Mating Behavior of *Xylotrechus pyrrhoderus* BATES (Coleoptera : Cerambycidae) I. Behavioral Sequences and Existence of the Male Sex Pheromone

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Observation and experimental analysis of the mating behavior of *Xylotrechus pyrrhoderus* were carried out. The mating behavior consists of at least two phases: (1) The flying female approaches the resting male, (2) The male is stimulated by the alighting female and copulates with her. Experimental analysis indicated that in the first phase, the female is attracted by the sex pheromone released by the male.

INTRODUCTION

The mating behavior of Cerambycid beetles has been studied by many investigators who have reported interesting facts (review by LINSLEY, 1959; MICHELSEN, 1964; HUGHES, 1979, 1981). However, most of these studies dealt with the behavior between sexes during or just prior to mounting, and little is known how males and females approach each other, and by what factor this approach is induced.

In some species, observations have suggested that the location of conspecific individuals of the opposite sex over long distances may be guided by olfaction (DUFFY, 1953; LINSLEY, 1959). In *Megacyllene robiniae* (GALFORD, 1977) and *Hylotrupes bajulus* (DOPPELREITER, 1979), it was shown experimentally that a kind of pheromone deposited by the female causes the male to spend time in staying and searching for it, resulting in an increased chance of a male encountering a female. Nevertheless, in most species the process still remains obscure.

The studies to be reported here will provide information about the sequence and temporal characteristics of mating behavior of *X. pyrrhoderus* and the signals involved.

MATERIALS AND METHODS

Animals. For field observations of the sequence of mating behavior, adults obtained from overwintering larvae collected from pruned vine shoots were allowed to emerge in an outdoor cage. For other observations and experiments performed under laboratory conditions, pupae were taken from vine shoots and sexed. Pupae and adults obtained from them were reared in the laboratory under a photoperiod of 14 hr light—10 hr dark at 25°C. For the assessment of sexual maturation adults were reared separately in plastic cups (6 cm × 6 cm) under both natural and the above stated conditions. For other experiments adults were put separately into a glass tubes

(1.5 cm $\times$ 3.5 cm) for a period of 16 days, the period determined for sexual maturation. For the experiments with a flight tunnel, ten adults were later put together in a glass bottle (3 liters) with wet absorbent cotton for four days.

Field observation of mating sequence. observations were performed in the field as well as in an outdoor cage (8 m $\times$ 3 m $\times$ 2.5 m) at the vineyard of Suntory Ltd., Yamanashi Winery, Kitakoma-gun, Yamanashi Prefecture, during August and September, 1979 and 1980. At least seventeen cases were closely observed.

Sexual maturation. Sexual maturation of females was evaluated by the number of mature eggs in ovaries and oviducts. Ten females reared under natural conditions were dissected every few days after adult eclosion and the emergence from pupal cell. Females reared under controlled conditions (25°C) were also dissected every two days after adult eclosion.

Daily mating activity. A male and a female kept in a small plastic box (20 cm $\times$ 30 cm $\times$ 10 cm) were observed to check their mating activity. Because copulation was presumed to take place whenever male and female came together in the daytime, the following three series of twenty couples each were prepared. Light was put on at 6:00 and off at 20:00, at 30°C. One 16-day-old male and a female of the same age were put together into the plastic box, either 1) at 6:00, i.e. at the time of light-on, 2) at 11:00, five hours after light-on, or 3) at 16:00, ten hours after light-on. Observation was made every 15 minutes therefrom until 22:00, i.e. two hours after light-off.

Observation and experiment in a flight tunnel. The flight tunnel used for observation of daily mating activity and for experimental analysis of signals involved was a clear plastic tube 150 cm long and 50 cm diameter, placed horizontally and illuminated evenly from all sides at a luminosity of about 10,000 lux with several fluorescent lamps. Smooth air flow of 0.15 m/s was supplied at 30°C and 70–75% RH.. About ten adults were released at the upwind or downwind side of the flight tunnel. Three adults separately confined in net cages 10 cm in diameter and 14 cm high were placed in the tunnel 100 cm away from the site of adult release. Net cages were covered with nylon gauze to keep the insects inside from sight. The number of adults approaching and landing on the net cages during thirty minutes was counted. Released adults were marked individually by white felt pen to avoid double counting.

RESULTS

Observation

The adults of *X. pyrrhoderus* were active on fine days from 8:00 or 9:00, when emergence from pupal cells took place most frequently, to about 16:00. Flying activity was marked between 11:00 and 15:00, when mating behavior was also observed to take place.

Mating behavior began with the approach of the female to the male. The flying female passed within 1–1.5 m of the male resting motionless on leaves or shoots of vines and approached him with a changing flight pattern. At a distance of about 20 cm from the male, she turned to the searching flight at a very low speed close to hovering.

Immediately thereafter the female alighted on leaves or shoots about 10 cm from the male. The male was stimulated and dashed toward her at high speed with

antennae vibrating. Sometimes she also walked toward him. When the female alighted at the opposite side of the leaf on which the male was resting and the two were out of sight of each other, both continued to walk for a while, the male at a higher speed than the female.

The male mounted the female from her front end. Then he turned around to the correct position and started the "licking" movement with his mouthpart applied to the female's pronotum or elytra, while the abdominal tip of the male protruded downward to come in contact with the genitalia of the female and copulation was established. Mounting lasted about 15 minutes on the average, and several bouts of copulation were observed in a mounting.

Sexual maturation

After eclosion the adults stayed for some time within the pupal cells inside a shoot. When they emerged from the pupal cells, their reproductive capacity was already fully developed, and copulation took place without interval.

Ovaries from females just after eclosion were still undeveloped showing no mature eggs. Under natural conditions in August (25–30°C), the number of mature eggs increased gradually and ovaries developed with time. Ovaries finally contained about 80 eggs at the age of 16 days, thus reaching the level of females just after emergence from pupal cells. The number of eggs remained relatively constant when females were kept separately and quiescent.

Under laboratory conditions of 25°C, mature eggs appeared in about 6 days, and in 14 days the number of eggs had reached a plateau of about 80. These results suggested that the duration of the female's stay in the pupal cell was about two weeks, and females two weeks after eclosion are already sexually mature with fully-developed mating capacity.

Daily mating activity

Copulation of a male and a female confined in a small box took place between

Fig. 1. Daily change in the number of mature eggs in a female, under outdoor (A) and laboratory conditions at 25°C (B). Mean of 10 females with standard deviations.

Fig. 2. Daily mating activity in 20 couples of 16-day-old males and virgin females confined separately in small boxes under a 14L-10D photoperiod. Arrows indicate the time at which the male and female were put together.

Fig. 3. Hourly change in response of 10 released females to male-containing cages in the flight tunnel. Circles show the percentage of females attracted to the cage. Average of at least three replications.

10:00 and 18:00, especially 10:00 to 14:00, regardless of the time when they were put together. During this period most couples repeated copulation.

In the flight tunnel, the cage containing a male was approached by females with typical searching flight. The number of females attracted to caged males in the flight tunnel showed a peak at about 14:00, i.e. 8 hours after the light-on. Little or no response was observed before noon.

Flight tunnel experiments for analysis of cues involved in the mating behavior

An analysis of signals involved in the mating behavior was carried out in the flight tunnel between 13:00 and 14:30 of the day mating behavior was most frequently observed during these studies. Experimental procedures and results are summarized

Table 1. Responses of females or males in a flight tunnel to small cages containing live insect or male extract

Contents	Location	Insect released	Total No. released	% Attracted	Hovering ^a
None	upwind	Female	30	6.7	—
Male	upwind	Female	30	76.7 ^b	++
Male	downwind	Female	29	20.7	+
Male extract	upwind	Female	40	35.0 ^c	++
Female	upwind	Female	41	14.6	—
Male	upwind	Male	30	13.3	—
Female	upwind	Male	29	3.4	—

^a ++ : Typical hovering-type, searching flight observed in most members.

+ : Hovering-type flight slightly shown by a part of the members.

— : Hovering-type flight was not observed.

^b Percentage differs significantly from control at $P < 0.001$ (χ^2 test).

^c Percentage differs significantly from control at $P < 0.05$ (χ^2 test).

in Table 2.

To the males confined in cages placed upwind, 76.7% of the females were attracted and showed typical searching flight including hovering. When empty cages were placed, the rate of approaching females was 6.7% and no hovering was observed at all. Such reduction of approach rate also appeared when the male-containing cages were placed downwind. Only a few cases of hovering were observed in this situation. This strongly suggested that females are attracted to males by some chemical cues.

Hexane (*n*-hexane) extract of fifteen males reared in the outdoor cage was prepared and a net cage containing a piece of filter paper soaked with this extract was placed in the flight tunnel. Thirty five percent of the females were attracted to this cage and hovering was also observed. Supplementary experiments with a higher concentration of male extract showed that all of the 10 females tested were attracted to the cage.

Similar experiments were carried out in various combinations. However, practically no attractance of females to females, males to males, or females to males was observed, as expected from the result of field observations.

When the males or the male extract were confined in a cage, remarkable behavior in addition to the searching flight was observed among the females. Most females alighting on the cage walked about along its surface without seeing the male. The female on or near the cage sometimes mounted another female, showing homosexual behavior.

DISCUSSION

This beetle is sun-loving like other Cerambycine species of the tribe Clitini, and copulation takes place in the daytime, especially between 10:00 and 14:00, as above.

Mating behavior consists of at least two phases: (1) flying female approaches a resting male, (2) the male is stimulated by the alighting female, and the encounter ends in mounting and copulation. In many insects, males are searching for mates and are attracted to chemical or visual cues originating from females. The Cerambycid

beetles, too, have been shown to be no exception. In the case of the Malaysia species, some flying males were attracted by a confined female, aided by olfactory organs in the male antennae (DUFFY, 1953). All members of the Cerambycinae of the tribe Callichromini produce scents detectable by man, and these scents are supposed to be sex attractants (DUFFY, 1953; LINSLEY, 1959). However, practically nothing is known about the means whereby the males are able to detect and recognize their mates, beyond the fact this is done partly with the aid of sense organs in the antennae (DUFFY, 1953). Recently, in *Megacyllene robiniae* (GALFORD, 1977) and *Hylotrupes bajulus* (DOPPELREITER, 1979) females were reported to deposit the pheromone on the host wood, and males tended to stay there longer, but no attraction of males to the pheromone-impregnated site was suggested.

The results obtained in the present work clearly show that in *Xylotrechus pyrrhoderus* the female actively searches out the male, and is attracted from a distance to the pheromone he releases. This is the first case of an airborne pheromone attracting over a distance, as well as of a male sex pheromone experimentally evidenced in Cerambycidae.

Although males of some insects are known to produce sex pheromones which stimulate females, most of them function at a very short range or as "aphrodisiacs" (JACOBSON, 1972; SHOREY, 1973). The case of *X. pyrrhoderus*, in which females are attracted from a distance to males releasing the sex pheromone, appears to be an exception. The effective sphere of the male sex pheromone of this beetle was estimated from the observations of female change in flight pattern to be about 1–1.5 m downwind. But it might be effective in other aspects at a longer distance.

In the second phase of the mating behavior, males were stimulated and dashed toward the alighting female, even if she was out of sight. It remains unsolved by which factor this behavior is induced. In the final contact of the sexes of some Cerambycid species, antennae of the male are important for female recognition (LINSLEY, 1959), and while the olfactory sense appears to guide the male to the immediate vicinity of the female, other senses may play a role in the final contact (LINSLEY, 1959). In *X. pyrrhoderus*, a mounting lasts for about 15 min. Among the Clytini a pair copulates several times in a period of 10 to 15 minutes (BUTOVITSCH, 1939). In the present study, several bouts of copulation were observed during a mounting, and the second mounting of the same pair occurred after a short break under the outdoor conditions. The same was observed in captivity. The male showed a licking movement with his mouthparts during mounting. MICHELSEN (1964) supposes this licking movement to be a movement to calm the female.

Most Cerambycidae require foods in the adult stage for egg maturation (LINSLEY, 1959) and thereafter lay eggs on dead or dying trees. The mating site is usually associated with the site for feeding or oviposition.

In Lepturinae and perhaps some species of Cerambycidae the male encounters the female on flowers which both visit for feeding and mating occurs there (LINSLEY, 1959). Mating of *Monochamus alternatus* takes place on the dying pine tree suitable for oviposition. In this species, orienting to the suitable tree is presumably guided by plant attractants which differ among trees according to their physiological conditions (YAMANE and ASADA, 1977). A similar situation is supposed to occur in *Monochamus notatus* and *Monochamus scutellatus* (DYER and SEABROOK, 1978), and in the latter species large numbers of adults of both sexes aggregate (HUGHES, 1979, 1981).

In *Xylotrechus pyrrhoderus*, the male and female are supposed to have fewer chances to encounter, because they do not have an aggregation site such as the flowers that Lepturinae has and the dying trees of the genus *Monochamus*. In such species, a distance pheromone will be useful for enabling the sexes to encounter and select each other. However, the reason the male pheromone instead of the female pheromone has developed is not clear. THORNHILL (1979) explained the relatively few cases where females respond to a male distance pheromone in terms of sexual selection, as follows. When a male has a nuptial offering, or is in possession of some other resource critical to female fitness such as territory or oviposition site, selection is stronger on females for response to any male odor than vice versa (THORNHILL, 1979). Further studies on the mating strategy of *Xylotrechus pyrrhoderus* are needed from this aspect.

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