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Courtship and Mating Behavior of the Chinese Chive Fly, *Bradysia odoriphaga* (Diptera: Sciaridae) and Evidence of Female Sex Pheromone

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ABSTRACT

The Chinese chive fly, *Bradysia odoriphaga* is a serious insect pest to damage vegetables, such as Chinese chives (*Allium tuberosum*) and mushrooms throughout China. To develop behavioral control method for adults of the pest, the observations on its courtship and mating behavioral activities were conducted under laboratory conditions. Virgin male flies performed obviously courtship which includes orienting, wings extending and vibrating, chasing, and curling abdomen before mating. However, female flies performed orienting, wings extending and up-down abdomen. Further, male flies performed chasing and mating more at night, and wing vibration more during the day. Courtship and mating mostly occurred within 3 days after emergence, and male courtship and mating activities declined significantly with age. The extracts of female body tails attracted male flies, in contrast, the extracts of male body tails did not attract females. Our results suggest that *B. odoriphaga* male performs obvious courtship behavior which may be evoked by the sex pheromone released by female flies. Our results may improve the program of integrated management of the pest by development of the use of sex pheromones in mating disruptions or trap-and-kill methods, to alternate the opposed one to employing insecticides in the soil to control larvae based on the understanding of the fly's courtship and mating behaviors.

Article Information

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Authors' Contribution

LZ designed the experiments and wrote the article. MKU performed the experimental work and collected data on courtship behaviour. X-WY performed T-maze bioassay and prepared Figure 1.

Key words

Bradysia odoriphaga,
Behavior, Courtship, Mating, Attract, Female tail extract

INTRODUCTION

The Diptera insects have developed diverse courtship and mating behaviours, which are the stereotypic sequence of behaviors evoked by the cues including visual, tactile, acoustic, and chemical stimuli (Ewing, 1983). Several reports have described Dipteran insects' courtship and mating behaviors (Benelli, 2014, 2015; Benelli *et al*., 2012; Carpita *et al*., 2012; Lees *et al*., 2014; Oliva *et al*., 2013, 2014). In *Drosophila*, the elements of courtship include the male orienting and tapping the female's body with his fore tarsi, and displaying mouthparts, forelegs, wing movements, and curling the tip of the abdomen (Wyatt, 2003). As an acceptance response, the female insect performs wing spreading and genital spreading to the male's tapping action.

For many insect species, the most important role for chemical communication is in courtship behavior (Wyatt, 2003). Because insect sexual behaviors are based on sex pheromones, these chemicals can be identified and used to manipulate insect behavior (Carde and Minks, 1995). In Diptera, sex pheromones play an important role in eliciting mating behavior (Casartel *et al*., 1971; McDowell *et al*., 1981; McKay and Hatchett, 1984; Nemoto *et al*., 1994; Uebel *et al*., 1975). Courtship behaviors of male flies to alkanes include wing fanning, ventral bending of the abdomen, and opening of claspers (Gotoh *et al*., 1999). In the genus *Bradysia*, female flies of *B. optata*, *B. difformis*, and *B. tilicola* are able to produce some sexual attractants (Frank and Dettner, 2008), while *B. impatiens* has been shown to produce a sex pheromone (Alberts *et al*., 1981; Liu *et al*., 2002).

The Chinese chive fly (*Bradysia odoriphaga*) is a serious Dipteran insect pest of Chinese chives (*Allium tuberosum*) and mushrooms (Hussey *et al*., 1969; Li *et al*., 2007; Yang and Zhang, 1985). Its larvae feed on the roots and stems of vegetables and cause serious economic damage. The most popular management method to date is the application of chemical pesticides into soil to control larvae, but this has low efficiency and the use of chemical insecticides is restricted in China (Fan and Cong, 2006; Gao *et al*., 2000). Therefore, it is necessary to develop an efficient and alternative method for controlling the pest. Both male and female adults of *B. odoriphaga* emerge from soil and mate on the ground. Therefore, as opposed

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to employing insecticides in the soil to control larvae, we suggest an alternate method targeting adults based on an understanding of the fly's courtship and mating behaviors and related sex pheromones. This may help to improve the program of integrated management of the pest by development of the use of sex pheromones in mating disruptions or trap-and-kill methods.

Present study was focused to describe in detail the elements of courtship and mating behaviors of the Chinese chive fly and provided an evidence of its sex pheromone. The results will help us to understand the courtship behavior of this species, as well as to develop behavioral control methods for insect pests.

MATERIALS AND METHODS

Insects

Chinese chive fly (*Bradysia odoriphaga*) pupae were collected from the Chinese chive field of ShunYi District in Beijing City, China 2013 and reared in the Key Laboratory of Biological Control of the Ministry of Agriculture, China Agricultural University (CAU). The insects were cultured in plastic Petri dishes (diameter: 8.5 cm, height: 1.5 cm) at 23±1ºC, 75±5% RH in incubators. The bottom of each dish was fitted with two pieces of round filter paper which were moistened with 3-4 ml of deionized water. One newly emerged both sexes adults (male and female) of *B. odoriphaga* were gently transferred into each Petri dish and 2 cm long stems of Chinese chive were provided as food into each Petri dish and were allowed to mate. After oviposition the eggs were transferred to a dark incubator at 12h: 12h (light: dark) photoperiodic regime to hatch according to the rearing method described by (Mu *et al*., 2003). Every two or three day's fresh chive stems and deionized water were provided to the larvae. Before adults emerged the female or male pupa were selected and transferred into new dishes separately for behavioral observation. Once adults emerged they were used for experiment immediately. The adults were provided with 10% honey water in cotton in each experiment.

Observation of courtship and mating behavior

For identification of courtship behavior, a behavioral assay on the virgins was conducted with five treatments, which were male flies only, female flies only, female and male pairs, male-male pairs and female-female pairs at room temperature (20±1ºC - 25±1ºC) and humidity (about 60% RH).

For observation of courtship and mating behavior, the activities of each individual or pair were directly observed in clean and numbered Petri dish for 30 min every 2 h for 1 daytime for examination of elements of courtship behavior, or 3 consecutive days for determination of rhythm of courtship and mating behavior. During the observation when dark periods, a red dim light was used, at room temperature (20±1ºC - 25±1ºC) and humidity (about 60% RH). Four replicates were in each treatment, and each replicate consisted of twenty pairs.

Besides direct observation on behavior, video camera was used to record the activities of virgin male and female flies. Newly emerged virgin male and female flies were introduced into a plastic Petri dish and then placed under a stereomicroscope (Optec, SMZ-B4, China) connected to a CCD digital camera (Lumenera's INFINITY2-2C-2.0 Megapixel color CCD Camera, Canada). Courtship activities and mating behavior was observed and recorded for 1 min.

T- maze bioassay

Ten newly emerged virgin male flies (or female flies) tails of abdomen were cut off and put in to a little glass tube, then immersed in mineral oil (140 µL) for 2 h, ground and filtrated similar to those described by (Frank and Dettner, 2008). The filtrated liquid was used for further behavioral experiments.

A T-maze device was made according to method described by (Zhang *et al*., 1996) for testing the effect of body extract. A piece of filter paper (0.38 cm^2) with insect extract liquid was placed in one arm of the device, while a control paper with mineral oil was placed in the other. One microliter of female or male extract liquid was placed on the filter paper, which was located at the same distance on each side from the middle. Then, a single virgin male or female was transferred from the entrance at the middle into the test arena. Insect behaviors were visually observed and recorded for 10 min.

Statistical analysis

For the observation on courtship behavior, the percentage of numbers of male flies performing elements of courtship behavior was calculated with the equation,

$$\% = \frac{\text{Number of male flies performing one element of courtship behavior}}{\text{Total No. of tested male flies}}$$

For T-maze olfactometer test, the percentage of number of flies responding to tail extract was calculated with the equation,

$$\% = \frac{\text{Number of male or female flies responding one side}}{\text{Total No. of tested male or female flies}}$$

And the data were analyzed by *t*-test (SPSS software, version 20.0) in three days consecutive observation and T-maze assays.

RESULTS

Courtship and mating behavior of Bradysia odoriphaga *adults*

In observation, male flies perform very obviously courtship. When paired with female flies, virgin male flies performed orientation, wing extension and vibration, chasing, and abdomen curling and trying to touch female's abdomen (Fig. 1A, Video data1). In contrast, female pairs, or males only, or female only performed orientation only, male pairs performed both orientation and wings extension. Therefore, wing vibration, chasing and tail curling are specific behaviors used in male courtship (Fig. 1B).

Male only, female only, or male-male and female-female pairs were not active when they were transferred to the test arena. In contrast, when a female-male pair was transferred into the arena, the male fly performed very actively.

The newly emerged male flies performed quick walking, as well as cleaning and rubbing the legs, head and antennae. When performed courtship behaviors the male fly oriented towards the female fly, extended and vibrated wings, cleaned the abdomen with hind legs, curled the abdomen and chased female with very quickly fanning wings. Unlike male, the newly emerged virgin female flies performed not very obvious courtship behavior. Their activities included orientation, abdomen up-down movement. They walked slowly, jumped, and cleaned antenna with forelegs.

Before commencing copulation, the male fly approached the female fly with wings vibrating and curling abdomen. The male moved around of the female, and attempted to mate with protruding genitalia. If the female fly rejected by kicking her hind legs, the male fly immediately moved away and kept his body backwards. If the female fly accepted him, she moved the tip of her abdomen slightly, and extruded the genitalia and ovipositors. During this period, the female fly lowered her antennae and body posture while remaining stationary. Once the female and male genitalia connected, the female's wings remained motionless covering her body and the male's wings remained extended without fanning until the end of mating. After mating, both sexes cleaned their mouthparts, heads, and antennae with the forelegs. The average mating duration was 10.5±1.37 min.

Rhythms of courtship and mating behaviors

Virgin male flies in a couple displayed differently within a single day (Fig. 2A). The mean number of individuals performing orientation and wing vibration showed similar pattern which maintains a high level but with no obvious peak. The trend of the mean number of individuals performing chasing and curling behaviors was similar to that of individuals performing mating, both were lowest at noon, increased gradually from 20:00 pm and reaching a peak from 02:00 am to 04:00 am. This indicates that the majority of the newly emerged adults mated at midnight, specifically from 22:00 to 04:00.

Within 72 h of a continuous observation, we found that male *B. odoriphaga* initiated immediately the courtship behavior and mating in the first day after emergence and reached a peak, then decreased gradually in the following 2 days (Fig. 2B). The mean number of flies performing all activities in the second or in the third days after emergence significantly decreased compared with those in the first day (*t*-test, $P<0.05$). There were significant difference between the mean number of orientation or wing extension and vibration of males in first day and second day, first day and third day, second and third. However, between the mean numbers of male flies performing chase and curl in second day and in third day there are no significant difference. So was the mean number of male flies performing mating events between the second and third day. After 3 days, most males were less active, and performed courtship and mating rarely.

Behavioral responses to female or male extracts in a T-maze test

Using a T-maze olfactometer, we tested possibility of presence of pheromones in the female body. When presented with female tail extract, most of male flies (73%) were significantly attracted to the extract (*t*-test, $P<0.001$). By contrast, the percentage of female flies (53%) responding to the extract showed no significant difference from that to the controls (Fig. 3A). In treatments with male tail extract, however, the percentage of female or male flies responding to the extract was not significantly different to that of male or female to the control (Fig. 3B).

DISCUSSION

In this paper the behavioral observations indicate that adult male performs in *B. odoriphaga* is more complex than that of female flies. It includes several elements, such as orientation, wing extension and vibration, chasing, curling abdomen, and tail touching. But significant elements of courtship behavior are wing vibration, chasing, abdomen curling and trying touching. Wing vibration in Dipteran species has been shown to have an important role in successful courtship and mating

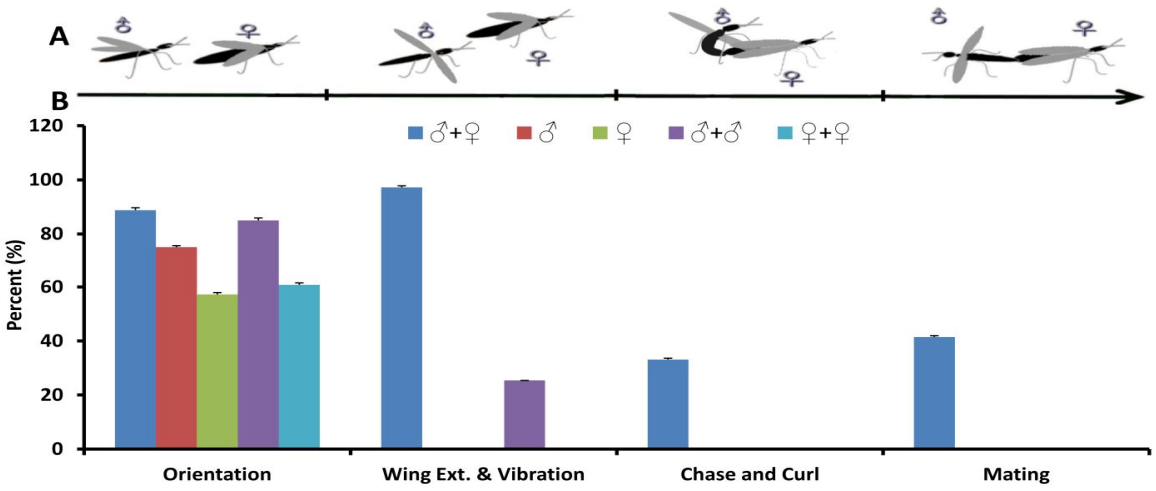

Fig. 1. Courtship and mating behavior of *B. odoriphaga*. A, the process of courtship and mating behaviours of both male and female in couple. B, a comparison between the performances of single virgin male (brown color box), singles virgin female (green color box), virgin male pair (purple color box), virgin female pair (light blue color box), and virgin male and female pair (dark blue color box) in arena.

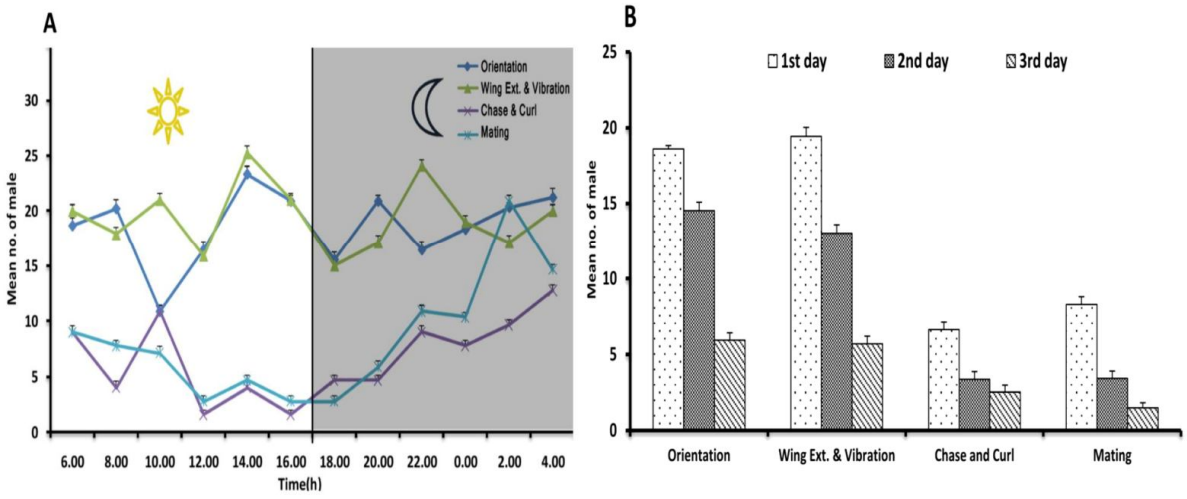

Fig. 2. Mean number of virgin male flies performing activities in couple with females within 24 h (A) and 72 h (B), the different upper characters on columns indicating significant difference at 0.05 level (*t*- test).

behavior of virgin male flies (Benelli *et al.*, 2012). *Bactrocera dorsalis* female flies are attracted to male flies whose wings vibrated, indicating that this behavior was a key signal in courtship activities (Poramarcom and Boake, 1991). Few studies, however, mentioned chasing and tail touching during courtship which may be the features of this species. Once male flies of the Chinese chive fly perform tail touching and are accepted, they may mate with their partners. Male mating behavior was highest at night in our study. It is possible that this is due to the rhythm of calling time of female flies who release their sex pheromone at higher amounts during that time. However, more study is required to support this hypothesis.

In 72-h observation period, we found that more flies performed orientation than mating. Orientation always occurs, even when male and female flies are alone, and this behavior has little association with mating. However, our observation indicated that wing vibration mostly occurs between couples of male and female flies. As stated above, several Dipteran species may use this as a key signal for courtship. Thus, wing vibrations appear to have a close relationship with mating behavior in the

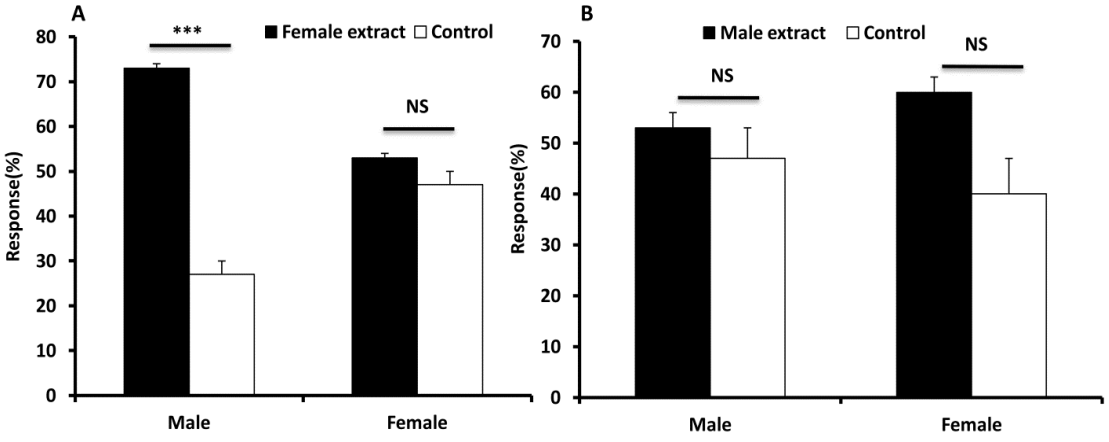

Fig. 3. One-day-old virgin male and female flies responding to female (A) or male (B) tail extract. The tests were carried out with 60 μL of extract and control (mineral oil); *** denotes significant difference (*t*-test, P<0.001); ns denotes no significance.

Chinese chive fly. However, our observations also showed that the number of flies performing wing vibrations was higher than that of flies performing mating.

These observations indicated that the number of male courtship activities decreased significantly with age over the 72-h period. This means that, on one hand, *B. odoriphaga* has a very short duration for courtship and mating. On the other hand, age is a key factor for the Chinese chive fly to successfully perform courtship and mating behaviors. Older female flies may release less sex pheromone and thus are not as attractive as newly virgin females to virgin male flies. Additionally, virgin male flies might spend or transfer more sperm than older male flies during mating (Partridge and Farquhar, 1983). This could likely explain why virgin male flies are accepted more frequently by virgin female flies for mating (Jones *et al*., 2000; Jones and Elgar, 2004).

Female insects release more sex pheromone and call virgin males for mating (Charlton and Carde, 1990; Girling and Carde, 2006), and male insects recognize the sex pheromone and then mate with females (Gotoh *et al*., 1999; McKay and Hatchett, 1984; Wicker-Thomas, 2007). In our experiments, male flies were significantly attracted to female tail extract, which implies that this part of the female fly may contain sex pheromones. However, whether different components of sex pheromone are responsible for different elements of courtship behaviors is interesting and warrants further study. Our study is the first report of courtship and mating behavior of the Chinese chive fly in detail. Therefore, our results may help to improve the program of integrated management of the pest by development of the use of sex pheromones in mating disruptions or trap-and-kill method, as the opposed one to employing insecticides in the soil to control larvae.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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