REPRODUCTIVE BEHAVIORS OF ASIAN LONGHORNED BEETLE

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ABSTRACT

There is a critical need for information on the reproductive behavior of the Asian longhorned beetle, *Anoplophora glabripennis* (Motschulsky), to provide the biological basis for predicting population dynamics, especially as the population size declines due to eradication efforts. A series of four experiments using three strains (New York, Illinois, and China) was designed to document the following: 1) the reproductive behaviors (both mating and oviposition) of a single pair until natural separation or over 6 hours, whichever came first; 2) the number of females a male can successfully mate in its lifetime and how male age affects mating success; 3) the frequency and duration of matings associated with sustained female fertility; and 4) the interactions of all possible combinations (male and female) and sequences of three individuals(MMF, MFM, FFM, FMF).

The reproductive behaviors of *A. glabripennis* are typical of diurnally active species of the subfamily Lamiinae. When a male contacted a female with his antennae, he generally would quickly attempt to mount her and mate. If the female was receptive (did not fight the mounting and allowed access to her genital chamber), he would mate with her immediately after mounting and initiate a prolonged pair-bond. Non-receptive females would exhibit one or more of the following behaviors: run away, kick with hind legs, hit with antennae, make quick turns, fall, or fly. In these cases, the male might abandon his attempt and separate or perform a short antennal wagging courtship behavior. Non-receptive females would generally become receptive after further contacts. During the entire time, the male continuously grasped the female with his front tarsi or both front and middle tarsi. Individual copulation events lasted an average of 2.8 minutes, and 1-10 copulations occurred per event, followed by a male refractory period averaging 95 and 60 minutes, for the New York and Illinois males, respectively. During copulations, the female held her genital chamber open while stationary, walking, or chewing the host for oviposition (the latter occurred more often during later copulation events). Between copulations, the female most often walked or attempted oviposition in the pit she had chewed. The average total time *in copula* was 34 minutes, and this resulted in an average hatch of 56 percent of eggs females laid over their lifetime. Oviposition (0-5 eggs per female) lasted an average of 12 and 10 minutes, for the New York and Illinois females, respectively, on bolts with bark 1-2 mm thick. After a female chewed a pit, she rotated 180°, extended her ovipositor, and used it to find the pit. She then inserted her ovipositor under the bark and used the sclerites at the tip of her abdomen to pry the bark up while lifting her body. After laying an egg, she wiped excretions across the opening using the tip of her abdomen. Females abandoned some pits at various points in the process.

Male beetles at 11-104 days of age were able to copulate successfully (≥ 3 minutes in duration). A copulation was considered successful if the female laid eggs that hatched. Males generally had one or more unsuccessful copulations prior to the first success. Male fertility (as measured by percentage egg hatch of females he copulated with) and copulation success peaked for the three males tested at 3-5 weeks of age then slowly declined until copulation stopped at about week 15. When the males reached 12 weeks of age, they were less agile and less able to grasp and hold a female, which in an unconstrained environment could result in less receptive females escaping. The shortest interval between two successful copulations was 5 minutes (when a second virgin female was presented immediately after a copulation ended). The most times a female successfully copulated with one male was 27, resulting in a total of 1,366 progeny.

On average, females that had copulated 10 times or more (each ≥ 3 minutes in duration) had a significantly higher percentage of viable eggs than did females with only 1 to 5 copulations, though fecundity was unaffected. The number of copulation events did not significantly affect female longevity. These results suggest that over 1 hour of total time *in copula* (excluding mate guarding time) is needed for maximum sustained fertility, as measured by percentage hatch. In nature, this time requirement for copulation could be satisfied by one or more matings of longer duration (multiple copulations), rather than through a series of short encounters.

During the experiment where three beetles (male and female) were put together in different combinations and sequences, our observations suggested that several semiochemicals are likely involved in the mating process. We observed that, when males were added after a female was present, one-third of the males walked slowly, palpating the bark as if following a chemical trail directly to the female, then mounted the female after his antennae contacted her. We also observed that approximately one-third of the females located the males and made the first contact, but were not mounted until after the male contacted the female with his antennae. If an aggressive male to male encounter occurred first, then the percentage of opposite sexes finding each other declined dramatically. When a male mounted a female, he remained with her and fought off both males and females that made contact with them, usually without dismounting. When males dismounted, they were able to relocate the females easily. The loser of a male-to-male encounter flew away 21 percent of the time and ran away 75 percent of the time, which tended to disperse the males and thus ensured that all females would be found. Twice, we observed a male treating another male (that had recently been with a female) like a female, even initiating copulation attempts. These observations suggest that once the two sexes are on the same tree, they will find each other, that a male will stay with a female long enough to ensure she will remain fertile the rest of her life, and that males will disperse themselves to reduce aggressive encounters, thus increasing the likelihood of locating females even when populations are low.