Bulletin of Entomological Research (2017) **107**, 812–819
doi:10.1017/S0007485317000335

Number of attacks by *Trichogramma dendrolimi* (Hymenoptera: Trichogrammatidae) affects the successful parasitism of *Ostrinia furnacalis* (Lepidoptera: Crambidae) eggs

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Abstract

In laboratories, the parasitism rate of *Ostrinia furnacalis* (Güenée) (Lepidoptera: Crambidae) eggs by *Trichogramma dendrolimi* Matsumura (Hymenoptera: Trichogrammatidae) is low; however, efforts to control *O. furnacalis* with *T. dendrolimi* in the field have been successful. In this study, the effects of the number of attacks by *T. dendrolimi* against *O. furnacalis* eggs and diet of *O. furnacalis* larva on wasp development were investigated. The results indicated that more attacks increased significantly not only the successful parasitism rate of *O. furnacalis* eggs by *T. dendrolimi*, but also the percentage of host eggs that failed to develop into either *O. furnacalis* larvae or *T. dendrolimi*. Both the size and female proportion of *T. dendrolimi* offspring decreased as the number of attacks increased. The number of *T. dendrolimi* eggs laid in per host egg increased significantly as the ratio of wasps to host eggs increased from 1:5 to 3:5. Host diet also significantly affected the developmental time of immaturity and the emergence rate of adults of *T. dendrolimi*. These results illustrate how inundative releases of *T. dendrolimi* can successfully control *O. furnacalis* despite the fact that pest parasitism by the subsequent wasp generation decreases sharply in the field. The suitability of *O. furnacalis* eggs to *T. dendrolimi* and the superparasitism effects on offspring of *T. dendrolimi* are discussed.

Keywords: *Trichogramma dendrolimi*, *Ostrinia furnacalis*, inundative release, attack number, successful parasitism, size, female proportion

(Accepted 13 March 2017; First published online 11 April 2017)

Introduction

The successful parasitization is related primarily to the suitability of a given host for the parasitoid, where, as first defined by Salt (1938), a suitable host is defined as one on which the parasitoid can generally produce fertile offspring. Later, Doutt (1959) proposed that two steps, host acceptance and parasitoid offspring development in the host, better reflect host suitability to a given parasitoid. Vinson (1976) and Vinson & Iwantsch (1980) reviewed the suitability of hosts by analyzing host acceptance and the development of parasitoid progeny, respectively. Larval parasitoids do not have enough time to determine the host quality when they encounter a strong response from host larva. In contrast, as egg parasitoids, *Trichogramma* have sufficient time to evaluate the host suitability before they oviposit in the host egg. The examination of suitability of a putative host by a *Trichogramma* wasp occurs at two steps: external examination with appendages including antenna, ovipositor and legs and internal

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examination with ovipositor. The suitability of host eggs affects the oviposition preferences of female *Trichogramma*, which may refuse to insert her ovipositor into unsuitable host eggs. Moreover, one ovipositor pierce does not necessarily result in an egg laid in a non-suitable host egg (Pavlík, 1993; Babendreier *et al.*, 2003). Studies have indicated that an experienced *Trichogramma* will generally refuse to oviposit in unsuitable host eggs such as eggs previously parasitized by itself or other individuals (van Dijken & Waage, 1987; Liu & He, 1991; Wang *et al.*, 2016), eggs in which the host embryo has developed past a certain stage (Ruberson & Kring, 1993; Godin & Boivin, 2000; Pizzol *et al.*, 2012), or eggs that are non-suitable at all (Babendreier *et al.*, 2003). However, superparasitism can occur when an inexperienced *Trichogramma* encounters a host even if that host has already been parasitized (Liu & He, 1991; Miura *et al.*, 1994; Wang *et al.*, 2016), or when host is in shortage (van Lanteren & Bakker, 1975; Narendran, 1985; Corrigan *et al.*, 1995).

The nutrient component of host eggs affects the development of immature *Trichogramma* (Nettles *et al.*, 1983; Barrett & Schmidt, 1991; Li *et al.*, 2008; Dias *et al.*, 2010). When heavy superparasitism occurs, nutrition competition can result in numerous detrimental fitness costs for the parasitoid offspring. Consequently, the number of *Trichogramma* eggs laid in a given host egg will affect the development, emergence, and even sex ratio of the wasp offspring (Klomp & Teerink, 1978; Grenier *et al.*, 2001; Boivin & Martel, 2012).

Corn is widely grown around the world. The Asian corn borer, *Ostrinia furnacalis* (Güenée) (Lepidoptera: Crambidae) is a serious corn pest that can cause heavy crop yield losses (Wang *et al.*, 2014). *Trichogramma ostriniae* Pang et Chen and *Trichogramma dendrolimi* Matsumura, two native *Trichogramma* species, have been released to control *O. furnacalis* in East Asia, especially in China (Wang *et al.*, 2014). Because the mass rearing of *T. dendrolimi* on large host eggs (such as those of *Antheraea pernyi* Guérin-Mèneville, which are easy to obtain en masse) is substantially more cost-effective that mass rearing of *T. ostriniae*, which can be reared only on small host eggs such as those of *Corcyra cephalonica* (Stainton), the former is mostly used as a biological agent to control the Asian corn borer in North-eastern China (Wang *et al.*, 2014). However, in laboratories, the percentage of female *T. dendrolimi* parasitized *O. furnacalis* eggs ranged within low rates (from 3.28% (Li *et al.*, 2002) to 30% (Liu *et al.*, 1998)), compared with that of nearly 100% by *T. ostriniae* (Li *et al.*, 2002; Zhang *et al.*, 2010). The data of field surveys in several Chinese provinces also indicated that *T. ostriniae* is the dominant egg parasitoid on *O. furnacalis* eggs (Zhang *et al.*, 1979, 1990). However, the parasitism rate of *O. furnacalis* eggs by *T. dendrolimi* reached >80% after inundative releases (30,000 wasps/667 m^2) (Feng, 1996). Later data of field surveys indicated that the parasitism rate of the subsequent (post-release) generation of *O. furnacalis* by *T. dendrolimi* decreased to <8% unless supplemented by continued releases of the parasitoid; without such releases, the dominant egg parasitoid of *O. furnacalis* switched to *T. ostriniae* (Zhang *et al.*, 1979, 1990; Feng, 1996). These results raise questions about what caused the failure of the laboratory results to conform to the field trial results and why the initial successful parasitism at release immediately drop off in subsequent generations.

Here, we hypothesize that the low rate of successful parasitism of *O. furnacalis* eggs by *T. dendrolimi* may have occurred because fewer to no wasp eggs were laid into each host egg resulting from low host suitability. A high ratio of wasps to host eggs results in high number of parasitoid encounters with the same host, increasing the probability of superparasitism and, thus, increasing the initial success rate of parasitoids in the field; however, the high ratio also results in the production of low-quality offspring, which, along with the low suitability of *O. furnacalis* eggs, contributes to a sharp decline in parasitism of the pest by the wasp of following generation. Thus, we investigated the relationship between the number of attacks and the number of parasitoid eggs oviposited into host eggs and the effect of the number of attacks on the successful parasitism rate of *O. furnacalis* eggs by *T. dendrolimi*. Furthermore, the *O. furnacalis* eggs used in laboratory are predominantly laid by adults that emerged from larvae fed on artificial food, whereas, the eggs in field are laid by adults emerged from larvae fed on corn. Several studies reported that host food affected the parasitization preference or bionomics of *Trichogramma* (Song *et al.*, 1997; Nathan *et al.*, 2006). Thus, we also investigated the effects of diet (plant vs. artificial) during host rearing. Additionally, to assess the effect of the quantity of *Trichogramma* released to control *O. furnacalis*, we investigated the number of parasitoid eggs laid per host egg under different ratios of parasitoids to hosts.

Materials and methods

Insect cultures

O. furnacalis larvae were collected from a corn field in the suburb of Nanjing, Jiangsu Province, China, in 2010 and separated into two populations that were fed on either corn (abbreviated as OfC) or a modified artificial diet (Zhou *et al.*, 1980) (abbreviated as OfA) under the following laboratory conditions: 25 ± 2°C, 70 ± 10% relative humidity (RH) with a photoperiod of 14:10 (L:D). For each population, pupae were collected and placed inside a cage (25 cm × 25 cm × 40 cm), the top of which was covered with an iron screen of 1 cm × 1 cm mesh. A piece of tracing paper was placed on top of the cage to collect *O. furnacalis* eggs, and the paper was replaced daily to obtain <24-h-old eggs. The paper was cut into pieces with one egg mass on each piece. Then, one or two egg masses were affixed to a 2 cm × 2 cm piece of paper to make an *O. furnacalis* egg card.

C. cephalonica were introduced from the Department of Pesticide of Nanjing Agricultural University and reared in the laboratory on ground wheat and corn at 25 ± 1°C. *Trichogramma dendrolimi* introduced into the laboratory from the Institute of Biological Control of Jilin Agricultural University in 2007 were thereafter continually reared on *C. cephalonica* eggs at 25 ± 2°C, 70 ± 10% RH and a photoperiod of 14:10 (L:D). Ten percent sucrose solution was provided as food when the wasps began to emerge. From this population, 24-h-old mated naive *T. dendrolimi* wasps were used in the following experiment.

Relationship between number of wasp eggs laid in the host and the number of attacks

An <24-h-old egg mass containing approximately six eggs of *O. furnacalis* (OfA) was used to make an egg card, which was placed in a glass tube (9.5 cm in length and 3.5 cm in diameter). One 24-h-old *T. dendrolimi* female wasp that had been exposed to *O. furnacalis* eggs for 1 h to learn the eggs was introduced into the tube. The attack behavior of the wasp was observed under a stereoscopic microscope (Nikon SMZ 800; Nikon Instrument Inc., Tokyo, Japan). An attack

https://doi.org/10.1017/S0007485317000335 Published online by Cambridge University Press

was determined according to the following wasp's behavior: (1) ovipositor insertion, (2) approximately 10 s of internal examination, (3) stability for several seconds, and (4) abdomen trembling and pulling out of ovipositor (Suzuki *et al*., 1984; Liu & He 1991). A new female wasp was introduced to replace any female that failed to parasitize the eggs (the wasp stops examining the eggs and rests). Thirty wasps were used in this experiment. All the parasitized eggs were dissected under a stereoscopic microscope to check for wasp eggs inside.

Number of attacks by T. dendrolimi, *the fate of* O. furnacalis *eggs, and wasp development*

One egg card was placed inside a glass tube (3.5 cm in diameter and 9 cm in length). Then, one 24-h-old mated female wasp was introduced into the tube. The behavior of the *T. dendrolimi* female wasp was carefully observed under a stereoscopic microscope. The egg-laying behavior of *T. dendrolimi* was evaluated according to the methods of Liu & He (1991). Female wasps that failed to parasitize were replaced by fresh wasps. The locations of all parasitized eggs were marked on a paper photocopy of the egg card (Chen *et al*., 2013).

After all the eggs in an egg mass had been parasitized, the eggs were classified according to the number of attacks they had experienced. The eggs were then placed in an incubator at 25 ± 1°C and 70 ± 10% RH with a photoperiod of 14:10 (L:D) and checked once every 12 h. On day 3, eggs containing a moving black head of *O. furnacalis* larvae were destroyed using a pin tip to avoid cannibalism and recorded. On days 6–7, the eggs that had been attacked once or twice were destroyed, while 30 eggs that had been attacked three times remained in the egg masses. A similar process was followed to obtain the egg masses with eggs that had been attacked once and twice.

The emerged wasps were moved to a new tube containing a 0.5 cm × 0.5 cm piece of wet filter paper to provide water. The adults were checked once every 12 h. Any dead wasps were sexed and the hind tibia was measured. To check the status of the host eggs, *O. furnacalis* eggs without emergence holes were dissected 3 days after the last wasp emerged. The successful parasitism rate was calculated by dividing the black eggs, which means the *Trichogramma* inside have developed into pupae, by the total number of host eggs that were attacked once, twice or three times. The emergence rate was calculated by dividing the number of host eggs from which wasps emerged by the number of black eggs.

To understand the difference in host suitability between larvae fed corn and those fed artificial diet, both OfC and OfA eggs were tested in this experiment. In total, there were six treatments: two host strains (OfC and OfA) multiplied by three attack times (attacked once, twice, or three times). We conducted five replicates for OfC eggs with one attack and four replicates for all the other treatments.

Ratio of T. dendrolimi *wasps to host eggs and number of wasp eggs laid in per egg of* O. furnacalis

To determine whether the number of wasp eggs oviposited into the host would increase with the ratio of wasp to *O. furnacalis* eggs, a 5-egg mass <24-h old was exposed to one, two or three mated and <24-h-old *T. dendrolimi* female wasps for 6 h. Ten percent sucrose solution was provided as food. Then, all the exposed eggs were dissected under a microscope, and the number of wasp eggs was counted. Thirty replicates were performed for each of three wasp:host egg ratios (1:5, 2:5 and 3:5). However, because some wasps died during the experiments (7, 9, and 10 wasps in the 1:5, 2:5, and 3:5 ratios, respectively), data from 23, 21, and 20 replicates for the 1:5, 2:5, and 3:5 wasp: egg ratios, respectively, were analyzed.

Data analysis

SPSS software (version 13.0; SPSS Inc., Chicago, IL, USA) was used to analyse the data. The proportions of *O. furnacalis* eggs that contained different numbers of *T. dendrolimi* eggs were subjected to one-way analysis of variance (ANOVA) tests. Development and parasitism rate data were subjected to two factors (number of attacks and host diet). The ANOVAs were based on a 3 (attack 1, 2 and 3 times) × 2 (corn fed host and artificial fed host) factorial design, and the effects of the interaction between number of attacks and host diet was the dependent variable. The means were analyzed by Tukey's HSD test. The percentages were arcsine square-root transformed before being subjected to ANOVA, and untransformed data are presented. All the graphs were constructed using GraphPad Prism 6 software (GraphPad Software, San Diego, CA, USA).

Results

Relationship between number of attacks and number of wasp eggs per host egg

In total, 59.56% of the *O. furnacalis* eggs contained one wasp egg after being attacked once by *T. dendrolimi*, while 57.41% of the *O. furnacalis* eggs contained two wasp eggs after being attacked twice. Among the host eggs that were attacked three times, only 29.00% contained three wasp eggs; this percentage was significantly lower than 59.56% and 57.41% (F = 27.70, df = 2,6, P < 0.001) (table 1) resulting from only one or two attacks. The number of wasp eggs per host increased significantly as the number of attacks increased (F = 55.24, df = 2,262, P < 0.001) (fig. 1).

Parasitism of O. furnacalis *eggs by* T. dendrolimi *and wasp emergence rate*

The parasitism of *O. furnacalis* eggs by *T. dendrolimi* increased as the number of attacks increased (for OfC: F = 28.98, df = 2,10, P < 0.01; for OfA: F = 12.69, df = 2,9, P < 0.01) (table 2). Except for those eggs with two attacks, there were no significant differences in the parasitism rate between attacks on the OfC and OfA eggs (three attacks: F = 0.22, df = 1,6, P = 0.65; two attacks: F = 1.16, df = 1,6, P < 0.01; one attack: F = 1.03, df = 1,7, P = 0.35). The interaction between host diet and number of attacks had no significant effect on the parasitism rate (F = 1.42, df = 2,19, P = 0.27).

Emergence rates ranged from 72.3 to 93.3%, and there were no significant differences among different numbers of attacks (for OfC: F = 3.11, df = 2,10, P = 0.09; for OfA: F = 1.54, df = 2,9, P = 0.27). However, the interaction between host diet and number of attacks significantly affected the emergence rate of *Trichogramma* (F = 4.17, df = 2,19, P = 0.032).

The number of wasps that emerged from per *O. furnacalis* egg increased with attack number for both OfC and OfA host eggs, there was no significant differences between OfC and OfA hosts with the same number of attacks (table 2), and the

Table 1. Percentage (mean ± SE) (%) of host eggs containing wasp eggs after different numbers of attacks by *T. dendrolimi*.

Wasp eggs contained	Attack number		
	1	2	3
0	40.44 ± 3.38 a	11.21 ± 3.74 b	5.87 ± 2.38 b
1	59.56 ± 3.38 a	31.39 ± 0.60 b	32.77 ± 1.13 b
2	0.00	57.41 ± 4.18 a	32.36 ± 3.39 b
3	0.00	0.00	29.00 ± 3.67

Note: Different lowercase letters in the same row indicate significant differences among the percentages (Tukey's test, $P < 0.05$).

effect of the interaction between host diet and the number of attacks was not significant ($F = 0.04$, df = 2,19, $P = 0.958$).

However, the number of attacks significantly affected the proportion of female offspring ($F = 27.01$, df = 2,19, $P < 0.01$). The proportion decreased as the number of attacks increased. The host diet did not significantly affect the proportion of female offspring ($F = 3.88$, df = 1,19, $P = 0.06$).

Development time of T. dendrolimi *offspring on* O. furnacalis *eggs*

For the same number of attacks, the offspring development time for the OfC eggs was significantly longer than the that for the OfA eggs (three attacks: $F = 21.25$, df = 1,75, $P < 0.01$; two attacks: $F = 11.62$, df = 1,75, $P < 0.01$; one attack: $F = 5.80$, df = 1,112, $P = 0.02$) (table 2). As the number of attacks increased, the development time of the wasp offspring increased (on OfC: $F = 78.79$, df = 2,109, $P < 0.001$; on OfA: $F = 26.90$, df = 2,107, $P < 0.001$). The interaction between host diet and number of attacks significantly affected the development time of *Trichogramma* ($F = 10.78$, df = 2,216, $P < 0.001$).

Size of T. dendrolimi *offspring*

The number of attacks also significantly affected the size of the emerged wasps, and the size of female offspring decreased as the number of attacks increased (on OfA: $F = 28.27$, df = 2,73, $P < 0.001$; on OfC: $F = 34.10$, df = 2,72, $P < 0.001$) (fig. 2). However, among the male offspring, the largest wasps emerged from host eggs that had suffered two attacks (on OfA: $F = 5.31$, df = 2,39, $P < 0.01$; on OfC: $F = 10.91$, df = 2,46, $P < 0.001$). There were no significant differences between host diets on the size of offspring of the same gender from eggs with the same number of attacks. No significant effects of the interaction between host diet and number of attacks were found on offspring size (for females: $F = 0.03$, df = 2,145, $P = 0.97$; for males: $F = 0.07$, df = 2,85, $P = 0.93$).

Fate of O. furnacalis *eggs attacked*

After being attacked by *T. dendrolimi*, some parasitized *O. furnacalis* eggs developed into *O. furnacalis* larvae (table 3). The percentage of *O. furnacalis* eggs that developed into host larvae differed significantly among attack numbers (for OfC: $F = 42.41$, df = 2,10, $P < 0.001$; for OfA: $F = 19.44$, df = 2,9, $P < 0.001$). There were no significant differences in the percentage of eggs that developed into host larvae between OfC and OfA except for eggs that were attacked twice (three attacks: $F = 0.26$, df = 1,7, $P = 0.63$; two attacks: $F = 1.95$, df = 1,6, $P = 0.04$; one attack: $F = 1.80$, df = 1,6, $P = 0.22$). The

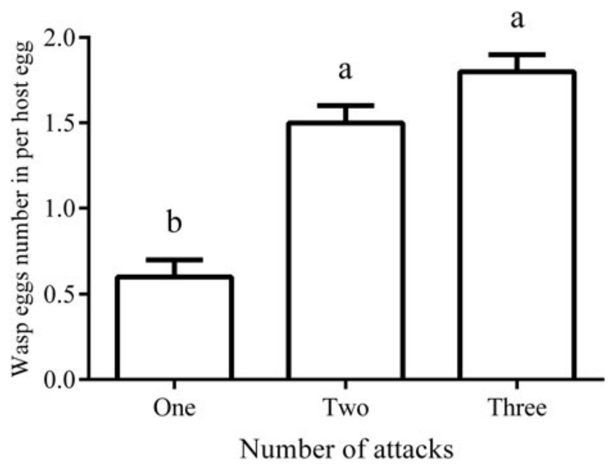

Fig. 1. The number of wasp eggs per *O. furnacalis* host egg after one, two or three attacks by *T. dendrolimi*; different letters indicate significant differences (Tukey's test, $P < 0.01$).

interaction between host diet and number of attacks was not significant ($F = 1.62$, df = 2,19, $P = 0.22$).

There were also eggs that failed to develop into *O. furnacalis* larvae and from which no *T. dendrolimi* emerged. Only light liquid was found inside these eggs at dissection. Both number of attacks and host diet significantly affected the percentage of undeveloped host eggs (attack number: $F = 21.33$, df = 2,19, $P < 0.01$; host diet: $F = 6.40$, df = 2,19, $P < 0.02$). However, no significant interaction effects were found ($F = 0.94$, $P = 0.41$).

Number of wasp eggs in host eggs after exposure for 6 h at different ratios of wasps to host eggs

There were 70, 65, and 64 host eggs containing wasp eggs at dissection in the 1:5, 2:5, and 3:5 treatments, respectively, and the ratio of wasps to host eggs significantly affected the number of wasp eggs per host egg after a 6-h exposure ($F = 35.61$, df = 2,196, $P < 0.01$) (fig. 3).

Discussion

Number of attacks of T. dendrolimi *and successful parasitism of* O. furnacalis *eggs*

Although *T. dendrolimi* female did not like laying eggs in *O. furnacalis* eggs and often performed several examinations before laying an egg into a host egg in the present experiments, we obtained *O. furnacalis* eggs that were attacked up to three times by placing one female together with an egg mass. *Trichogramma* females will lay one egg in a small suitable egg with each attack (Klomp & Teerink, 1962; Suzuki *et al.*, 1984). In experiments of counting number of attacks, we observed all the parasitization behavior before carefully scrutinizing *Trichogramma* eggs under stereomicroscope. Intact wasp eggs were readily to be distinguished from the background substrate. However, we cannot ensure that no wasp eggs were destroyed at dissecting *O. furnacalis* eggs, and the destroyed wasp eggs would mix with debris of host eggs chorion and thus be missed. Nevertheless, the number of eggs increased with the number of attacks. In addition, up to nine the wasp eggs were laid in one egg of *O. furnacalis*. These

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Table 2. Parasitism success rate, developmental time (day) and emergence number of *T. dendrolimi* after different numbers of attacks on *O. furnacalis* eggs.

Host	Number of attacks	Parasitism rate (%)	Emergence rate (%)[1]	Developmental time (day)	Number of emerged wasps per host[2]	Female proportion
OfC	1	6.7 ± 1.7 cA	93.3 ± 7.5	9.13 ± 0.03(71) cA	1.1 ± 0.1 b	0.95 ± 0.05 aA
	2	24.2 ± 4.0 bB	85.3 ± 5.8	10.06 ± 0.12(31) bA	1.2 ± 0.1 ab	0.77 ± 0.02 bA
	3	42.5 ± 7.3 aA	72.3 ± 6.2	10.60 ± 0.22(10) aA	1.6 ± 0.2 a	0.67 ± 0.04 bA
OfA	1	13.3 ± 7.9 bA	76.7 ± 13.1	9.01 ± 0.05(43) bB	1.1 ± 0.2 b	0.91 ± 0.10 aA
	2	44.2 ± 4.3 aA	86.7 ± 2.4	9.61 ± 0.07(46) aB	1.4 ± 0.0 ab	0.65 ± 0.03 bB
	3	45.8 ± 4.3 aA	93.2 ± 3.2	9.64 ± 0.10(21) aB	1.7 ± 0.1 a	0.44 ± 0.03 bB

Note: The values are means ± SE; the lowercase letters denote comparisons among different number of attacks of the same host type, and the uppercase letters indicate comparisons between OfC and OfA with the same number of attacks. Data followed by different letters are significantly different (Tukey's test, $P < 0.05$).
[1]No significant differences were found among different numbers of attacks or different host diets.
[2]No significant differences were found between host diets with the same number of attacks.

results indicated that superparasitism can occur when a wasp repeatedly encounters a host egg, even if the egg is not well suitable for the *Trichogramma* wasp (van Dijken & Waage, 1987; Liu & He, 1991; Miura *et al.*, 1994; Wang *et al.*, 2016). Superparasitism may be common in egg parasitoids when suitable host are insufficient (Narendran, 1985; van Alphen & Visser, 1990) or under inter/intraspecific competition (van Dijken & Waage, 1987; Dasilva *et al.*, 2016).

As the number of attacks increased, the rate of successful parasitism of eggs of *O. furnacalis* by *T. dendrolimi* increased significantly. Furthermore, the percentage of eggs that failed to result in either parasitoid or host larvae emergence increased as the number of attacks increased. This can be considered a control effect of *Trichogramma* (Abram *et al.*, 2016). When the ratio of wasps to host eggs increased to 3:5, which is close to that 3:4–6 in the field after an inundative release (Shen *et al.*, 1986; Gu *et al.*, 1989; Wang *et al.*, 2000; Feng *et al.*, 2011; Bai *et al.*, 2014; Zhou *et al.*, 2014), the number of eggs per host egg increased to 4.6 (fig. 3), which is far above the average (1.8) from three attacks, resulting in the death of the host egg. These results likely explain the high rate of successful parasitism of *O. furnacalis* eggs by *T. dendrolimi* after an inundative release, which results in successful borer control.

Effects of the number of attacks on Trichogramma offspring

As the number of attacks increased, the proportion of female offspring decreased (table 2). One model to explain the sex ratio of haplodiploidy hymenopteran is local mate competition (LMC) (Hamilton, 1967), which predicts that the increase in number of female would results in increasing in proportion of male offspring. In our experiments, different females may have been used to parasitize same one egg mass. Thus, a female introduced into the arena might have detected the trail of previous females, potentially representing unidirectional intraspecific competition, which is similar to competition in the LMC model. A modified model by Suzuki *et al.* (1984) may be more specific to explain the sex ratio in our experiment, when eggs were attacked by more than one female, the female offspring suffered higher mortality than male offspring, and thus, superparasitism would result in lower female proportion. Even after being attacked thrice, that the emerged wasps from per host egg were less than two individuals indicated the superparasitism did occur in present study. Martel & Boivin (2004) observed similar phenomena in *Trichogramma pintoi*. A decrease in female proportion caused by superparasitism has also been reported in *Trichogramma evenescens* (Waage & Ming, 1984) and *Trichogramma minutum* (Corrigan *et al.*, 1995). A low female offspring proportion means that fewer *T. dendrolimi* females would emerge to parasitize the eggs of the next *O. furnacalis* generation. Furthermore, as the extent of superparasitism increase, the size of emerged female wasps decrease (Waage & Ming, 1984; Grenier *et al.*, 2001; Boivin & Martel, 2012), and smaller *Trichogramma* females contain fewer eggs (Honěk, 1993; Durocher-Granger *et al.*, 2011; Huang *et al.*, 2015). These results most likely contribute to the sharp decline in the parasitism rate of *O. furnacalis* eggs by *T. dendrolimi* that are observed in the field after an inundative release (Zhang *et al.*, 1979; Feng, 1996).

Effects of host diet on host suitability

Although the artificial diet did not affect the biological characteristics of the Asian corn borer (Zhou *et al.*, 1980; Wang & Zhou, 1998), our results indicated that the diet affected the eggs' suitability for *T. dendrolimi*: among eggs that experienced two attacks, the parasitism rate of OfC eggs was significantly different from that of OfA eggs (table 2). In a choice test, Song *et al.* (1997) found that *T. minutum* parasitized more eggs from *Choristoneura fumiferana* Clemens that were reared on artificial diets than those reared on balsam fir. The authors thought that host diet affected volatile and contact kairomones that repel parasitoids from egg masses of *C. fumiferana* that were reared on balsam fir or attracted parasitoids to *C. fumiferana* reared on an artificial diet. In the present study, we did not find obvious differences in the parasitization behavior of *Trichogramma* between OfC eggs and OfA eggs; however, we did not investigate whether there were differences in the egg laying after each attack between the two host strains because we did not dissect the OfC eggs. Nathan *et al.* (2006) found that the diet (millet, wheat, rice, and sorghum) of *C. cephalonica* affected the *Trichogramma chilonis* emergence rate but not the host parasitism rate. Here, the diet probably affected the quality of *O. furnacalis* eggs and, thus, the successful parasitism and development of immature *Trichogramma*. The mechanism of the influence of host diet on egg parasitism by an egg parasitoid remains unclear.

Fate of host eggs attacked by wasps

Some host eggs failed to develop into host larvae or wasps: only yellow liquid was found in these eggs. Furthermore, among eggs that were attacked twice, 11.21%

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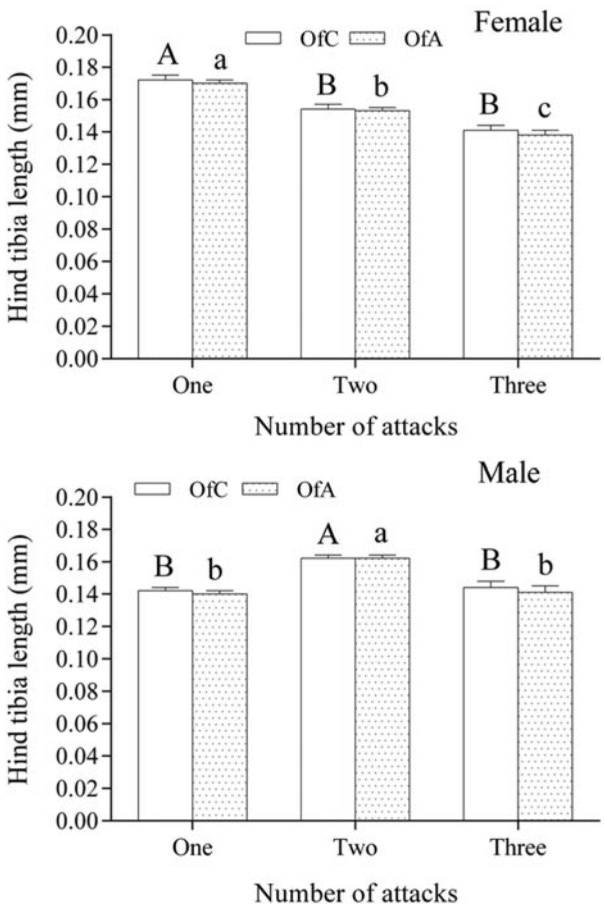

Fig. 2. Hind tibia length (mean ± SE) of *T. dendrolimi* adults that emerged from *O. furnacalis* eggs that were attacked different numbers of times. The uppercase letters denote the comparison results from OfC eggs with different numbers of attacks, and the lowercase letters denote the comparison results from OfA eggs with different numbers of attacks. Different letters indicate significant differences (Tukey's test, $P < 0.01$). There were no significant differences between OfC and OfA eggs with the same number of attacks.

did not contain wasp eggs, while 65.0% of the OfC eggs and 51.7% of the OfA eggs developed into *O. furnacalis* larvae. These findings indicate that host eggs may be capable of suppressing wasp egg development. It was found that the activities of immulectin-V, prophenoloxidase, prophenoloxidase activating protease I, and proparalytic peptide increased in fresh *Manduca sexta* (Linnaeus) eggs after the eggs were parasitized by *Trichogramma evanescens* Westwood, and the decrease in successful parasitism rate of *M. sexta* eggs was supposed to be related to this change (Abdel-latief & Hilker 2008). The immune response to parasitoid eggs by *M. sexta* eggs even could be improved through transgenerational immune priming (Trauer-Kizilelma & Hilker, 2015). Such a mechanism might explain the low suitability of *O. furnacalis* eggs to *T. dendrolimi*. *Trichogramma* females may secret parasitization factors to confront the host's immune response (Takada *et al.*, 2000; Jarjees & Merritt, 2004). Zhu *et al.* (2014) reported that the venom of *T. ostriniae* females significantly increased the successful parasitism of *O. furnacalis* eggs

Table 3. Fate of *O. furnacalis* eggs that did not turn into black after being attacked by *T. dendrolimi*.

Host	Number of attacks	Percentage of eggs that developed into *O. furnacalis* larvae (%)	Percentage of hosts that failed to result in either parasitoid or host larvae (%)
OfC	1	92.0 ± 0.9 aA	1.3 ± 1.2 b
	2	65.0 ± 4.8 bA	10.8 ± 3.3 a
	3	40.8 ± 8.2 cA	16.7 ± 3.1 a
OfA	1	85.8 ± 7.4 aA	0.8 ± 0.9 b
	2	51.7 ± 3.3 bB	4.2 ± 2.4 ab
	3	45.0 ± 5.1 bA	9.2 ± 1.8 a

Note: The values show means ± SE; data in the same column were analyzed by Tukey's tests at $P < 0.05$. The lowercase letters denote comparisons among different numbers of attacks of the same host type and the uppercase letters denote comparisons between OfC and OfA with the same number of attacks. Different letters indicate significant differences.

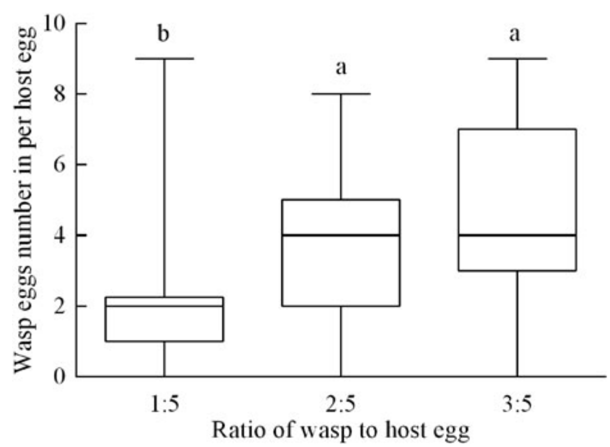

Fig. 3. Box plot of the number of *Trichogramma* eggs per host after 6 h exposure at different ratios of wasps to host eggs. Different letters indicate significant differences (Tukey's test, $P < 0.01$).

by *T. dendrolimi*. This suppression and anti-suppression may determine the suitability of a given host to a *Trichogramma* species. Regardless of the underlying mechanism, some attacked *O. furnacalis* eggs that failed to produce parasitoids or host larvae, and the percentage of such non-producing eggs increased significantly as the number of attacks increased, an outcome that also contributes to the control effects (Abram *et al.*, 2016).

Conclusions

In conclusion, we found that despite the low suitability of *O. furnacalis* eggs to *T. dendrolimi*, successful parasitism increased with the number of attacks by *T. dendrolimi*. The attack increase resulted in a lower proportion and smaller size of female offspring. Increasing the ratio of *Trichogramma* females to *O. furnacalis* eggs resulted in a high level of superparasitism, which resulted from more attacks. These results partially illustrated the high rate of successful parasitism of *O. furnacalis* eggs in field after an inundative release of *T. dendrolimi* and the sharp decrease in parasitism rate by *T. dendrolimi* without repeated wasp releases.

Acknowledgements

The authors thank Dr Yin-Quan Liu of Zhejiang University for his constructive suggestions on an early draft of the manuscript. Funding was provided by the National Basic Research Program of China (Grant No. 2013CB127605) from the Chinese Ministry of Science and Technology and the National Natural Science Foundation of China (Grant No. 30871671), and these institutions are gratefully acknowledged.

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