Effects of Thelytokous Parthenogenesis-Inducing Wolbachia on the Fitness of Trichogramma dendrolimi Matsumura (Hymenoptera: Trichogrammatidae) in Superparasitised and Single-Parasitised Hosts

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Thelytokous Wolbachia-infected Trichogramma species have long been considered as biological control agents against lepidopteran pests in agriculture and forestry. Wolbachia has been suggested to increase the probability of the superparasitism of Trichogramma, but the fate of infected offspring in the superparasitised host is still unknown. The present study aimed to evaluate the fitness of thelytokous Wolbachia-infected (TDW) and bisexual Wolbachia-free (TD) Trichogramma dendrolimi Matsumura (Hymenoptera: Trichogrammatidae) lines in superparasitised or single-parasitised hosts. The results showed that irrespective of whether Trichogramma wasps were developed from superparasitised or single-parasitised hosts, the TDW line was characterized by reduced fitness, including lower fecundity, shorter longevity, and smaller body size of F1 offspring, and lower emergence rate of F2 offspring than the TD line. This was not true for the survival rate and developmental time of F1 offspring. Additionally, the fitness parameters of T. dendrolimi that developed from superparasitised hosts were lower compared with that of T. dendrolimi that developed from single-parasitised hosts. Interestingly, Wolbachia-infected females had higher dispersal capacity than bisexual females when they developed from superparasitised hosts. The results indicated that Wolbachia negatively affects fitness of T. dendrolimi, but enhance dispersal capacity of T. dendrolimi females in superparasitism condition. Further studies need to be carried out to select the best line that will allow Wolbachia and their host Trichogramma to be better adapted to one another.

Keywords: Wolbachia, Trichogramma dendrolimi, superparasitism, biological control, thelytokous parthenogenesis, intraspecific competition
INTRODUCTION

The egg parasitoids *Trichogramma* spp. have been widely used worldwide to control lepidopteran pests in agriculture and forestry (Li, 1994; Smith, 1996; Wang et al., 2019; Zhou et al., 2019a,b; Zang et al., 2020). In general, the sex determination of *Trichogramma* wasps is haplodiploidy, as haploid males develop from unfertilized eggs and diploid females develop from fertilized eggs (Cook, 1993; Werren et al., 2008). However, certain strains of *Trichogramma* spp. only produce females, even without fertilization, in a process known as thletyoky. Thletyoky caused by parthenogenesis-inducing (PI) *Wolbachia* has been found in at least 15 *Trichogramma* species (Vavre et al., 2004; Zhang, 2009; Ma and Schwander, 2017).

Thletyokus *Trichogramma* has advantages in biological control programs including easier colonization without mating and less costly to rear en masse (Stouthamer, 1993; Zhou et al., 2019b, 2020). Rahimi-Kaldeh et al. (2017) found that *Wolbachia* infection increases fecundity of thletyokus *Trichogramma brassicae* Bezdenko. Vavre et al. (1999) also reported the presence of the A subdivision of *Wolbachia* increases fecundity of *Trichogramma bourarachae* Pintureau and Babault. However, others showed *Wolbachia* has negative effects on host fitness in most occasions, including lower emergence rate, shorter longevity, smaller body size, and lower fecundity, when compared to their uninfected counterparts (Stouthamer and Luck, 1993; Hohmann et al., 2001; Tagami et al., 2001; Miura and Tagami, 2004; Russell and Stouthamer, 2011; Zhou et al., 2020). Previous studies also found that *Wolbachia* is the factor that causes the occurrence of intersex (Bowen and Stern, 1966; Beserra et al., 2003; Tulgtske and Stouthamer, 2012; Ning et al., 2019). In these cases, although thletyokus *Trichogramma* have many advantages in biological control programs, the *Wolbachia* infection may have potential negative consequences on the production of thletyokus *Trichogramma*.

Nevertheless, our previous studies and others indicated that infected *Trichogramma* females show a higher probability of superparasitism, which parasitoid females lay a second clutch of eggs on a host that has been parasitised by the same species (Harvey et al., 2013; Farahani et al., 2015; Huang et al., 2017a; Liu et al., 2018; Zhou et al., 2019b). According to Lack’s hypothesis as applied to gregarious parasitoids by Lack (1947) and Charnov and Skinner (1984), parasitoid females always allocate an optimal clutch size to a host to maximize offspring fitness. In superparasitised hosts, parasitoid offspring compete with each other for limited resources, leading to reduced individual fitness and increased mortality (Devescovi et al., 2017; Tunca et al., 2017; Duval et al., 2018). Thus, superparasitism had long been viewed as a maladaptive mistake of parasitoids (van Dijken and Waage, 1987; van Alphen and Visser, 1990). It is worth noting that some pathogens or symbionts can be horizontally transmitted among parasitoids when superparasitism occurs (Martínez et al., 2012; Parratt et al., 2016). *Wolbachia* has been found to transmit horizontally from infected to uninfected individuals as the offspring share a superparasitised host (Schilthuizen and Stouthamer, 1997; Huigens et al., 2004a; Zhang, 2009).

Some authors argue that a genomic conflict may exist between the PI *Wolbachia* and their host's genome, because the nuclear genes favor a population with at least some males, while PI *Wolbachia* favor a 100% female population (Stouthamer, 1997). In such situations, an “arms race” ensue between the *Wolbachia*, which try to enhance their transmission, and host nuclear genes, which try to suppress *Wolbachia* and their effects (Stouthamer, 1997). Consequently, a higher fitness cost is expected in infected individuals (Huigens et al., 2004b). However, Herre (1993) hypothesized that *Wolbachia* and the host may be better adapted to one another due to their common evolutionary fate, as the transmission of *Wolbachia* is largely vertical. In other words, *Wolbachia* quickly spreads in the host population as the host produces more offspring. Considering these two opposite hypotheses, an important question that should be tested arises: is the effect of *Wolbachia* on the fitness of *Trichogramma* offspring positive or negative in superparasitised hosts? Although previous studies have shown that *Trichogramma* infected by *Wolbachia* have higher probability for superparasitism (Huigens et al., 2004b; Farahani et al., 2015; Zhou et al., 2019b, 2020), the fate of *Trichogramma* offspring in the superparasitised host is still unknown.

*Trichogramma dendrolimi* Matsumura has been described as an effective biological control agent against many lepidopteran pest species, including *Ostrinia furnacalis* (Gueneé) (Wang et al., 2014; Zhang et al., 2018; Zhou et al., 2019a), *Mythimna separata* Walker (Huang et al., 2017b; Du et al., 2018), and *Mamestra brassicae* (Linnaeus) (Takada et al., 2001). The adults of *Trichogramma* spp. can deposit several to hundreds of eggs on a relatively large lepidopteran egg, such as those of *Antheraea pernyi* Guérin-Méneville, *Samia cynthia ricini* Donovan, *Helicoverpa armigera* (Hübner), and *M. brassicae* (Takada et al., 2001; Wang et al., 2015, 2020; Iqbal et al., 2019, 2020). However, on small eggs such as those of *Cocytia cephalonica* (Stainton), the females of *Trichogramma* spp. often deposit only one egg by a single oviposition event (Li et al., 2008; Wang et al., 2015, 2019; Du et al., 2018). Owing to the easier and more exact determination of the superparasitism of host eggs, the eggs of *C. cephalonica* were used as the host eggs in this study.

To determine whether the effect of *Wolbachia* on the fitness of *T. dendrolimi* offspring is positive or negative in single-parasitised or superparasitised hosts, we tested the survival rate, body size, and developmental time of F1 offspring; fecundity, adult longevity, and dispersal capacity of F1 female offspring; and emergence rate of F2 offspring of a thelytokous *Wolbachia*-infected line (TDW) and a bisexual *Wolbachia*-uninfected line (TD) of *T. dendrolimi* in superparasitised or single-parasitised hosts. The single-parasitised host eggs were determined as one offspring being allocated to a *C. cephalonica* egg. The superparasitised host eggs were determined as two offspring being allocated to a *C. cephalonica* egg. As the effects of *Wolbachia* on the fitness of *Trichogramma* have rarely been examined in superparasitism condition, the results bridge some gaps in the interaction of *Wolbachia* and host *Trichogramma*, and
provide the reference for the application of *Wolbachia*-infected thelytokous *Trichogramma*.

**MATERIALS AND METHODS**

**Insects**

The insects used in this study, including the TD and TDW of *T. dendrolimi* and their host *C. cephalonica*, were maintained in the Pest Biological Control Laboratory, Shenyang Agricultural University. All insects were reared at 25 ± 1°C, 70 ± 5% RH and a L16:D8 photoperiod. Both *T. dendrolimi* lines were originally obtained from the same bisexual isofemale line. The TDW line was first obtained by artificially transferring *Wolbachia* derived from infected females of *Trichogramma embryophagum* Hartig into developed pupae of an TD female by micro-injection (Zhang, 2009). Thereafter, the females of the TDW line produced offspring that were nearly 100% female-biased even without mating. The infection of *T. dendrolimi* individuals by *Wolbachia* was detected and determined by specific primers (81F: 5'-TGGTICAAATAAGTGATGAAAGAAC-3' and 691R: 5'-AAAAATTAAAACGCTACTCCA-3') for the wsp gene of PI *Wolbachia* (Braig et al., 1998; Liu et al., 2018). The host, *C. cephalonica*, was reared on a semi-artificial diet (Yang et al., 1990). The *C. cephalonica* eggs were collected in groups of ca. 300 and then glued onto a 10 mm × 40 mm card using Arabic gum solution (Arabic gum powder:water = 1:6). To prevent the hatch of *C. cephalonica* larvae, the host eggs were then killed by UV-irradiation for 30 min. Both *T. dendrolimi* lines were reared on *C. cephalonica* eggs by generations in the laboratory.

The active host eggs (1-day-old, without UV-irradiation) were collected in groups of 30 and glued on a white card (10 mm × 10 mm) using the Arabic gum solution. The egg cards were used in this experiment.

**Experimental Procedure**

The egg card containing 30 glued host eggs was transferred into a small Durham glass tube (8 mm diameter, 10 mm length, stopped with cotton balls). Thereafter, a group of 10 TD or TDW wasps were introduced into the tube. The oviposition behaviors were observed under an anatomical lens. A single egg from the female parasitoid was successfully deposited into the host egg when a single oviposition behavior occurred with a time of fluctuating abdominal movement (Guo, 1993). The egg locations on the egg card were marked by a surgical skin marking pen (0.5 mm tips, T3023; Tondaus, Dongguan, China) quickly as the oviposition behavior occurred. Ten egg cards were supplied to TD or TDW wasps for 30 min, after which the wasps were removed. The host eggs were then reared until the eggs blackened, which occurs during the *Trichogramma* prepupal stages (Flanders, 1937). The blackened host eggs were cut off and transferred singly into a new Durham glass tube for emergence. Thereafter, the host egg was reared until the wasp(s) either emerged or not.

After wasp emergence, the blackened host eggs were dissected to determine the existence of a dead body of a *Trichogramma* offspring. The survival rate of F1 offspring was calculated by the number of wasps that emerged and the total number of offspring deposited. The single-parasitised host eggs were determined as one offspring being allocated to a *C. cephalonica* egg. The superparasitised host eggs were determined as two offspring being allocated to a *C. cephalonica* egg. Every unmated female wasp was supplied with a host egg card with approximately 300 eggs for parasitization and replaced daily. The wasps were fed with 10% honey solution via a cotton thread daily. Female lifespan and fecundity were recorded daily. The body size of the females was measured as the length of the left hind tibia.

Thirty TD or TDW females that emerged from superparasitised hosts or single-parasitised hosts, were randomly selected to test their aerial dispersal capacity by recording the flying distance according to the method of Zboralski et al. (2016). A single female was introduced into a 100 µL pipette tip (50 mm height, and the wide mouth side stopped with cotton). The tip was erected in the center of an A2 paper (594 mm × 420 mm) as the diving tower and the tip of the tower was coated with Vaseline to prevent the female from walking along the tip. The females had to fly from the tower and their landing positions were marked by the position of the female glued on the paper coated with vaseline. The flying distance of the female was recorded.

**Statistical Analysis**

Two factors were considered in this study: the parasitism type (superparasitised host eggs or single-parasitised host eggs) and the *T. dendrolimi* line (TD and TDW). The binary logistic regression model was used to estimate the survival rate of F1 offspring and the emergence of F2 offspring as influenced by the parasitism type and the *T. dendrolimi* line (Walker and Duncan, 1967). The log-linear model was used to estimate the effects of the *T. dendrolimi* line and the parasitism type on the fecundity and developmental time of *T. dendrolimi* (Berk and MacDonald, 2008). A generalized linear model with Gaussian distribution was applied to analyze the body size and flying distance as influenced by the parasitism type and the *T. dendrolimi* line.

Cox’s proportional hazard model (hereafter “Cox model”) was applied to quantify the adult longevity of F1 female offspring by the hazard rate, which can be interpreted biologically as the death risk at different age. The Cox model are well suited to time-to-event data (e.g., longevity of organisms), which are seldom normally distributed and cannot be made to fit the assumption of linear models with transformations. The hazard death rate at wasp’s age $t$ is given by:

$$h(t) = h_0(t) \exp \left\{ \sum_{i=1}^{n} \beta_i Z_i \right\}$$

Where $h_0 (t)$ is the baseline hazard function of adult death depending only on the age when all covariates $Z_i$ are set to zero, and $\beta_i$ is the regression coefficient that give the relative contribution of the n covariates $Z_i$ (t). If hazard ratio of adult death, expressed by $\exp \{ \Sigma \beta_i Z_i \}$, is reduced, resulting in an increase of the adult longevity. The instantaneous death risk describe the increasing rate of mortality increased with the age. The Kaplan-Meier plot was applied to describe the curve of cumulative hazard of adult death increased with the age (Cox, 1972; Sheng et al., 2014).
All analyses were carried out in R ver. 4.0.2 (R Core Team, 2020).

RESULTS

The survival rate of F1 offspring was not affected by the parasitism type \((\chi^2 = 2.19, df = 1, P = 0.14)\), the *T. dendrolimi* line \((\chi^2 = 3.68, df = 1, P = 0.055)\), or their interaction \((\chi^2 = 0.073, df = 1, P = 0.79)\) (Figure 1).

The body size of F1 female offspring was significantly affected by the parasitism type \((\chi^2 = 205.59, df = 1, P < 0.001)\) and the *T. dendrolimi* line \((\chi^2 = 7.08, df = 1, P = 0.0078)\), but it was not affected by their interaction \((\chi^2 = 0.39, df = 1, P = 0.53)\). The body size of the females that emerged from superparasitised hosts (TD: 120.35 ± 2.29 μm; TDW: 115.76 ± 2.02 μm) was significantly smaller than that of those that emerged from single-parasitised hosts (TD: 154.03 ± 2.42 μm, z = 14.98, P < 0.001; TDW: 146.64 ± 2.26 μm, z = 14.34, P < 0.001). The body size of TD females was significantly higher (z = 2.66, P = 0.0078) than that of TDW females (Figure 2-S1).

Fecondity of F1 female offspring was significantly affected by the parasitism type \((\chi^2 = 167.56, df = 1, P < 0.001)\) or the *T. dendrolimi* line \((\chi^2 = 65.86, df = 1, P < 0.001)\). Regardless of the *T. dendrolimi* line, the fecondity of the females that emerged from superparasitised hosts (TD: 71.29 ± 4.27; TDW: 48.44 ± 2.13) was significantly lower than that of those that emerged from single-parasitised hosts (TD: 142.90 ± 7.55, z = 3.64, P < 0.001; TDW: 92.37 ± 4.39, z = 2.70, P = 0.0069). The fecondity of the TD females was significantly higher than that of the TDW females in superparasitised (z = 4.50, P < 0.001) and single-parasitised (z = 6.68, P < 0.001) hosts (Figure 2-S2).

The developmental time of F1 offspring was not affected by the parasitism type \((\chi^2 = 0.083, df = 1, P = 0.77)\), the *T. dendrolimi* line \((\chi^2 = 2.99, df = 1, P = 0.084)\), or their interaction \((\chi^2 = 0.0010, df = 1, P = 0.99)\) (Figure 2-S3).

The emergence rate of F2 offspring was significantly affected by the interaction of the parasitism type and the *T. dendrolimi* line \((\chi^2 = 48.57, df = 1, P < 0.001)\). The emergence rate of TDW offspring that emerged from superparasitised hosts (61.78 ± 2.12%) was significantly lower than that of those that emerged from single-parasitised hosts (85.38 ± 1.28%; z = 8.38, P < 0.001), but the difference was non-significant in TD offspring (superparasitised host: 70.20 ± 2.13%; single-parasitised host: 70.41 ± 2.13%; z = 0.41, P = 0.68). The emergence of TDW offspring was significantly higher than that of TD offspring (z = 7.22, P < 0.001) in single-parasitised hosts, but was significantly lower than that of TD offspring (z = 2.65, P = 0.0082) in superparasitised hosts (Figure 2-S4).

The adult longevity of F1 female offspring was significantly affected by the parasitism type \((\chi^2 = 18.37, df = 1, P < 0.001)\) or the *T. dendrolimi* line \((\chi^2 = 4.66, df = 1, P = 0.031)\), but it was not influenced by their interaction \((\chi^2 = 0.71, df = 1, P = 0.40)\). Regardless of the *T. dendrolimi* line, the adult longevity of females that emerged from superparasitised hosts [instantaneous death risk: 1.37 ± 0.22 (TD), 1.77 ± 0.30 (TDW)] was significantly shorter than that of those that emerged from single-parasitised hosts (TD: 0.48 ± 0.087, z = 3.81, P < 0.001; TDW: 0.85 ± 0.14, z = 3.52, P < 0.001). The adult longevity of TD females was significantly higher (z = 2.12, P = 0.035) than that of TDW females (Figure 3).

The flying distance of F1 female offspring was significantly affected by the interaction of the parasitism type and the *T. dendrolimi* line \((\chi^2 = 15.42, df = 1, P < 0.001)\), but it was not affected by the parasitism type \((\chi^2 = 3.43, df = 1, P = 0.064)\) or the *T. dendrolimi* line \((\chi^2 = 2.09, df = 1, P = 0.15)\). The flying distance of TDW females in superparasitised hosts (32.21 ± 2.81 mm) was significantly higher than that in single-parasitised hosts (19.56 ± 2.64 mm; single-parasitised host: 25.53 ± 2.56 mm; z = 1.67, P = 0.094). The difference in the flying distance between TD and TDW females was non-significant in superparasitised hosts (z = 1.24, P = 0.20) or single-parasitised (z = 1.41, P = 0.16) hosts (Figure 4).

DISCUSSION

Our results showed that the survival rate of F1 offspring was not affected by the parasitism type or the *T. dendrolimi* line. However, the fitness parameters including body size, adult longevity, and fecondity of *T. dendrolimi* females that developed from superparasitised hosts were reduced compared with those of *T. dendrolimi* females developed from single-parasitised hosts, regardless of the *T. dendrolimi* line.

Generally, the outcome of intrinsic competition depends on the host usage strategies of the parasitoids (Harvey et al., 2013). Once the hosts are superparasitised, parasitoid offspring compete for the host resources by scramble competition for the acquisition of shared host nutrition, or by contest competition for monopolization of host resource through excluding competitors. Larvae of solitary parasitoid species often destroy their competitors and gregarious parasitoid larvae often share the host resources with other individuals in a superparasitised host. *T. dendrolimi* can be considered a facultative gregarious species (Martel and Boivin, 2010). These tiny wasps often deposit only one egg on a small host egg, such as that of *C. cephalonica*, but they can also deposit a clutch of several to hundreds of eggs on a large host egg, such as those of *M. separata, M. brassicaceae*, and *A. peryii* (Kong et al., 1988; Takada et al., 2001; Wang et al., 2015). However, the larvae of *Trichogramma carverae* Oatman and Pinto have been observed to siphon and kill their siblings (Heslin and Merritt, 2005). So far, *T. dendrolimi* larvae have not been observed to initiate aggressive attacks. If the larva shows a form of scramble competition, most offspring would emerge, but with reduced fitness. Our previous study and others revealed that infected parasitoids have a higher probability of parasitizing host eggs that had been previously parasitised (Farahani et al., 2015; Liu et al., 2018). In superparasitised hosts, *Wolbachia* gains the opportunity for horizontal transmission from infected *Trichogramma* individuals to uninfected *Trichogramma* individuals (Huigens et al., 2004;
FIGURE 1 | Survival rate of TDW or TD F1 offspring in superparasitised (cyan bars) or single-parasitised (white bars) hosts. Bars indicate Means. Error bars indicate the interval of Mean ± Standard Error. The same uppercase letters indicate non-significant difference between TD (n = 35) and TDW (n = 38) in superparasitised hosts. The same lowercase letters indicate non-significant difference between TD (n = 39) and TDW (n = 35) in single-parasitised hosts. “ns” indicates non-significant difference between superparasitised hosts and single-parasitised hosts. Texts labeled on the bars indicate sample size and values of Mean ± Standard Error.

FIGURE 2 | Body size of F1 female offspring (S1), fecundity of F1 female offspring (S2), developmental time of F1 offspring (S3), and emergence rate of F2 offspring (S4) of TDW or TD line in superparasitised (cyan bars) or single-parasitised (white bars) hosts. Bars indicate Means. Error bars indicate the interval of Mean ± Standard Error. The different uppercase letters indicate significant difference between TD and TDW in superparasitised hosts. The different lowercase letters indicate significant difference between TD and TDW in single-parasitised hosts. “ns” or “***” indicates non-significant difference or significant difference at $P < 0.001$, respectively, between superparasitised hosts and single-parasitised hosts. Texts labeled on the bars indicate sample size and values of Mean ± Standard Error.
Farahani et al., 2015; Liu et al., 2018). Thus, superparasitism may have a positive effect on the spread of Wolbachia.

Our results also showed that the infected line of *T. dendrolimi* had reduced fitness, including lower fecundity and a shorter longevity, than the uninfected *T. dendrolimi* line, regardless if it was developed in superparasitised or single-parasitised hosts. However, the survival rate of F1 offspring did not vary among parasitism types or *T. dendrolimi* lines. The results
support the genomic conflict hypothesis that exists between the PI-Wolbachia and their host's genome. Superparasitism does not seem to be an adaption of T. dendrolimi wasps to PI-Wolbachia.

Similar to our results, other studies also reported that the infected Trichogramma offspring exhibit greater mortality, shorter longevity, and smaller body size (Tagami et al., 2001; Huigens et al., 2004b). PI-Wolbachia can convert the haploid eggs of Trichogramma into diploid embryos through an aborted first mitotic division. This mechanism is called gamete duplication. Consequently, homozygous individuals develop as females. In contrast, the nuclear genes of the host favor a sex ratio with at least some males developed from haploid eggs and try to suppress Wolbachia and their effects. This arms race between the nuclear genes of hosts and PI Wolbachia may lead to a higher fitness cost in infected individuals (Stouthamer et al., 2010). First, the conflicts between Wolbachia and host Trichogramma may lead to delayed hatching in the early mitotic stage of Trichogramma embryo. Hohmann and Luck (2000) reported that the infected offspring of Trichogramma kayakai Pinto and Stouthamer took approximately half a day longer than uninfected offspring to hatch. Tagami et al. (2001) found that most embryos of uninfected Trichogramma could develop to the cellular blastoderm stage 6 h after oviposition, while approximately 35% of embryos of infected Trichogramma still remained at the mitotic stage even 48 h after oviposition. Owing to the delayed hatching, the Trichogramma offspring may be unable to obtain sufficient nutrition from the host eggs especially in superparasitism conditions. A second factor suspected to affect the fitness of Wolbachia-infected Trichogramma larvae, is their requirement for more nutrients when compared to that of uninfected counterparts (Huigens et al., 2004b). During the mitotic proliferation of host organisms, Wolbachia divides rapidly and rely on nutrients and energy sources provided by the host (Landmann et al., 2010; Grote et al., 2017). Rahimi Kaldeh et al. (2017) implied that the presence of Wolbachia may cause energy reduction when facing stresses. Although potential quality risks exist in PI Wolbachia-infected Trichogramma wasps, the PI Wolbachia-infected thletytokous Trichogramma line will allow a predictable performance in fields and could be used as a population to help maintain a typical genotype and traits without change. Genetic improvement methods should be encouraged to select the best line in the future. For example, Ebrahimi et al. (2019) tried to create a set of completely homozygous Wolbachia-infected recombinant lines by hybridizing Wolbachia-infected Trichogramma wasps and bisexual wasps.

Though the infected Trichogramma offspring is less competitive relative to uninfected offspring when competing in superparasitised hosts, we found Trichogramma offspring benefit for survivorship from Wolbachia in single-parasitised hosts. Our previous study also observed a higher emergence of Wolbachia-infected offspring than that of uninfected offspring when the wasps emerged from A. pernyi eggs (Zhou et al., 2020). One potential explanation is that Wolbachia protect hosts against a broad range of pathogens by induction of host innate immune responses or competing with pathogens for nutrients (Brownlie and Johnson, 2009; Zhang et al., 2020). Some studies doubted that the abortion of Trichogramma offspring may be caused by the vertical transmitted pathogens like Nosema spp. and pathogenic bacteria in host eggs (Pu, 1983; Ruan et al., 2000; Qin, 2015; Zhao et al., 2019). Therefore, further investigations need to be carried out to bridge the gaps in knowledge pertaining to the pathogenicity of the pathogens in Trichogramma. Another explanation is that Wolbachia has evolved nutritional mutualism for the survival of host organisms (Braquart-Varnier et al., 2015). For example, Wolbachia is the supplier of B vitamins for their hosts (Hosokawa et al., 2010; Ju et al., 2020). Although Wolbachia protect immature Trichogramma offspring against death, Wolbachia also taxes resources of infected Trichogramma offspring, necessitating a longer developmental time and requirement for more nutrients (Huigens et al., 2004b). Thus the infected Trichogramma offspring is less competitive for limited resources in intra-specific competition.

Interestingly, our results showed that Wolbachia-infected females had a higher dispersal capacity than bisexual females when they developed from superparasitised hosts. Similar results were also found in Laodelphax striatellus (Fallén) (Sun et al., 2015), Coccy trenys dactyliperda Fabricius (Tremmel et al., 2020), and Aedes aegypti (Linnaeus) (Evans et al., 2009), Caragata et al. (2011) found that Wolbachia infection increases the recapture rate of field-released Drosophila melanogaster Meigen. Hoffmann et al. (2007) revealed that Wolbachia infection results in size differences in flies and, consequently, affects their dispersal distances. Nevertheless, infecting nervous tissues with Wolbachia could also influence the locomotor activities of insects (Strunov et al., 2013). While in this study we showed that the increased dispersal capacity of infected wasps might be driven by superparasitism, the mechanism behind this phenomenon was not explained. Future studies should be encouraged to examine the dispersal behaviors and motivation of infected females under superparasitism conditions.

Although Wolbachia and the host will be better adapted to one another as Wolbachia quickly spreads in the host population when the host produces more offspring, Wolbachia-infected T. dendrolimi wasps could not benefit from superparasitism according to our results. However, the experiments were conducted in laboratory conditions. The Wolbachia-infected and uninfected lines have been separately reared on intermediate hosts for over 40 generations. This makes it a little challenging to be confident that the effects were directly attributable to Wolbachia. It is critical to clarify the effects of Wolbachia on the intraspecific competition of Trichogramma offspring in field conditions. In biological control programs, genetic improvement methods should be encouraged to select the best line that will allow Wolbachia and the host to be better adapted to one another in the future.

**DATA AVAILABILITY STATEMENT**

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.
AUTHOR CONTRIBUTIONS
L-SZ, J-CZ, and HD: conceptualization and funding acquisition. QZ, S-ML, L-XH, ZX, DS, J-CZ, and HD: data curation. J-CZ and QZ: original draft writing. J-CZ, QZ, HD, and L-SZ: review and editing. All authors contributed to the article and approved the submitted version.

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