Correlations between Sexual Development, Age, Maturation Feeding, and Mating of adult *Anoplophora glabripennis* Motsch. (Coleoptera: Cerambycidae)*

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ABSTRACT The results of research on artificial rearing, adult anatomy, and mating trials of *Anoplophora glabripennis* showed that newly emerged males had mature sperm and were able to mate effectively. Supplemental feeding was required to maintain normal vigor. Although females mated after emergence, ovaries did not become fully developed until supplemental feeding was involved. Mating improved ovary maturation significantly under the conditions of supplemental feeding, whereas no obvious effect of mating on ovary maturation was observed for adults without supplemental feeding. Ovaries matured gradually within 10 d after emergence. Supplemental feeding was also beneficial to the success of adult mating.

Key Words: *Anoplophora glabripennis*; sexual maturation; supplemental feeding; mating

Previous studies showed that supplemental feeding is vital to the successful reproduction of *Anoplophora glabripennis* after emergence. However, there are no reports on the effects of supplemental feeding on reproductive activity and the correlations between sexual development, adult age, supplemental feeding, and mating. Therefore, we designed studies to better understand adult activity, sexual development, mating, and reproduction to improve decision-making during management of this pest.

Materials and Methods

Adult Rearing. Adults used in this study were collected from poplar (*Populus opera*) forests near the campus of Ningxia Forest College. Newly cut poplar logs were placed inside cages for adult emergence. Adults emerging within the cages were collected hourly from dawn to dusk during the emergence period. Number, sex, and time of emergence were recorded. Adults were reared individually in ventilated, lighted 12 × 5 cm (diameter × height) transparent plastic boxes at 25.8 ºC. First year leafy poplar (*P. opera*) branches were provided as food for those with supplemental feeding. Rearing boxes and poplar branches were replaced every other day. Distilled water was provided through wet filter paper for those without supplemental feeding. In treatments where food was provided, males and females typically fed before mating. There were 8 treatments for female adults in the rearing tests (Table 1).

Anatomic Examination of the Sexual Development of Males. The spermatheca of male *A. glabripennis* of different ages (days after emergence, same below) was removed to examine sexual development. A small portion of the spermatheca tissue was placed on a glass slide with a few drops of 75% physiological salt solution and examined under an optical microscope at × 400 for the presence of mature sperm and their activity. The sexual development of male *A. glabripennis* at different ages was determined through anatomic examination.

Anatomic Examination of Female Sexual Development. The development of eggs inside both ovaries under different supplemental feeding treatments was examined and

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compared by dissecting five or more females from each age group to determine the correlations between ovary maturation, adult age, supplemental feeding, and mating.

Table1. Treatments of supplemental feeding for female *A. glabripennis*

No.	Treatment	No.	Treatment
a	feeding + no mating	*e*	feeding + mating 6 - 8 d after emergence
b	no feeding + no mating	*f*	no feeding + mating 6 - 8 d after emergence
c	feeding + mating 0 - 1 d after emergence	*g*	start feeding + mating 6 - 8 d after emergence
d	no feeding + mating 0 - 1 d after emergence	*h*	stop feeding + Mating 6 - 8 d after emergence

Mating Trials. Males used in this study were relatively active, 0-12 d old, whereas females were 0-2 d or 6-9 d old with or without supplemental feeding.

Paired adults were kept in a 12 × 5 cm (diameter × height) rearing box for about 30 min. Mating behavior and responding time were recorded for each adult. Sexual excitement was recorded when a male chased a female, or male mounted a female with bending abdomen. Successful mating was recorded when the male finished the mating by inserting its genital into the female. Differences in the rate of sexual excitement for the males and the rate of successful mating in respond to females at different ages and supplemental feeding conditions were analyzed.

Results and Analysis

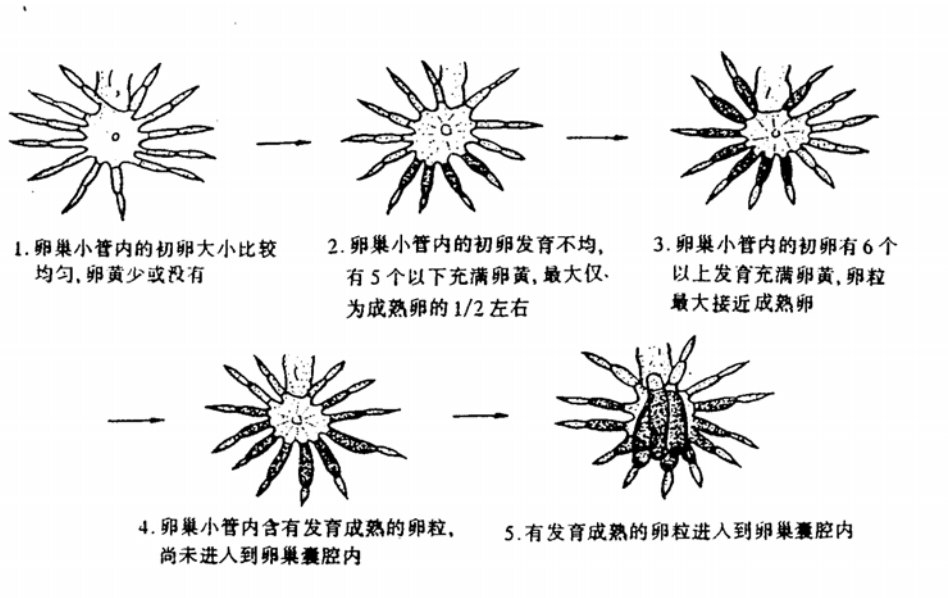
Correlation between Sexual Maturation, Age, Supplemental Feeding and Mating for Males. A total of 55 males between the ages of 0-24 d were dissected to determine development of the reproductive system. The results showed that male *A. glabripennis* contained mature, active sperms and were able to mate with females effectively the day following emergence. In addition, it was also shown that supplemental feeding was essential for males to maintain their vigor.

Correlation between Sexual Maturation, Age, Supplemental Feeding, and Mating for Females. Development of the female ovary could be divided into five stages (Fig. 1) based on the degree of maturation obtained from dissection. Ovary development for females in different treatments was showed in Fig. 2 *a* ~ *h*, in which Y – axis represents the average degree of development, with an error bar indicating the maximum and minimum value. The correlation between sexual development, adult age, supplemental feeding, and mating was analyzed as following:

(1). Supplemental feeding was the key factor that determined the development of female ovaries. Ovaries generally failed to reach maturity without supplemental feeding. Data of ovary development in Figs.2*b*, 2*d*, 2*f* showed that very few ovaries developed fully when supplemental feeding was not provided. Results of statistic analysis also showed that there was no significant difference among different age group within treatment *b* (Duncan's test), *d* (Duncan's test), and *f* (Aspin-Welch test). For those very few females that reached sexual maturity without supplemental feeding, sufficient nutrition could have been accumulated before their eclosion, and therefore could support egg development by consuming their own nutrition.

(2). Significant changes in ovary development started to appear 5 d after initial supplemental feeding, as indicated by comparing the development at different ages between Figs.

2*a* and 2*b*, and 2*f* and 2*g*. Results of statistic analyses showed that the difference in ovary development for females between Figs. 2*a* and 2*b* was not significant at 5 d after their emergence, but significant at 10 d (Duncan's test). For Figs 2*f* and 2*g*, significant difference was observed 15 d after emergence, but not at 10 d (Aspin – Welch test).



1. Early eggs in ovarioles, similar in size, with no or least amount of yolk
2. Ovarioles with unevenly developed early eggs. Less than 5 filled with yolk, half the size of mature eggs
3. Early eggs inside ovarioles, more than 6 filled with yolk, size close to mature eggs
4. Mature eggs inside ovarioles, yet to reach ovisac
5. Mature eggs reach ovisac

Fig. 1. Developmental stages of female ovary (one side) of *A. glabripennis*

(3). Female ovaries matured about 10 d after emergence when *P. opera* branches and leaves were served as supplemental food. This trend was well demonstrated in Figs. 2*a*, 2*c*, 2*e*, and 2*g*.

(4). Mating had a positive effect on development of ovaries under the conditions of supplemental feeding, whereas no obvious effect was observed when supplemental feeding was not provided, as indicated by the difference in Figs.2*a* and 2*c*, and 2*b*, 2*d*, and 2*f*. In addition, analyses showed there were significant differences in ovary development between Figs. 2*a* and 2*c* at10 d (Aspen-Welch test). No difference was observed between Figs. 2*b* and 2*d* at any adult age.

(5). Continuous feeding was needed for the normal development and reproduction of female *A. glabripennis*. Ovary development stopped several days (ca. 8 d) later when supplemental feeding was terminated, as indicated in Figs. 2*e*, and 2*h*. In addition, no significant difference in ovary development was observed among different ages within the treatment of *h* (Duncan's test). No difference was found between treatment *e* and *h* at 10 d as well. However, significant difference was observed between treatment *e* and *h* at 15 d after emergence.

Sexual Response of Males when Live with Females under Different Conditions.

Statistical analysis showed that sexual excitement was significantly higher for males that lived with females with supplemental feeding, compared to those without supplemental feeding (Table 2). There was no significant difference between the males that lived with younger (0-2 d) vs. those that lived with older females (6-9 d), although males appeared to be more excited sexually when lived with younger females (Table 2). The rate of successful mating followed the same trend as the sexual excitement rate. The results of this study indicated that supplemental feeding was beneficial to mating, in addition to individual development.

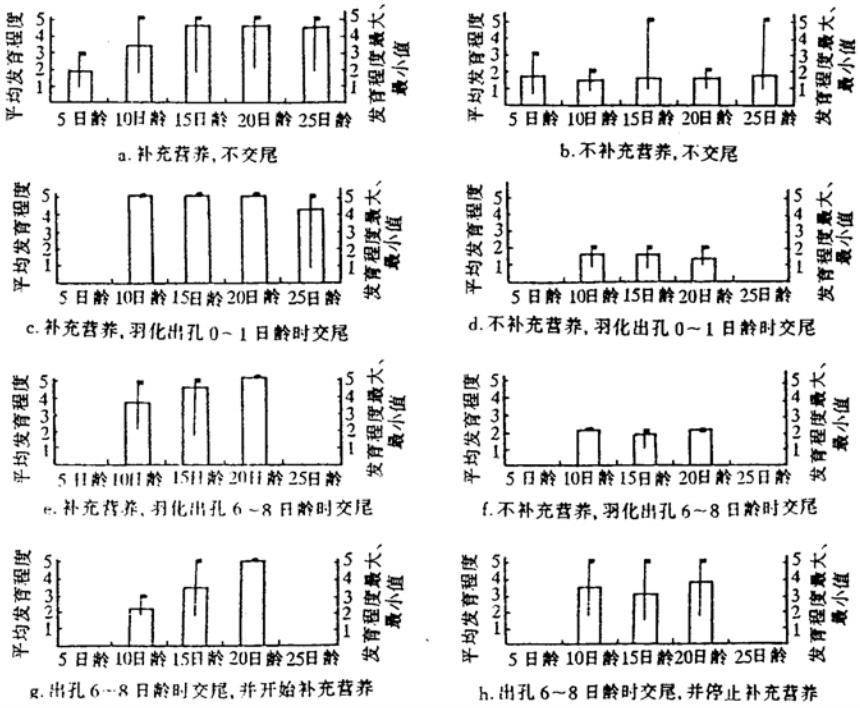


Fig. 2. Ovary development of female *A. glabripennis* under different treatments. X - axis represents adult age (5, 10 15, 20, 25 d after emergence). Y - axis represents the average degree of development (left), minimum and maximum value of development (right)

Table 2. Results of adult mating

Rearing conditions	Female age	Pairs of adults	Rate of male excitement (%)	Rate of successful mating (%)
Supplemental feeding	0-2 d	43	60.5	41.9
	6-9 d	107	44.9	30.8
	Total	150	49.3	34.0
No supplemental feeding	0-2 d	65	40.0	24.6
	6-9 d	102	32.4	11.8
	Total	167	35.3	16.8

Discussion

The results of this study demonstrated that supplemental feeding was the key factor to the reproduction of *A. glabripennis* not only because it supported the development of female reproductive system, maintained vigor for adults of both sexes, but also because it affected mating behavior. It is therefore very important to utilize the supplemental feeding features of *A. glabripennis* in the management of this pest.

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