

# Mate choice and host discrimination behavior of the parasitoid *Trichogramma chilonis*

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## Abstract

*Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) is an important natural enemy of many species of lepidopterous pests and a widely used biological control agent. Detailed knowledge about its mate choice and host discrimination behavior is lacking. In this study, we studied the mate choice and host discrimination behavior of *T. chilonis* in experimental arenas through video tracking. Males' mate recognition capacity was realized by perceiving the sex pheromone of females. When offered two females of different species, male could distinguish the conspecific female from *Trichogrammatoidea bactrae* Nagaraja (Hymenoptera: Trichogrammatidae), a species that has overlapping hosts with *T. chilonis*. When placed with two females of different mating status, male preferred mating with the virgin female to the mated female. *T. chilonis* females could distinguish unparasitized host eggs from parasitized ones (parasitized by conspecific females or heterospecific females). They preferred to stay on and lay eggs in unparasitized host eggs. When *T. chilonis* females were only provided with parasitized host eggs (parasitized by *T. chilonis* and *T. bactrae* females), conspecific superparasitism occurred more often than heterospecific superparasitism. Furthermore, the host egg discrimination ability of *T. chilonis* females was mainly achieved through antennal perception.

**Keywords:** *Trichogramma chilonis*, mate choice, host discrimination, parasitoid, biological control

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## Introduction

Parasitoids in the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) are important natural enemies of many species of lepidopterous pests and are successfully used in inundative and inoculative biological control program in the world (Hassan, 1993; Smith, 1996). Mass rearing of *Trichogramma* is necessary during the inundative biological control program. Field fitness of *Trichogramma* wasps is related to the host egg searching and parasitic ability of females. Thus,

the study of their mate choice and host discrimination behavior is very crucial for mass rearing and releasing of these parasitoids in biological control program.

Mate finding is influenced by many factors, such as low population densities and their limited ability of dispersal (Hopper & Roush, 1993; Bellamy & Byrne, 2001). It is known that the chemical cues released by females are important to males in searching for suitable mates (Fauvergue *et al.*, 1995; Bernal & Luck, 2007). Pompanon *et al.* (1997) studied the substrate-borne pheromone released by *Trichogramma brassicae* Bezdenko females. This pheromone not only induces *T. brassicae* male searching in an area where virgin females had walked, but also attracts males from a short distance. Thus it may also increase mating opportunity of virgin females at their emergence sites (Pompanon *et al.*, 1997). In addition,

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this pheromone's emission by *T. brassicae* females is significantly affected by the mating status of females, since the naive males are indifferent to the areas where mated females have walked (Pompanon *et al.*, 1997). When exposed to conspecific and heterospecific pheromones, *Trichogramma semblidis* (Aurivillius) males present a clear preference to conspecific pheromones (Dupont *et al.*, 2010).

The response of a male to a female is related to his expected benefits and costs (Bonduriansky, 2001). *Trichogramma* are prospermatogenic species: males have all their spermatozooids mature at emergence and do not produce additional spermatozooids during their adult life (Boivin *et al.*, 2005; Damiens & Boivin, 2005). In order to maximize their expected fertilization success from each mating, males prefer to copulate with the higher quality females, which are associated with high fecundity or low sperm competition intensity (Bonduriansky, 2001). Damiens & Boivin (2006) reported that virgin *Trichogramma evanescens* Westwood females could store more than 50 sperms when mating first with a virgin male, and when mating with another male after that, the number of sperms contained in the spermathecae is not significantly increased. Thus, for *T. evanescens* males, selecting the virgin females seems very important for fathering more offspring during their short life span (5.7 days) (Lund, 1938).

Host discrimination is a capacity of female parasitoids to distinguish between parasitized and unparasitized hosts, it helps female parasitoids save their eggs and time, and reduce the mortality of their offspring (Bakker *et al.*, 1985; Ardeh *et al.*, 2005). For *Trichogramma* females, the host egg discrimination capacity is influenced by their oviposition experience: *Trichogramma* females which had no oviposition experience could not distinguish between parasitized and unparasitized host eggs; however, the experienced females clearly discriminate the parasitized host eggs from the unparasitized ones (Suzuki *et al.*, 1984; Miura *et al.*, 1994). Conspecific discrimination behavior means that a female recognizes hosts parasitized by females of the same species, and interspecific discrimination refers to a female ability to distinguish hosts parasitized by females of other species (Mackauer, 1990). Generally, females could discriminate a previously parasitized host egg because *Trichogramma* females imprint on their host twice during oviposition, first externally and then internally (Vinson, 1998). And the imprinting that serves as olfactory marks could be perceived by females through antennal drumming and/or ovipositor inserting (Salt, 1937; Le Ralec & Wajnberg, 1990).

*Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) has been used for controlling several lepidopterous pests on corn, cotton, rice and vegetables (Wührer & Hassan, 1993; Chang *et al.*, 2001; Ballal *et al.*, 2009; Preethe *et al.*, 2009). *Trichogrammatoidea bactrae* Nagaraja (Hymenoptera: Trichogrammatidae) is an important natural enemy of diamondback moth, *Plutella xylostella* (L.) (Wührer & Hassan, 1993; Vasquez *et al.*, 1997). Huang *et al.* (2002) have investigated the population dynamics of *T. chilonis* (= *Trichogramma confusum* Viggiani) and *T. bactrae* in the same field in Shenzhen (China) from 15 September 1999 to 15 March 2000, reported that both of these two species could parasitize the eggs of diamondback moth, and the population of *T. chilonis* is always significant larger than *T. bactrae* during the investigation period. In addition, these two *Trichogramma* species used in our experiments were collected from the parasitized diamondback moth egg masses from the same location. Therefore, it is highly likely that *T. chilonis* males have the

opportunity to encounter both *T. chilonis* and *T. bactrae* females at the emergence site. However, whether *T. chilonis* males rely on female sex pheromone for locating mates is unclear. The relationship between female species and mating status and male mate choice in *T. chilonis* is also unknown. In addition, host eggs which were parasitized by *T. bactrae* females may be encountered by *T. chilonis* females. There have been some studies on the oviposition behavior (Suzuki *et al.*, 1984; Mills & Kuhlmann, 2004) and host egg discrimination (Miura *et al.*, 1994; Delpuech & Leger, 2011; Delpuech & Delahaye, 2013) of *Trichogramma* species. However, there is no report on the interspecific host egg discrimination in *T. chilonis*.

The objectives of this study were to investigate: (1) whether *T. chilonis* males rely on female sex pheromone for locating mates; (2) mate choice behavior of *T. chilonis* males; and (3) host egg discrimination behavior of *T. chilonis* females.

## Materials and methods

### Insects

*T. chilonis* and *T. bactrae* populations originated from parasitized diamondback moth egg masses (about 200 eggs) collected in April 2009 from cabbage (*Brassica oleracea* L.) fields near Guangzhou, China. The stock populations were reared on eggs of *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) at 25 ± 1°C, 80 ± 5% relative humidity (RH) and a 14:10 (L:D) photoperiod in the laboratory, except some of *T. chilonis* which used in male mate choice experiment were reared at 18 ± 1°C, 80 ± 5% RH and a 14:10 (L:D) photoperiod. *C. cephalonica* was reared following the procedure described in Wang *et al.* (2012). Eggs of *C. cephalonica* were collected daily, and were stored at 4°C. The host eggs were killed by UV radiation before parasitoids rearing and experiments. Adults of *T. chilonis* and *T. bactrae* were fed with 25% honey–water solution.

To obtain virgin *T. chilonis* and *T. bactrae* adults, parasitized *C. cephalonica* eggs were placed individually in glass vials (10 mm internal diameter, 55 mm internal height; one egg per vial) the day before emergence. After emergence, males and females were sexed and fed with 25% honey–water solution before experiment. Parasitoids used in all experiments were less than 24 h old. All experiments were carried out at 25 ± 1°C, 60 ± 5% RH from 12:00 to 18:00 h and experimental arenas were lighted with a circular LED lamp (400 lux, Micro-shot Technology Ltd., Guangzhou, China) in the laboratory.

### Sex pheromone perception by males

Perception of sex pheromone by *T. chilonis* males was recorded following the approach by Dupont *et al.* (2010). Cylindrical arena (30 mm diameter, 1 mm height) that was made by drilling a 30 mm diameter hole on a Plexiglas sheet (50 × 50 × 1 mm<sup>3</sup>) was used as experimental arena. Both of its top and bottom were covered with glass sheets. Each arena was divided into two equal parts by a silicon bar (chemically inert). Two females of same age (a virgin *T. chilonis* female and a virgin *T. bactrae* female; or a virgin and a mated *T. chilonis* female) were introduced into each arena (one female per half of the arena). The mated females were obtained 1 h before experiment. Each female was allowed to walk on half of the arena for 5 min to ensure the pheromone marking of the entire half of the arena (Delpuech *et al.*, 1998; Dupont *et al.*, 2010). Then, both females and the silicon bar were removed. A virgin

male was introduced into each arena by a glass sheet, and its walking path during 5 min was recorded with a digital camera (Model CANON G10, Canon Inc., Tokyo, Japan). Each treatment was repeated 29 or 30 times. The positions of two halves of the arena which were marked by female pheromone were switched after each observation. Time spent by male on each half of the arena was calculated.

#### Male mate choice

To determine whether male *T. chilonis* discriminate between females of different species or mating status, glass vials (10 mm internal diameter, 55 mm internal height; closed with cotton balls) were used as experimental arenas. Two experiments were carried out: in experiment 1, a virgin male was placed with a virgin *T. chilonis* female and a virgin *T. bactrae* female; in experiment 2, a virgin male was placed with a virgin *T. chilonis* female and a mated *T. chilonis* female. Females were introduced first in a random order, and then a male was introduced right after. Observation started as soon as the male was introduced in the glass vial and ended after the first mating happened, or after 10 min if no mating occurred. The mated females were obtained 1 h before experiment by holding a virgin female with a virgin male in a glass vial until copulation was observed. Each female combination was replicated 50 times.

*T. chilonis* females have different morphological characteristics when they are reared at different temperatures: females reared at 15–20°C have brown metascutellum; however, the ones reared at 25°C have their abdomens in brown, and there is a dull yellow transverse region on the abdomen (Lin, 1994). In experiment 1, the parasitoids were reared (rearing method was same as above) at two different conditions in the laboratory, one part of them at 25 ± 1°C, 80 ± 5% RH and a 14:10 (L:D) photoperiod, the other part of them at 18 ± 1°C, 80 ± 5% RH and a 14:10 (L:D) photoperiod. And we could identify them easily with the naked eye during study: the ones reared at 18 ± 1°C were darker than the ones reared at 25 ± 1°C. The mating status of these two kinds of *T. chilonis* females were distributed randomly. Our preliminary experiment indicated that females mating behavior were not affected by their colors. *T. bactrae* females can be distinguished easily from *T. chilonis* females with the naked eye: the former were darker than the latter, both of them were reared at 25 ± 1°C, 80 ± 5% RH and a 14:10 (L:D) photoperiod.

#### Host egg discrimination

The host (*C. cephalonica*) egg discrimination ability by experienced and inexperienced *T. chilonis* females was tested separately in this experiment. Females used in this experiment were fertilized. Each 'Experienced' *T. chilonis* female had one oviposition experience on a host egg 1 h earlier. 'Inexperienced' females had no prior experience of oviposition on host eggs. An egg sheet was provided to each *T. chilonis* female. On each sheet, two groups of six eggs were glued onto cardboard strip. Eggs with the same parasitic status (unparasitized or parasitized by the same species of parasitoids) were glued next to each other (glued in the same group). The two groups of host eggs which have different parasitic status were approximately 1 mm apart. Within each group, each two adjacent eggs were also approximately 1 mm apart (fig. 1). Three different combinations of eggs were used: (1) six unparasitized eggs and six eggs parasitized by *T. chilonis*; (2) six unparasitized eggs and six eggs parasitized by *T. bactrae*; (3) six eggs parasitized by *T.*

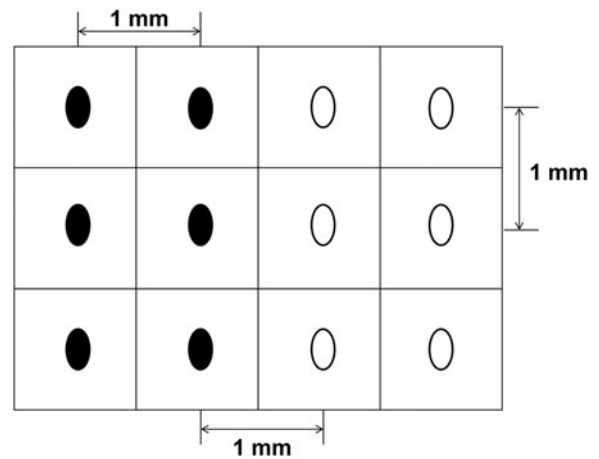


Fig. 1. The mode of host eggs arrangement. Each oval indicates a host egg. Different colors (black and white) denote the different parasitic status of host eggs.

*chilonis* and six eggs parasitized by *T. bactrae*. Both the parasitized and unparasitized eggs were the same age (48 h after being laid by *C. cephalonica*). The first two combinations were replicated 20 times. The third combination was replicated 12 times. The positions of two groups of eggs in experimental arena were switched after each observation. The parasitized host eggs were obtained before the experiment by leaving about 400 *C. cephalonica* eggs with 100 *T. chilonis* or *T. bactrae* females in a glass tube (23 mm internal diameter, 74 mm internal height) for 12 h until the use of eggs for the experiment (preliminary experiments showed that the percentage of host eggs' parasitism by *T. chilonis* and *T. bactrae* were 71.2 and 87.7%, respectively).

The cylindrical arena (12 mm diameter and 3 mm height) was used as experimental arena. Both the top and bottom were covered with glass sheets (fig. 2). For our experiment, the egg sheet was placed in the center of the experimental arena, and then a mated female (experienced or inexperienced) was introduced by a piece of glass sheet. The female's behavior was immediately video-recorded for 15 min. In this experiment, the digital camera used for recording female's behavior was connected to a dissecting microscope (Zeiss Stemi 2000-CS, Shanghai, China) (fig. 2). Antennal rejection was recorded when a female encountered a host egg, examined its surface with her antennae and moved to another host without parasitizing it. Ovipositor rejection was recorded when a female drilled a host egg but left without laying an egg (Delpuech & Leger, 2011; Delpuech & Delahaye, 2013).

#### Statistical analysis

The male mate choice data were compared using chi-square ( $\chi^2$ ) test. Paired samples *t*-test were used to analyze the percentage of time spent by *T. chilonis* males on either half of the arena in the sex pheromone perception experiments, and the percentage of time spent by *T. chilonis* females on parasitized and unparasitized host eggs. Wilcoxon signed-rank test was used to compare the *T. chilonis* female choice (percentage of host eggs parasitized) between parasitized and unparasitized host eggs, and the difference between antennal and ovipositor rejection by *T. chilonis* females on parasitized host eggs.

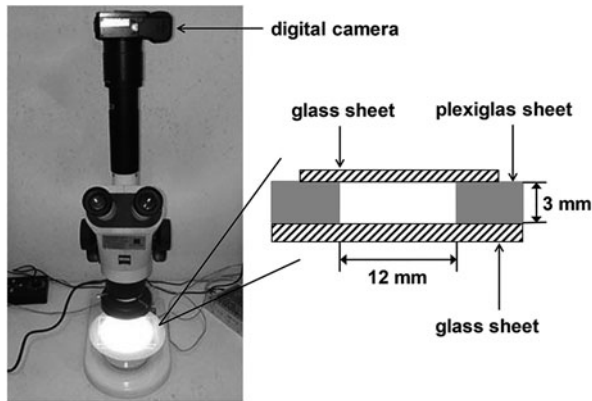


Fig. 2. Side view of the experimental arena and digital camera used in the experiment of host egg discrimination.

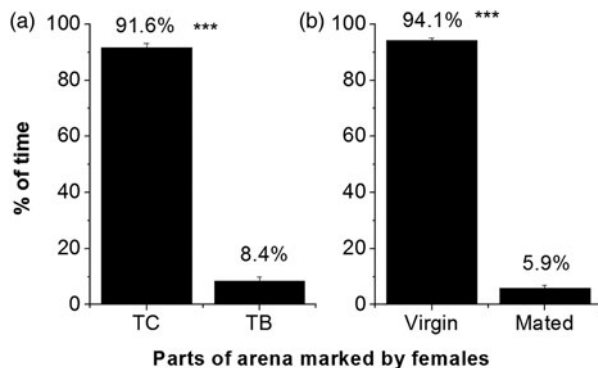


Fig. 3. Mean percentages ( $\pm$ SE) of time spent by *Trichogramma chilonis* males on part of the arena marked by sex pheromone from females. (a) Arena marked by virgin *T. chilonis* female (TC) and virgin *T. batrae* female (TB); (b) arena marked by virgin *T. chilonis* female and mated *T. chilonis* female.

In the host egg discrimination experiments, the difference between experienced and inexperienced *T. chilonis* females in measured variables were compared using independent samples *t*-test for analyzing the percentage of time spent on parasitized or unparasitized host eggs, and Mann–Whitney *U* test for analyzing the differences in the percentage of host eggs parasitized by experienced and inexperienced wasps, and the antennal or ovipositor rejection on parasitized host eggs. All statistical analyses were conducted using SPSS version 13.0 (SPSS, Chicago, IL, USA).

## Results

### Sex pheromone perception by males

In the first experiment, *T. chilonis* males spent significantly more time on the half of the arena marked by conspecific females than the half marked by *T. batrae* females ( $t = 28.3$ ,  $df = 29$ ,  $P < 0.001$ ) (fig. 3a). In the second experiment, *T. chilonis* males spent significantly more time on the half marked by virgin *T. chilonis* females than the half marked by mated *T. chilonis* females ( $t = 43.2$ ,  $df = 29$ ,  $P < 0.001$ ) (fig. 3b).

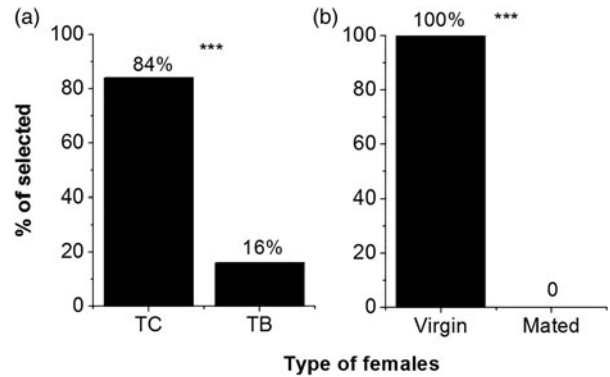


Fig. 4. Percentage of females chosen by *Trichogramma chilonis* males. (a) Virgin *T. chilonis* female (TC) and virgin *T. batrae* female (TB). (b) virgin *T. chilonis* female and mated *T. chilonis* female.

### Male mate choice

When exposed to virgin females of two different species, *T. chilonis* males significantly preferred conspecific females to heterospecific females ( $\chi^2 = 23.1$ ,  $df = 1$ ,  $P < 0.001$ ) (fig. 4a). When exposed to two *T. chilonis* females of different mating status, all of the *T. chilonis* males chose to mate with the virgin female (fig. 4b).

### Host egg discrimination

There are no significant differences between experienced and inexperienced females in all measured variables (mean time spent and number of eggs parasitized by *T. chilonis* females on each group of host eggs, and antennal and ovipositor rejection rate by *T. chilonis* females on parasitized host eggs; all  $P > 0.05$ ); thus the results of experienced and inexperienced females were combined. *T. chilonis* females spent significantly more time ( $t = -13.0$ ,  $df = 39$ ,  $P < 0.001$ , fig. 5a;  $t = -9.9$ ,  $df = 39$ ,  $P < 0.001$ , fig. 5b) and parasitized more eggs ( $Z = -5.4$ ,  $P < 0.001$ , fig. 6a;  $Z = -5.3$ ,  $P < 0.001$ , fig. 6b) on unparasitized host eggs than on parasitized ones regardless of the host eggs combinations. When exposed to the two groups of parasitized host eggs, females significantly preferred to stay on ( $t = 11.6$ ,  $df = 23$ ,  $P < 0.001$ , fig. 5c) and parasitize ( $Z = -4.1$ ,  $P < 0.001$ , fig. 6c) the host eggs which have been parasitized by their conspecific females.

*T. chilonis* females made significantly more antennal rejections than ovipositor rejections on the parasitized host eggs ( $Z = -5.0$ ,  $P < 0.001$ , fig. 7a;  $Z = -4.7$ ,  $P < 0.001$ , fig. 7b).

## Discussion

Sex pheromones are usually used by male insects to locate and select females (Bonduriansky, 2001). In our studies, *T. chilonis* males tend to restrict their walking in the area where their conspecific females or virgin females have been walking. Dupont *et al.* (2010) confirmed that pheromone specificity exists between *T. semblidis* and *T. evanescens*: males of each species tend to walk on the part of the arena visited by their conspecific females. In *Aphidius ervi* Haliday (McClure *et al.*, 2007) and *Trichogramma turkestanica* Meyer (Martel *et al.*, 2008), males strongly discriminate between virgin and

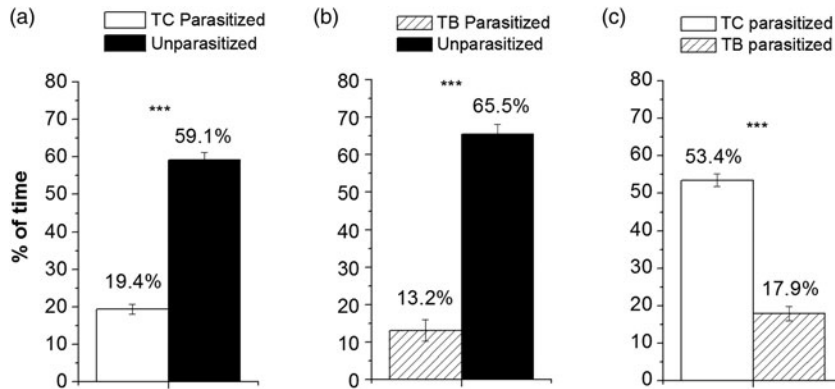


Fig. 5. Mean percentage ( $\pm$ SE) of time spent by *Trichogramma chilonis* females on each group of host eggs. Host eggs combinations are: (a) six *T. chilonis* (TC) parasitized and six unparasitized; (b) six *T. bactrae* (TB) parasitized and six unparasitized; (c) six *T. chilonis* (TC) parasitized and six *T. bactrae* (TB) parasitized.

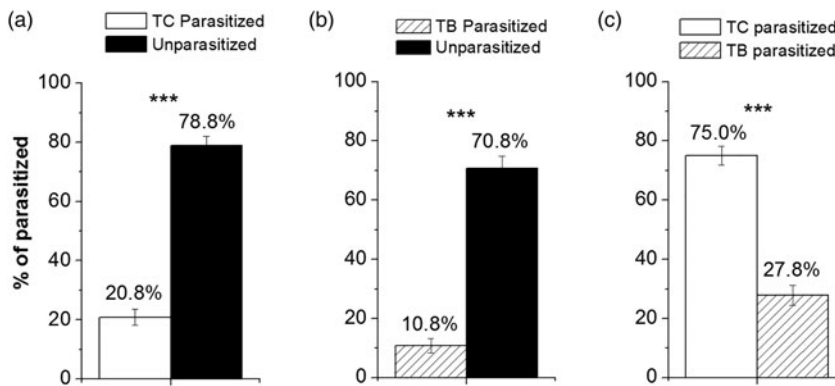


Fig. 6. Mean percentage ( $\pm$ SE) of eggs parasitized by *Trichogramma chilonis* females on each group of host eggs. Host eggs combinations are: (a) six *T. chilonis* (TC) parasitized and six unparasitized; (b) six *T. bactrae* (TB) parasitized and six unparasitized; (c) six *T. chilonis* (TC) parasitized and six *T. bactrae* (TB) parasitized.

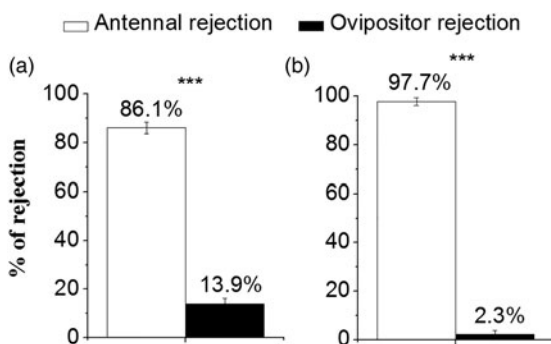


Fig. 7. Mean percentage ( $\pm$ SE) of antennal and ovipositor rejection by *Trichogramma chilonis* females on parasitized host eggs. (a) *T. chilonis* parasitized, n = 34; (b) *T. bactrae* parasitized, n = 24.

mated females. This phenomenon is due to a post-mating decrease of the sex pheromone, as observed for other parasitoids (McNeil & Brodeur, 1995; Pompanon *et al.*, 1997). In *Trichogramma*, sex pheromones are released by females (Pompanon *et al.*, 1997). They are composed of two putative

compounds in *T. turkestanica* (Van Beek *et al.*, 2005). In *T. chilonis*, the composition of its sex pheromone is still unknown and needs to be further studied.

To maximize the fertilization success during each mating, *T. chilonis* males preferred to copulate with conspecific and virgin females. Interspecific mating decreases the fecundity of females and makes males suffer an important loss of time and fertilization opportunity (Stouthamer *et al.*, 2000; Delpuech *et al.*, 2010). Although females which copulate with males from a close relative species could be inseminated and use the sperm to fertilize their eggs, the fertilized eggs would not survive (Stouthamer *et al.*, 2000; Delpuech *et al.*, 2010). Copulating with a virgin female could father more progeny during the female's first bout of oviposition (Polak *et al.*, 2001). Thus, it would be detrimental for males to copulate with mated females, since the number of offspring fathered by non-discriminating males would be reduced (Simmons & Siva-Jothy, 1998; Damiens & Boivin, 2006). In our experiment, the mated *T. chilonis* females were obtained 1 h before the experiment. However, the mated females would be varying in their post-mating durations in the field. It is regretful that mated *T. chilonis* females with different post-mating duration were not studied in male mate choice experiment. In our recent

experiment examining male mate choice in the common bed bug, *Cimex lectularius* L. (Hemiptera: Cimicidae), we found the virgin males could discriminate between virgin females and females mated 1 h, 1 or 3 days earlier; however, they could not discriminate between virgin females and females mated 7 days earlier (Wang *et al.*, unpublished data). Therefore, whether the different post-mating duration affects male mate choice behavior in *T. chilonis* need to be further studied. In our experiment, copulation was the only measurement of male mate choice. However, Martel & Boivin (2011) reported that other factors, such as female rejection and mate competition also affect male's mate selection. Thus, whether these factors affect male mate choice behavior should be further studied.

Recognition of unparasitized hosts from the parasitized ones is an important ability of parasitoid females. It allows parasitoid to avoid involuntary superparasitism. Suzuki *et al.* (1984) reported that inexperienced *T. chilonis* females cannot discriminate unparasitized *Papilio xuthus* L. eggs from the parasitized ones. In our study, both experienced and inexperienced *T. chilonis* females preferred to spend time on and oviposit in the unparasitized host eggs. However, the 'inexperienced females' used in our study were not the real inexperienced ones. In our study, each inexperienced female was supplied twelve host eggs (six unparasitized and six parasitized eggs, or 12 parasitized eggs); in this way if the first oviposition happened, the inexperienced females would become the experienced ones. Thus, no significant difference of host discrimination behavior between experienced females and the 'inexperienced' ones. In our experiment, the parasitized host eggs were obtained 0–12 h before experiment; however, in the field, the parasitized eggs would be in any conditions (with different post-parasitization duration) when they were encountered by parasitoids. The time of the previous oviposition (post-parasitization duration) plays an important role in the success of the second oviposition (superparasitism). If both eggs were laid 'simultaneously', both of them would have the equal chances to survival. Miura *et al.* (1994) reported that the survival rate of offspring laid by a second female *T. chilonis* in the host egg within 10 min is almost equal as that the first female. As time passed, eggs laid by the previous females would have developed, the response of *T. chilonis* females to the parasitized eggs would be likely to gradual change. Tena *et al.* (2008) reported that *Metaphycus flavus* (Howard) females often oviposit on parasitized hosts after killing some eggs and larvae laid by themselves or others when they superparasitized. However, whether this infanticide behavior existed in *T. chilonis* is still unclear. The effect of post-parasitization duration on female host discrimination behavior in *T. chilonis* should be further studied. In addition, Miura *et al.* (1994) reported that female host discrimination of *T. chilonis* is time-dependent, with greater acceptance of parasitized eggs after 24 h of no exposure to host eggs. In our experiment, we only studied the host discrimination of experienced females after 1 h of no exposure to host eggs. Thus, further studies should be carried out on the host discrimination behavior of experienced females with different post-oviposition duration.

When the *T. chilonis* females had a choice between host eggs parasitized by conspecific females and eggs parasitized by heterospecific parasitoids (*T. bactrae*), they preferred to stay on and oviposit in the host eggs which were parasitized by conspecific females. This interspecific host discrimination behavior was rarely observed (Bakker *et al.*, 1985; Van Baaren *et al.*, 1994). It has been found mostly when one of

the species has a marked competitive advantage (McBrien & Mackauer, 1991; Scholz & Höller, 1992). In our experiment, we only studied the host discrimination behavior of *T. chilonis*. The host discrimination behavior of *T. bactrae* was not researched, thus whether *T. bactrae* had a competitive advantage over *T. chilonis* was unclear. Our preliminary experiment indicated that only one *T. chilonis* adult could successfully emerge from a *C. cephalonica* egg; therefore, this conspecific superparasitization of *T. chilonis* would be harmful to their offspring's development. However, this superparasitism could have selective advantages under specific circumstances, such as when the number of unparasitized host eggs is limited, and if the offspring of the second female has high chances of winning the competition against the first larva (Bakker *et al.*, 1985). In addition, when a parasitoid finds an egg patch in which most eggs have been parasitized, it might be more favorable for the parasitoid to superparasitize on the parasitized eggs than to leave the egg patch and search for unparasitized host eggs (Bakker *et al.*, 1985).

In the host discrimination experiment, we could not assess the reliability of the parasitization of all the hosts prior to the recording of the oviposition behavior of *T. chilonis*. Since 71.2 and 87.7% of hosts are usually parasitized by *T. chilonis* and *T. bactrae* through our procedure, it is safe to say that the majority of eggs identified as parasitized were indeed parasitized. Since a lesser degree of parasitism of the 'parasitized eggs' would have led to a higher acceptance by the test female, our results confirm that this methodological limitation was not problematic.

*Trichogramma* females can perceive the host marking pheromone deposited on the host egg surface with their antennae (Salt, 1937). They can also detect the pheromone inside the host eggs by their ovipositor (Le Ralec & Wajnberg, 1990). Our results showed that *T. chilonis* females made significantly more antennal rejections than ovipositor rejections on the parasitized host eggs. Thus, its host discrimination ability was mainly achieved by antennal perception. However, whether the egg rejection pattern of *T. chilonis* females would be affected by their post-oviposition duration and host eggs' post-parasitization duration should be further studied.

In summary, we found *T. chilonis* males could discriminate their mates accurately, and it was related to their ability to perceive sex pheromone. For *T. chilonis* females, they mainly used their antennae to detect the condition of host eggs. These behavior studies were necessary for mass rearing and releasing of *T. chilonis* in biological control program. However, in order to maximize the success of inundative release of *T. chilonis* in the field, many further studies should be carried out. Under field conditions, many factors such as ambient temperature, humidity and insecticide residues can influence the oviposition behavior and parasitic ability of *Trichogramma* (Delpuech & Leger, 2011; Wang *et al.*, 2012; Delpuech & Delahaye, 2013; Wang *et al.*, 2014). Therefore, further investigations evaluating the effects of environmental factors on the mate choice and host discrimination behavior of *T. chilonis* would be beneficial for biological control programs using *Trichogramma* species.

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