Mating Behavior of *Ascogaster reticulatus* WATANABE (Hymenoptera: Braconidae), an Egg-Larval Parasitoid of the Smaller Tea Tortrix Moth, *Adoxophyes* sp. (Lepidoptera: Tortricidae)
I. Diel Patterns of Emergence and Mating, and
Some Conditions for Mating

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Ascogaster reticulatus WATANABE is an egg-larval parasitoid of the smaller tea tortrix moth, *Adoxophyes* sp. Diel patterns of adult emergence and mating in this parasitoid were observed in the laboratory, and the effect of adult age on the mating was also analyzed. Most of the adult parasitoids emerged within a few hours after light-on, and the emergence peak of the male was earlier than that of the female by one day. High mating activity of these parasitoids was observed in the latter half of a light period when the walking activity of both sexes was also high. Premating period of males was longer than females. The combination of 3-day-old males and 1- to 2-day-old females was most appropriate for mating. High mating rate was obtained from the density of up to 5 pairs in a container (ca. 2 l). In addition to the conditions for mating, mating behavior is briefly described.

INTRODUCTION

Ascogaster reticulatus WATANABE is an egg-larval, solitary endoparasitoid of the smaller tea tortrix (STT) moth, *Adoxophyes* sp. This parasitoid has been reared by the method of KAINOH and TAMAKI (1982), but fewer females than males were obtained. Since *A. reticulatus* is an arrhenotokous species (KAINOH, unpublished), the low rate of female adults in the rearing may be due to the low rate of mating.

In the present study, the conditions for mating in this parasitoid were examined to increase the rate of mating and to obtain a sufficient number of females for laboratory culture. Diel patterns of emergence and walking activity, which are important factors in mating, were also observed in the laboratory.

MATERIALS AND METHODS

Insects. Both the parasitoid, *A. reticulatus*, and its host, *Adoxophyes* sp., used for this study were from a stock culture in the University of Tsukuba. The rearing method for both insects was a modified one from KAINOH and TAMAKI (1982), and the mated female parasitoids were used for oviposition. Emerged adult parasitoids were separated by sex and less than 20 insects were put into a clear plastic container (15 cm in diam.,

12 cm high) to which honey and wet cotton were provided. All the rearing and experiments were conducted at $25 \pm 1^\circ\text{C}$ and $60 \pm 20\%$ RH with a 16L-8D photoperiod.

Adult emergence. Mated 4-day-old females of *A. reticulatus* were allowed to oviposit on the 3-day-old egg masses of STT. These parasitized egg masses were reared as previously described. A total of ca. 1010 cocoons was obtained from 14 parasitized egg masses. These cocoons were individually put into test tubes (1.2 cm in diam., 7.5 cm long) and a cotton plug was inserted to each tube. Emerged adults were collected and sexed every hour in light periods throughout 5 emergence days.

Diel pattern of mating. Since matings of *A. reticulatus* were frequently observed in 3-day-old pairs in preliminary experiments, this age was adopted in the present experiment. In order to determine the time of high mating activity, matings of 40 to 48 virgin pairs were observed at different times of day. Immediately before the observation, these 3-day-old virgin pairs were separately put into clear plastic containers (8 cm in diam., 4 cm high) to which wet cotton was provided. Their behavior was observed for 20 min, which was enough to obtain the maximum number of matings (KAINOH, unpublished). These observations were conducted at eight 2-hr periods during the light period. Matings in the dark period were differently analyzed; 20 pairs of 3-day-old adults were separately put into the containers in dim light soon after light-off. The males were removed soon after light-on, and then each female was allowed to oviposit on a 3-day-old host egg mass. The egg mass was reared to determine the mating status from the sex of progeny.

Adult age and mating. To determine the effect of maturity of the two sexes on mating, each of 50 to 60 virgin pairs of various age combinations (0- to 7-day-old males and females) was put into a clear plastic container (8 cm in diam., 4 cm high) and their mating behavior was observed for 20 min. The observation period was between 16:00 and 20:00 (8:00 light-on; 24:00 light-off) when the previous experiment had shown the mating activity to be high. The duration of copulation was also recorded.

Mating and adult density in a container. To determine the optimum adult density for mating, different numbers (1, 2, 5, 10, and 20) of 3-day-old pairs were put into a clear plastic container (15 cm in diam., 12 cm high) immediately before light-off. These pairs were allowed to mate for 24 hr, and the female parasitoids were taken out immediately before next light-off and allowed to oviposit on 3-day-old host egg masses. The parasitized egg masses were separately reared and the rate of mating was determined from the sex of progeny.

Diel pattern of walking activity. Since the mating activity seemed to correlate with the active searching of the parasitoids, their behavior (walking or flying) was observed mostly during the light period. Ten 3-day-old parasitoids of each sex were separately put into plastic containers (15 cm in diam., 12 cm high) immediately before light-off. Honey was streaked on the inside wall and wet cotton was provided on the bottom. Observations were begun at 1 hr before light-on and ended at 30 min after light-off. The activity was expressed as the maximum number of insects walking or flying simultaneously within an observation of 1 min. The observations were replicated with different insects of the same age.

RESULTS

Adult emergence

A total of 613 adult parasitoids (312 males, 301 females) emerged during a 5 day period. Their diel emergence pattern is shown in Fig. 1. No difference in the emergence pattern was observed between the two sexes. The maximum number of emerged adults per hour (27.6%, 32.2% of the total males and females, respectively) was recorded 1 hr after light-on, and thereafter the number of adults gradually decreased. No adults emerged within 3 hr before light-off or in the dark period.

Daily emergence of *A. reticulatus* is shown in Fig. 2. On the 1st day of emergence, all the emerged adults were males (7.1%). On the 2nd and 3rd days, more males emerged than females, but females predominated after the 4th day. Emergence peak of males was earlier than that of females by one day (difference: 0.93 day, $t=12.1$, $p<0.001$).

Observation of mating

When a 3-day-old pair was put into a container, both the male and female walked actively. When a male contacted a female with his antennae, he became excited and began to pursue her by walking while occasionally vibrating his wings. If a male approached a female from behind, she escaped from him. The contact between male and female antennae often quieted the female movement and the male easily mounted her and then copulated. The male continued to tap the dorsal part of the female with his antennae while in copula. The mean duration of copulation of 3-day-old adults was 64.0 ± 1.9 sec ($\bar{x} \pm \text{S.E.}$, $n=51$). Soon after copulation some males tried to copulate again with the same female, but in most cases were rejected.

Diel pattern of mating

The diel pattern of mating activity of *A. reticulatus* is shown in Fig. 3. For a few hours after light-on, the parasitoids were stationary and the mating rate was rather low. From 4 hr after light-on, the rate of mating increased over 40% and reached the maximum (65%) during the 10–12 hr after light-on (18:00–20:00), then decreased slightly thereafter. Both males and females moved actively on the wall of containers in the latter half of the light period and matings were often observed. Two out of 20 pairs mated in the 8-hr dark period (mating rate: 10%).

Adult age and mating

On the day of emergence, both males and females were stationary and only a few

Fig. 1. Diel emergence pattern of *A. reticulatus*.

Fig. 2. Daily percent emergence of *A. reticulatus*.

Fig. 3. Diel mating pattern of *A. reticulatus* in a light period. Vertical bars indicate the standard errors of the means. Different letters above circles indicate significant difference at the 5% level (χ^2 -test). No. of pairs observed: 40-48.

Fig. 4. Effect of age on the rate of mating in *A. reticulatus*. Based on the data from pairs of the same age. Vertical bars indicate the standard errors of the means. Different letters above circles indicate significant difference at the 5% level (χ^2 -test). No. of pairs observed: 60.

Fig. 5. Effect of male age on the rate of mating in *A. reticulatus*. Based on the data from 3-day-old females. Vertical bars indicate the standard errors of the means. Different letters above circles indicate significant difference at the 5% level (χ^2 -test). No. of pairs observed: 40-50.

Fig. 6. Effect of female age on the rate of mating in *A. reticulatus*. Based on the data from 3-day-old males. Vertical bars indicate the standard errors of the means. Different letters above circles indicate significant difference at the 5% level (χ^2 -test). No. of pairs observed: 40-50.

matings were seen (Fig. 4). The rate of mating increased as the adults aged, and reached its maximum at the age of 3 days (60%), then decreased gradually. The highest rate of mating among 3-day-old females and males of various ages was observed in 3-day-old males (Fig. 5). The highest rate among 3-day-old males and females of various ages was observed in 1- to 2-day-old females (Fig. 6).

Mating and adult density in a container

The effect of adult density on the rate of mating is shown in Table 1. The highest rate (61.1%) was observed at 2 pairs/container, then decreased as the density increased. The lowest mating rate (26.8%) was recorded at 20 pairs/container.

Diel pattern of walking activity

Immediately before light-on, both males and females were stationary but soon after light-on, some of them, especially males, walked a little (Fig. 7). The activity of females and males increased sharply during the 4-5 hr after light-on (12:00-13:00) and during the 6-7 hr after it (14:00-15:00), respectively. The maximum activity

Table 1. Effect of adult density on the rate of mating in *A. reticulatus*

No. of pairs/container	Replication	Total no. of females available ^a	Mating (%)
1	19	19	56.5
2	9	18	61.1
5	5	21	57.1
10	5	48	41.7
20	3	56	26.8

^a Females that were alive at the time of oviposition after mating. Only their progeny could be used to determine the mating success.

Fig. 7. Diel pattern of walking activity of *A. reticulatus* in a light period. Each value is total of 2 replications. No. of males or females observed: 10/replicate.

of females and males was observed during the 10–11 hr after light-on (18:00–19:00) and during the 9–10 hr after it (17:00–18:00), respectively. Walking activity of females was more constant than that of males. All the adults were completely stationary 30 min after light-off and seemed to remain in the same state throughout the dark period.

DISCUSSION

The diel pattern of emergence of *A. reticulatus* (Fig. 1) suggested that its adult emergence is stimulated by a light-on signal and is prevented by darkness (KAINOH, unpublished). Both males and females of this parasitoid may emerge early in the morning in the field. The emergence peak of males was earlier than that of the females by one day (Fig. 2), as seen in most parasitic Hymenoptera (DOUTT, 1964).

Courtship and mating behavior of *A. reticulatus* were similar to those of other braconids (MATTHEWS, 1974), and copulatory position of both sexes was very similar to that of *Chelonus eleaphilus* (ARAMBOURG, 1968). Male pheromone on the antennae in *Aphytis* spp. has a combined effect of quieting the female and making her receptive (RAO and DEBACH, 1969). In *A. reticulatus*, chemical and/or tactile stimuli seem to be important in female recognition by a male and in quieting the female to receive the male, since behavioral changes were observed after contact with each other.

There was a correlation between the diel pattern of mating activity (Fig. 3) and walking activity pattern (Fig. 7), suggesting that the chances of mating in the field depend on the walking activity of both sexes. If female parasitoids actively search for hosts in the daytime, their matings may occur in daytime, when males also actively

search for females. At night, they may be motionless and no matings occur. Matings in the 2 out of 20 pairs in darkness may have been accidental when they were put into containers in dim light.

A larva of *A. reticulatus* egresses from a dispersed 4th-instar host larva, then makes a cocoon after external feeding (KAWAKAMI, 1985). Thus the adult parasitoid emerges solitarily where the 4th-instar host larva has ceased feeding and has spun a cocoon between the leaves. In addition, the mating activity of the adult male was rather low on the day of emergence (Figs. 4 and 5). Therefore, the mating of males soon after emergence may not occur in *A. reticulatus*, while most braconid parasitoids are ready to mate upon emergence from the cocoon (MATTHEWS, 1974). The longer premating period of *A. reticulatus* males than that of females may be compensated by the early emergence of male adults. In the egg-larval braconid parasitoid, *Chelonus inanitus*, mean premating period of females was 0.9 day (range: a few hours to 4 days) (HAFEZ et al., 1977), though that of males was not noted. In the gypsy moth parasitoid, *Apanteles melanoscelus*, older males (>1 week) did not mate readily, but old females were as receptive as young ones (WESELOH, 1977). As in the case of *A. melanoscelus*, the male age of *A. reticulatus* seems to be important, at least under laboratory conditions. To mate as many females as possible, combinations of 3-day-old males and 1- to 2-day-old females are preferable in *A. reticulatus*.

From the results of the experiments on adult density and mating (Table 1), up to 5 pairs in a container (ca. 2 l) is appropriate to obtain a sufficient number of mated females for an artificial rearing.

The diel pattern of walking activity (Fig. 7) may have been amplified, since 10 adults were confined in a container (ca. 2 l) and they interfered with each other when they moved actively. The first activity peak soon after light-on may be due to the response to the light and second activity peak in the latter half of the light period correlated with the high mating activity (Fig. 3). Therefore, the active movement of males may be their search for females. The optimum time for an experiment on mating behavior is during the 8–12 hr after light-on (16–8 LD photoperiod). If the active movement of females is caused by the search for hosts, the experiment of host-finding behavior and the bioassay of the kairomone (KAINOH et al., 1982) should be conducted in the latter half of the light period. The diel pattern of parasitoid behavior in the field was not determined by this study. Outdoor conditions, e.g., temperature, humidity and light intensity, may influence their behavior. In *Chelonus inanitus*, adults were active in the morning and in the late afternoon in the field (RECHAV, 1975). To determine the diel pattern of searching of *A. reticulatus* in the field, field observations must be made.

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