SEXUAL RECOGNITION AND MATING BEHAVIOR OF THE POPULUS LONGICORN BEETLE, *BATOCERA LINEOLATA* (COLEOPTERA: CERAMBYCIDAE)

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ABSTRACT: We studied the sexual recognition and mating behavior of *B. lineolata* by video tracking capture system (EthoVision 3.1) and field observation, for laying the foundation of the effective control of *Batocera lineolata* Chevrolat (Coleoptera: Cerambycidae) and enrich the reproductive behavior of the insect. The result showed that, during the encountering, the intersection time of two tracks and net relative movements for female-male group were longer than female-female group or male-male group; while the reaction time was shorter than the latters. A complete mating of *B. lineolata* included three stages, i.e. Pair-bonding, Mating and Post-copulatory guarding. The average time of Pair-bonding is 2.16 mins, and 10.28 mins, 5.37 mins for Mating and Post-copulatory guarding, respectively. In mating experiments with different sex ratios, the mating number fluctuated regularly, and it was greatly different in mating number for male or female individuals. The virgin female or male, takes more total mating time longer duration for single mating, higher insert genital frequency than un-virgin ones. The mating on the host plant had higher mating rate and longer duration time than on the open ground. The mating behavior in the field test was similar with that in the laboratory experiment.

KEY WORDS: *Batocera lineolata*, Mating, Sexual recognition, Behavior analysis.

Longhorn or longicorn beetles are a large group of beetles with more than 35,000 species (Lawrence, 1982) and the reproductive strategy of species is variant. However, only about 80 species have been studied in the aspect of reproductive behavior so far (Hanks, 1999). The longhorn beetle has a nature of multiple mating. For example, *Anoplophora glabripennis* (Motsoh) fed in the cagecan copulate 38 times through the whole life at most , and 28.25 times averagely (Zhang et al., 1997). Before the mating, there is no complicated courtship. When female and male individuals encountered, it is easy for them to mate. But the duration of single mating is different - (Hanks, 1999; He & Huang, 1993). During the study on the behavior of longicorn beetle, He and Huang (1993) found that the mating behavior of adult *A. glabripennis* involved five stages, i.e. Lingering, Approaching, Taking place, Wing licking and Mating. Those experts such as Yang et al. (2007) said that a complete mating process of *Monochamus alternatus* (Hope) included three stages, i.e. Encountering pair-bonding, Mating and Post-copulatory guarding. Li (1999) found that non-contact olfactory recognition did not play a leading role in the process of spouse seeking of *A. glabripennis*, and the mating action of adults generally started with the vision stimulation of female adults to the male adults. Through the observation in details at each stage from the encountering to mating display of *Philus antennatus* adults, Yin (1996) found that male adults were active and could recognize the female within a short distance, and the mating process involved five stages, i.e. Chasing, Mounting dorsally, Insertion of genetalia, Abdominal spasms and Ejaculation. Wang (2002), Wang and Davis (2005) stated that the sexual pheromone was not used during the mating behavior of *Zorion guttigerum* (Westwood)*,* and host features played an important role in their mating.

Batocera lineolata Chevrolat (Coleoptera: Cerambycidae) is an important wood-boring insect that damages many hardwoods, such as *Populus* spp., *Juglans regia* (L.), *Fagus engleriana* (Seem), *Rosa multiflora* (Thunb), *Viburnum awabuki* (K.), *Betula luminifera* (H.Winkl), and *Ligustrum lucidum* (Ait) (Sun & Zhao, 1991). *B. lineolata* mainly distributed in China, Vietnam, Japan, India, Burma (Chen et al., 1959). *B. lineolata* is the main wood-boring insect on *Populus* spp.in the central and southern part of China, and badly affects the construction of forestry eco-environment and the development of *Populus* spp. Industry (Chen & Luo, 2001). In recent years, *J. regia* in the north part of China also seriously suffered from the damage. More than 70% trees have been attacked, which becomes an important factor that restrains the development of *J. regia* (Wang et al., 2004).

B. lineolata adult is the unique insect with behavior-orientation expressed in obvious taxis to the host plant, and the taxis is to meet nutrition needs for reproduction activities (Yan et al., 1997). So, after-emergence of *B. lineolata* is the key stage both for reseach and preventing them from large areas. And the research on the mating behavior of *B. lineolata* not only can lay the foundation for effective control of *B. lineolata*, but also can enrich the study of insect reproductive behavior.

MATERIAL AND METHOD

Insect source

Adult virgin *B. lineolata*: collected from *Populus* in Luojiang County in Deyang, China in late April 2010. These adult longicorn beetles had just finished their emergence and had not mated. Put these beetles in two cages (60 cm x 60 cm x 60 cm, stainless steel mesh) at room temperature (25 ± 2) by female and male separately until mating experiment, feeding them with *R. multiflora*.

Sexual recognition

There were three groups in the experiment, i.e. two females, two males, one female and one male (no size difference between two *B. lineolatas* and no malformation). In automation of behavioral experiments, we placed two *B. lineolata* of one group into a fixed area, and use video tracking system to record the activities. Then use behavior analysis software (EthoVision3.1) (Noldus Information Technology Co. Ltd. located in Netherland) to compare the encountering situations of *B. lineolata* among different groups. Repeat the experiment for 8 times, and record for 20 s each time.

Laboratory mating

At the room temperature (25 ± 2), 4 pairs of collected *B. lineolata* with same emergence time (no size difference between females and males, who have been fed for 8 days after the emergence) was marked by different numbers - on the abdomen of the female and the backside of the male, placed in one cage (60 cm x 60 cm x 60 cm, stainless steel mesh) with *R. multiflora*. And put fresh pieces of

Populus spp. (Length: 100 cm; Φ: 15 cm) in the cage to lure the oviposition. Change the *R. multiflora* and pieces of *Populus* spp. - every 2 days.

Using the method of Ji's (Ji et al., 1996) to identify mated *B. lineolata*. Record the duration of first-copulate and re-copulate of each individual.

Every day, observed from 8:00-20:00. After 20:00 removed the male from the cage, fed separately, then put them back to the original cage next morning to continue the experiment.

The experiment continued 4 days. Repeated for 4 times.

Field mating

In late April, 2010, in the *Populus* in Luojiang County in Deyang, China, we observed mating *B. lineolata* on *R. multiflora* twigs from 8:00-20:00 every day. The observation lasted for 10 days.

Multiple mating

After the emergence, *B. lineolata* gathered on the feeding plant for extra nutrition, seeking partner and mating (Yan et al., 1997). This random gather will cause different mating ratios between male and female adults; as a result, mating number will be different.

In this experiment, *B. lineolata* adults from homochronous emergence was placed into mating cage (30 cm x 30 cm x 30 cm, stainless steel) with female-male ratio of 1:1, 2:2, 3:3, 4:4 and feed them by *R. multiflora* at room temperature (25 ± 2). Observe their behaviors from 8:00-20:00 every day. Observe for 4 days continuously and repeat the experiment for 3 times.

Statistical analysis

Statistical analysis was performed using the SPSS 10.0 statistical package (SPSS Inc., Chicago, IL). A one-factor randomized complete block analysis of variance (ANOVA) was conducted on the behavior analysis instrument data. Fisher`s protected least significant difference (LSD) multiple comparison procedure was used in the behavior analysis instrument of sexual recognition of *B.lineolata* adults. Independent sample *T* was used to check and compare the influences from the different mating experience of the females and males on mating behavior.

RESULT AND ANALYSIS

Sexual recognition

Encountering between a female and another female (Fig. 1-1,2,3,4): There is always certain distance between two females, and they will change their crawling direction to avoid each other after antennae or leg touching. It is rarely for them to stand against each other and fight.

Encountering between one male and another male (Fig. 2): in most cases, they will avoid contact with others (Fig. 2-2,3). In few cases, they will stand against each other and display their strength by friction sound between rear part of the prothorax and medithorax (Fig. 2-4). In extremly few cases, they will fight when the conflict is intensified. During the fighting, their antennas and legs will be bitten off by the opponent. Finally, the loser will flee (Fig. 2-1).

Encountering between a male and a female (Fig. 3): if the male has no intention to copulate, they will change their crawling direction to avoid each other (Fig. 3-1). Otherwise, it will follow the female and contact her body with his antennae. When he gets closer to the female, he will use his maxillary palps and labial palps to lick the pronotum and elytra of the female constantly, clasp and ride on her back and bend his abdomen. If the female defies, the mating cannot be made although the male has an obvious abdomen spasms, but if the female obeys, they can mate successfully (Fig. 3-2,3,4).

The result of sexual recognition behavior experiment indicated that intersection time for two tracks (F=61.70, P<0.01) and net relative movement (F=274.20, P<0.01) for a female and male are significantly longer than that for two females or two males. In above three groups, it is clear that reaction time for female-male group (F=147.89, P<0.01) is significantly shorter than that for the other two groups. But there is no significant difference in the velocity of three groups (F=1.05, P>0.05) (refer to Table. 1).

Mating behavior

The observation of mating behavior in the laboratory reveals that the entire mating process of *B. lineolata* includes three stages, i.e. Pair-bonding (male and female contact each other until the female agrees to mate), Mating (the genital of male mated with the genital of the female) and Post-copulatory guarding (after mating completion and the sepeartion of their genitals, their bodies still kept to be contacted). The average time required for a complete mating is 17.81 minutes, including average time of 2.16±0.015 (mean±SE) min for pair-bonding (n=121), 10.28±0.191 min for total mating (n=169), 23.4±0.059 s (n=955) for single mating and 5.37±0.035 min for post-copulatory guarding (n=121) (Table 2).

The observation of field mating behavior continued for 10 days. All mating behaviors were observed on host plant, including the average time of 3.69±0.04 min for pair-bonding, 32.54±0.564 min for total mating time (n=5), 65.62±1.423 s for single mating (n=5) and 15.73±0.342 min of the post-copulatory guarding (n=5) (Table 3).

Multiple mating

In the mating experiments with different sex ratios (Table 4), it was found that mating frequency fluctuated as the female and male ratio varied. When the sex ratio of female-male is 3:3, the frequency of mating can reach the maximum, i.e. female is 12.33±2.19 (mean±SE, range: 8-18 times) times, male is 18.00±2.85 (range: 18-5 times) times; while the number down to the minimum, i.e. female is 9.33±2.40 (range: 21-4 times) times, male is 9.07±0.88 (range: 20-6 times) times if the ratio is 4:4.

Influences from different mating experiences on mating behavior

If it is the first mating both for the female and male, the total time of mating (female: F=4.10, df=2, P<0.05; male: F=4.20, df=2, P<0.05) and post-copulatory guarding (female: F=2.59, df=2, P<0.05; male: F=11.47, df=2, P<0.05) shall be longer than that for those females or males who experienced previous mating(s). And mating number of the former shall be far more than that for the latter (female: F=5.23, df=2, P<0.05; male: F=4.92, df=2, P<0.05). But the pair-bonding for experienced females (F=14.66, df=2, P<0.05) or males (F=10.14, df=2, P<0.05) is far more than that for the males or females who take the first mating(Table 5).

Influences from different mating places on mating behavior

When *B. lineolata* mate on the host plant (F=5.10, df=4, P<0.05), the mating rate and duration time shall be higher than that for on the open ground (Table 6).

DISCUSSION

During the study on mating behavior of *B. lineolata*, many scholars regard the process from the insertion of internal sac to ejaculation completion as a complete mating process (Hanks, 1999; Zhang et al., 2006). In fact, the mating of longicorn beetle is a complicated process that is related to several stages before and after the mating (Yang et al., 2007). This study found that the complete mating process of *B. lineolata* included three stages: Pair-bonding, Mating and Post-copulatory guarding. And there is no obvious courtship. When a male meets a female, he will use the maxillary palpi and labial palpi to lick the pronotum and elytra of the female constantly, clasp and ride on her back and bend his abdomen. If the female obeys, the mating will be completed successfully. There are many factors which can affect the mating of *B. lineolata*, but the most important environmental factor is the temperature. When the temperature is low, the mating usually happens between 10:00-14:00; when it is high, it usually happens in the evening or at dawn (Yan et al., 1997).

It was discovered from the multiple mating test of *B. lineolata* that the male will choose some to mate when there are enough females for them. Xiang and Yang (2008) thought that the male may want to devote their limited resources to those females who have stronger reproduction abilities. When one female stays with more than 2 males or one male stays with more than 2 females, their mating capacities will be increased a lot. The result shows sex ratio can play a great influence upon the mating capacity of the female and male *B. lineolata*.

Generally, remote gather of *B. lineolata* adults will be achieved through attraction function of the host plant. During their habitation activity on the host plant, both female and male adults will approach for close gather caused by common taxis to micro habitat of host plants in specific areas. Mostly, when male *B. lineolata* meet females, they will crawl to the female promptly and make the mating. In mating activities, contact semiochemicals in female *B. lineolata* is very important for contact stimulation in males (Yang, 2008). During the mating process of *B. lineolata* adults, the direct contact will inspire the sexual excitation of the male to chase the female, clasp and ride on her back, bend his abdomen, and finally insert his internal sac to finish the mating. In this paper, it is indicated that the host plant not only plays an important role in the remote communication between different sexes of *B. lineolatas*, but also plays an obvious effect on the success rate of the mating. It is demonstrated by the study that the odor of host plant can strengthen the reactions of *Aphis glycines* (Matsumura), *Agrotis segetum* (Denis & Schiffermüller), *Trichoplus ni* (Hübner) and *Cyrtotrachelus buqueti* (F.) to the semiochemicals (Du et al., 1994; Hansson et al., 1989; Landolt et al., 1994; Yang et al., 2010). Further study is required to confirm the existence in *B. lineolata*.

During mating experiments of *B. lineolata*, many pair-bonding activities between males were observed. Usually males started pair-bonding with other males and tried to mate regardless of the sex. And the male who were pair-bonded would not defy but kept motionless, and the male who made pair-bonding would perform some mating behaviors such as abdomen spasms. During the experiment, it was seldom to find pair-bonding between females, which was only observed once. Pair-bonding with the same sex is also reported in the study on *M. alternatus* (Fauziah et al., 1987; Kim et al., 1992; Yang et al., 2007; Zhang et al., 2006). The reason for it needs further study.

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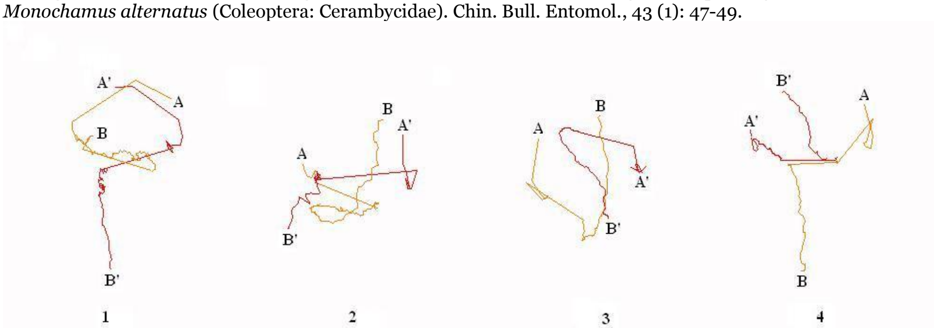

Figure 1. Encountering tracks of two females. 1-4. Behavior track acted unruly. Note: A-B and A'-B' are movement tracks of two *B. lineolata*, which starts from point A and A' and ends with point B and B'. Sic passim.

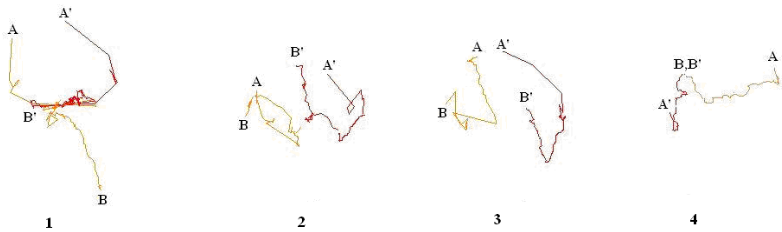

Figure 2. Encountering tracks of two males. 1.Two tracks contacted temperately, and then separated; 2-3. Parallel track; 4. Two tracks adjacency.

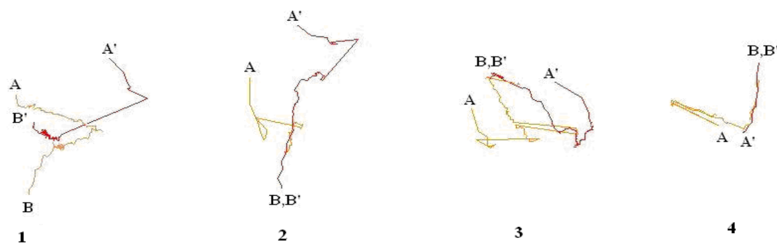

Figure 3. Encountering tracks of one female and one male. 1. Two tracks crossed and then separated; 2-4. Two tracks combined to be a line.

Table 1. Analysis upon sexual encountering behavior of *Batocera lineolata*.

	Intersection time for two tracks (s)	Velocity (cm/s)	Net Relative Movement (cm)	Reaction time (s)
Two Females	0.13±0.05B	9.78±0.56A	0.01±0.01C	1.68±0.03A
Two Males	0.10±0.06B	8.38±0.39A	0.08±0.01B	1.03±0.16B
One Female and One Male	2.50±0.24A	9.93±1.83A	0.28±0.14A	0.40±0.15C

Note: The data in the table indicate mean±SE, and different capital letters followed in the same line means the significant difference (P<0.05), ANOVA followed by LSD.

Table 2. Duration at each stage of mating behavior of *Batocera lineolata* in the laboratory.

	Number of Observations	Maximum	Minimum	Mean±*SE*
Pair-bonding (min)	121	16.72	0.05	2.16±0.015
Total time of mating (min)	169	82.85	0.12	10.28±0.191
Mating frequency	98	27	1	7.60±0.110
Duration of single mating (s)	955	257.00	2.00	23.40±0.059
Post-copulatory guarding (min)	121	86.52	0.03	5.37±0.035

Table 3. Duration at each stage of mating behavior of *Batocera lineolata* in the field.

	Number of Observations	Maximum	Minimum	Mean±SE
Pair-bonding (min)	5	7.25	0.13	3.69±0.040
Total time of mating (min)	5	60.82	4.25	32.54±0.564
Mating frequency	5	16	1	8.50±0.152
Duration of single mating (s)	5	127.00	4.24	65.62±1.423
Post-copulatory guarding (min)	5	31.23	0.22	15.73±0.342

Table 4. Mating number of *Batocera lineolata* at different sex ratios.

Sex ratio	Female			Male		
(♂:♀)	Mean±SE	Maximum	Minimum	mean±SE	Maximum	Minimum
1: 1	10.00±0.58B	10	8	10.00±0.33C	10	8
2: 2	11.67±0.33A	14	10	14.00±1.73B	23	1
3: 3	12.33±2.19A	18	8	18.00±2.85A	18	5
4: 4	9.33±2.40B	21	4	9.15±0.88D	20	4

Note: The data in the table refer to mean±SE, and different capital letters followed in the same line means obvious difference (P<0.05), ANOVA followed by LSD.

Table 5. Influences from different mating experiences on duration at each stage of the mating behavior of *Batocera lineolata* in the laboratory (mean±SE).

		Pair-bonding	Total time of mating	Post copulatory guarding	Mating frequency
Female	(n=16) First mating	0.64±0.08	17.76±2.14*	5.91±1.43*	9.60±0.45*
	(n=48) Mating again	2.23±0.30*	8.10±1.22	3.73±0.42	7.41±0.22
Male	(n=16) First mating	1.01±0.04	20.56±2.14*	7.34±2.41*	10.42±0.60*
	(n=48) Mating again	2.19±0.18*	6.49±0.89	3.88±1.32	7.54±0.20

*P<0.05 (t-test)

Table 6. Influence from different mating places on mating behavior of *Batocera lineolata* (mean±SE).

Mating Place	Mating Rate (%)	Minimum (min)	Maximum (min)	Mean±SE (min)
Host	67.30	6.00	52.00	33.29±2.94*
Open Ground	44.20	0.50	26.00	8.29±1.65

*P<0.05 (t-test)