalent (FE)] was applied in a straight line (9 cm long) across the center of 9-cm glass petri dishes. After the solvent was air-dried at room temperature, a 3-day-old male was released into the capped dish. Behavioral response of the male was observed for two min and scored based on the total walking distance occurring during antennal searching along the treated line as follows; score 0: no response, score 1: less than 9 cm, score 2: 9–18 cm, score 3: more than 18 cm. Twenty insects were used for each concentration. New insects and clean petri dishes were used for each replicate.

Trace left by females. Several tea branches (ca. 20 cm long) collected in a field were bundled and put into a jug and covered with an inflated plastic bag. The neck of the bag was tied with a string. Five males were introduced one at a time through a small hole at the top of the bag, and the behavior of each male was observed for two min after the introduction. The number of leaves on which they stayed or showed a characteristic antennal movement ("Antennal Searching" in Figs. 1, 2) and the time spent searching or resting on each leaf were recorded. After observation with the 5th male all the males were removed from the bag. Ten 3-day-old females were introduced into the bag, allowed to walk for 10 min and removed. Five new males were then introduced and observed as above. This experiment was replicated three times.

RESULTS

Observation of mating behavior

From the observations of paired males and females in plastic containers, the behavioral sequence leading to copulation was schematized in Fig. 1. A male released in a container usually began to walk soon thereafter (Fig. 1 "Walking") and sometimes showed a characteristic searching behavior by palpating a certain point of the container surface with his antennae (Fig. 1 "Antennal Searching", also see Fig. 2). After random walking, a male orientated a female from a distance less than ca. 3 cm (Fig. 1 "Orientation"). In the case of a male encountering a walking female, males engaged in pursuit from a 1–2 cm distance (Fig. 1 "Pursuit"). When a male orientated a stationary female, he approached her slowly (Fig. 1 "Approach") and often showed intermittent wing vibration at a 0.5–1 cm distance from the female. The male then antennated the female by slightly vibrating his antennae. Position of male and female bodies in antennal contact ranged from face-to-face (180°) to perpendicular (90°). This antennal contact by the male was not always conducted. Next, the male quickly mounted the female (Fig. 1 "Mounting"), extended the aedeagus and copulated (Fig. 1 "Copulation"). At that time, the male touched the dorsal part of the female with his antennae. While in copula, the male raised his fore-legs and mid-legs, supported himself with his hind-legs and outstretched wings, and stroked the female's dorsal part with his antennae (Fig. 3). Copulation lasted ca. 1 min as reported in KAINOH (1986) concluding with the movement of a female or dismounting of the male.

Fig. 1. Behavioral sequence in mating behavior of *A. reticulatus*. Solid lines show behavioral transition, and dotted lines possible influence of factors of one sex eliciting behavior from the other.

Fig. 2. Antenatal searching behavior of a male *A. reticulatus* in a 9 cm petri dish (Courtesy of Mr. T. KASHIMA).

Fig. 3. A pair of *A. reticulatus* in copulation posture (Courtesy of Mr. T. KASHIMA).

Fig. 4. Transition of mating behavior observed in *A. reticulatus* males ($n = 67$). Decimal numbers and corresponding thickness of bands are the rate of transition occurring between 2 behaviors. Behavior flows from left to right in the upper open bands and right to left in lower solid bands. Numerals in parentheses indicate the rate of males without further behavioral transition, and those in brackets the total rate of other transitions (frequency lower than 0.10).

Male courtship behavior was analyzed for quantitative behavioral transition from 67 observations as shown in Fig. 4. More than 40% of males doing "Random Walking" changed their behavior to "Orientation". About a half of the males performing "Orientation" pursued females. Thirty eight percent of them approached the female.

Fig. 5. Dose-response relationship between the hexane extract of females and antennal searching behavior of *A. reticulatus* males.

Table 1. Effect of trace left by female *A. reticulatus* on male searching on tea leaves

Male release ^a	Mean no. of leaves searched ^b	Mean time spent on one leaf (sec) ^b
A	1.1 ± 0.1]*	62.5 ± 12.5]*
B	6.3 ± 1.6]	6.7 ± 1.1]

^a A: males were released into the arena before female introduction, B: males released after female introduction.

^b $\bar{x} \pm \text{S.E.}$ ($n=15$) * Significantly different ($p < 0.05$) by MANN-WHITNEY *U*-test.

Some of these approaching males (16%) contacted a female before mounting. Forty percent of them did not conduct "Antennal Contact". The rate of pairs reaching copulation was 31% in 67 pairs.

Bioassay of female extract

The males individually released into the treated petri dishes walked along the line while showing antennal searching within a certain concentration range (Fig 5). The highest response of males was obtained at 3.2×10^{-3} FE/line. At higher concentrations, the antennal searching response decreased, and other components of mating behavior (e.g. "Approach" with wing vibration) were not observed. Males were often indifferent to the line when they passed by.

Trace left by females

The first five males introduced into the arena before the introduction of females were not so active and spent most of the time (62.5 sec) grooming or motionless on a single leaf (Table 1). They seldom performed antennal searching. Conversely, most of the males introduced after removal of females walked actively on leaves; antennal searching was often observed. They searched on significantly more leaves (6.3) and spent shorter periods of time on one leaf (6.7 sec) than the first group (1.1 leaf/62.5 sec, $p < 0.05$).

DISCUSSION

Most of the behavioral components in mating behavior observed in *A. reticulatus* males (Fig. 1) were common in braconid parasitoids (MATTHEWS, 1974). However,

the antennal searching of the male of certain parts of a substrate surface where a female adult had walked was a marked characteristic in the sequence of mating behavior in *A. reticulatus*. The female body extract applied in a line on the bottom of a petri dish elicited a very similar male behavior. Also, this male behavior was observed on tea leaves where females had walked (Table 1). These results indicate that the male's antennal searching is elicited by a chemical factor which is contained in and probably left as a trace on a substrate by the females. The chemical factor may be a sex pheromone, since the antennal searching of the male can be considered as one of the components of mating behavior (Fig. 4). Females did not respond to the female extract in our preliminary experiment. Males' responses seemed to be elicited by contact with the pheromone source.

Although males followed the line treated with female extract on the bottom of petri dishes they responded more sporadically to the substrates where females had walked, both in containers and on tea leaves, suggesting that the trace of female sex pheromone was occasionally deposited rather than a continuous trail. There may be some specific female behavior of pheromone deposition, but it could not be discerned in our experiment. These deposits may help the males to search for females. Males may persistently search for females in an area where many deposits are present. The role of these deposits is considered to be clearly different from "trail pheromone" in ants (WILSON, 1971), because the deposits by females are intermittent and may give males no trail cue, but only an indication of their mate(s) in the vicinity.

Optimum concentration of female extract to elicit the antennal searching of males was obtained at 1.0×10^{-3} – 3.2×10^{-3} FE (Fig. 5). The low response of males at higher concentrations may be due to impurities in the extract. If so, purification of the extract would restore the activity of pheromone in higher concentrations.

In the case of *Apanteles glomeratus* L., wing vibration of the male was elicited by female-produced sex pheromone (OBARA and KITANO, 1974), but mounting and copulatory response was elicited by the addition of visual stimuli (black or brown) to the sex pheromone (KITANO, 1975). In a pteromalid, *Anisopteromalus calandrae*, the female posture relative to the substrate acts as the visual stimuli to initiate the male courtship display (YOSHIDA and HIDAHA, 1979), in addition to the female sex pheromone which initiates approach and wing vibration of the male (YOSHIDA, 1978). In our observation, only antennal searching behavior was observed in response to the extract. Other behavioral components of mating, i.e., wing vibration and mounting, could be elicited by an addition of visual and/or tactile stimuli. The impurities in the extract may be inhibitory to elicit behavioral components other than antennal searching.

In order to elucidate the complete stimuli necessary to initiate a complete sequence of mating behavior in *A. reticulatus*, purification of the pheromone and additional experiments with various models as visual and/or tactile stimuli should be conducted in the future. Also, the role of the sex pheromone deposited on leaves as a cue for males should be confirmed in both the laboratory and the field.

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