



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

OPEN ACCESS

Edited by:

Nikolaos Papadopoulos,
University of Thessaly, Greece

Reviewed by:

Ahmad Pervez,
Radhey Hari Govt. Post Graduate
College, India

Jun-Ce Tian,
Zhejiang Academy of Agricultural
Sciences, China
Xiao-Yue Hong,
Nanjing Agricultural University, China

***Correspondence:**

Hui Dong
biocontrol@163.com
Li-Sheng Zhang
zhangleesheng@163.com

† These authors have contributed
equally to this work and share first
authorship

Specialty section:

This article was submitted to
Population, Community,
and Ecosystem Dynamics,
a section of the journal
Frontiers in Ecology and Evolution

Received: 25 June 2021

Accepted: 28 September 2021

Published: 18 October 2021

Citation:

Zhou J-C, Zhao Q, Liu S-M,
Shang D, Zhao X, Huo L-X, Dong H
and Zhang L-S (2021) Effects
of Thelytokous
Parthenogenesis-Inducing *Wolbachia*
on the Fitness of *Trichogramma*
dendrolimi Matsumura (Hymenoptera:
Trichogrammatidae)
in Superparasitised
and Single-Parasitised Hosts.
Front. Ecol. Evol. 9:730664.
doi: 10.3389/fevo.2021.730664

Jin-Cheng Zhou^{1,2†}, Qian Zhao^{2†}, Shi-Meng Liu², Dan Shang², Xu Zhao²,
Liang-Xiao Huo², Hui Dong^{2*} and Li-Sheng Zhang^{1*}

¹ Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China, ² College of Plant Protection, Shenyang Agricultural University, Shenyang, China

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 thelytoky. Thelytoky 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).

Thelytokous *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-Kaldehy et al. (2017) found that *Wolbachia* infection increases fecundity of thelytokous *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; Tulgetske and Stouthamer, 2012; Ning et al., 2019). In these cases, although thelytokous *Trichogramma* have many advantages in biological control programs, the *Wolbachia* infection may have potential negative consequences on the production of thelytokous *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 (Martinez 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* (Guenée) (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 *Corcyra 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 $25 \pm 1^\circ\text{C}$, $70 \pm 5\%$ 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'-TGGTCCAATAAGTGATGAAGAAAC-3' and 691R: 5'-AAAAATTAAACGCTACTCCA-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 \times 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 \times 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 \times 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 β_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 $\exp \{ \sum \beta_i Z_i \}$, 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: $120.35 \pm 2.29 \mu\text{m}$; TDW: $115.76 \pm 2.02 \mu\text{m}$) was significantly smaller than that of those that emerged from single-parasitised hosts (TD: $154.03 \pm 2.42 \mu\text{m}$, $z = 14.98$, $P < 0.001$; TDW: $146.64 \pm 2.26 \mu\text{m}$, $z = 14.34$, $P < 0.001$). The body size of TD females was significantly higher ($z = 2.66$, $P = 0.0078$) than that of TDW females (Figure 2-S1).

Fecundity 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 fecundity 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 fecundity 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 ($61.78 \pm 2.12\%$) was significantly lower than that of those that emerged from single-parasitised hosts ($85.38 \pm 1.28\%$; $z = 8.38$, $P < 0.001$), but the difference was non-significant in TD offspring (superparasitised host: $70.20 \pm 2.13\%$; single-parasitised host: $70.41 \pm 2.13\%$; $z = 0.41$, $P = 0.68$). 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 (19.28 ± 1.28 mm; $z = 4.01$, $P < 0.001$), but the difference was non-significant in TD offspring (superparasitised host: 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 fecundity 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. brassicae*, and *A. pernyi* (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., 2004a;

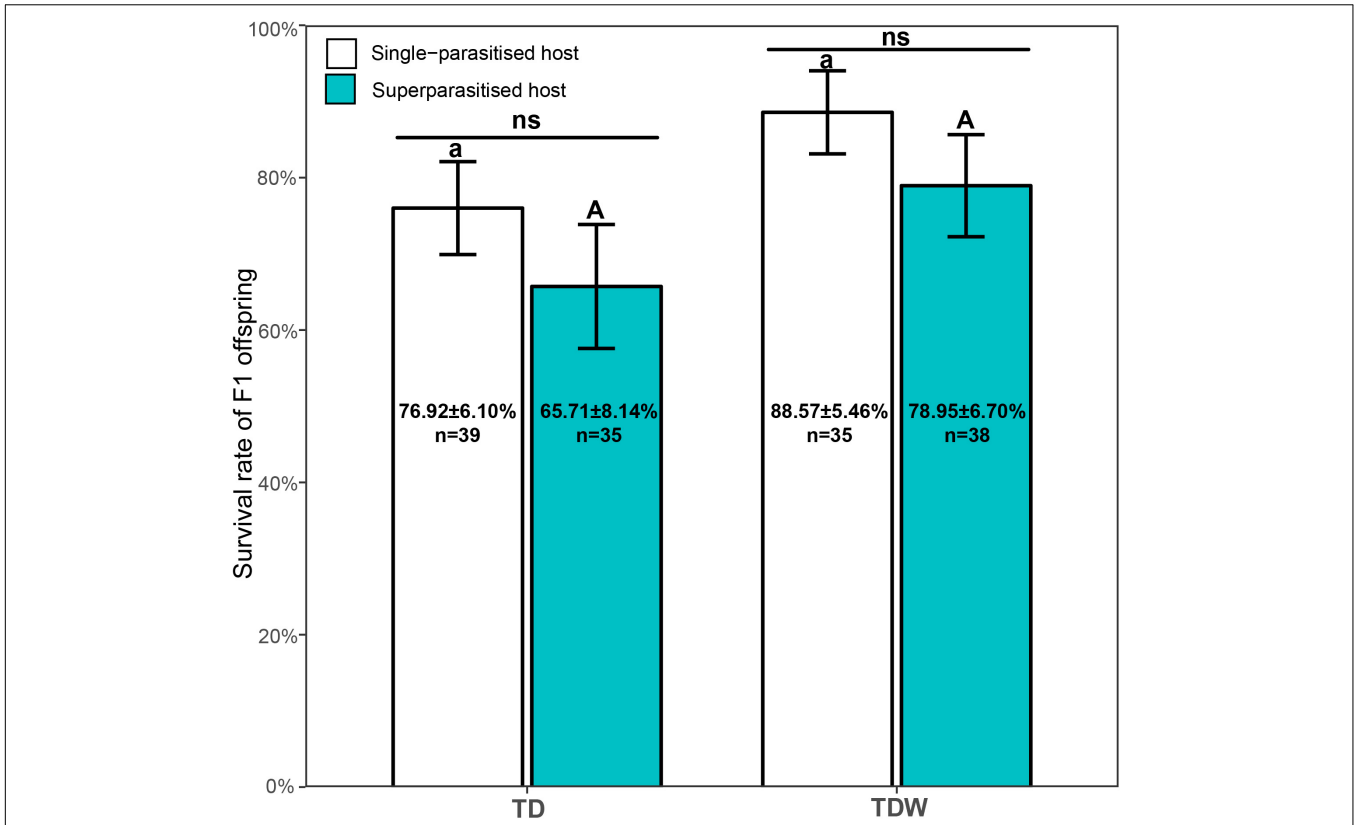


FIGURE 1 | Survival rate of TDW or TD F1 offspring in superparasitised (cyan bars) or single-parasitised (white bars) hosts. Bars indicate Means. Error bars indicate the interval of Mean ± Standard Error. The same uppercase letters indicate non-significant difference between TD (n = 35) and TDW (n = 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.

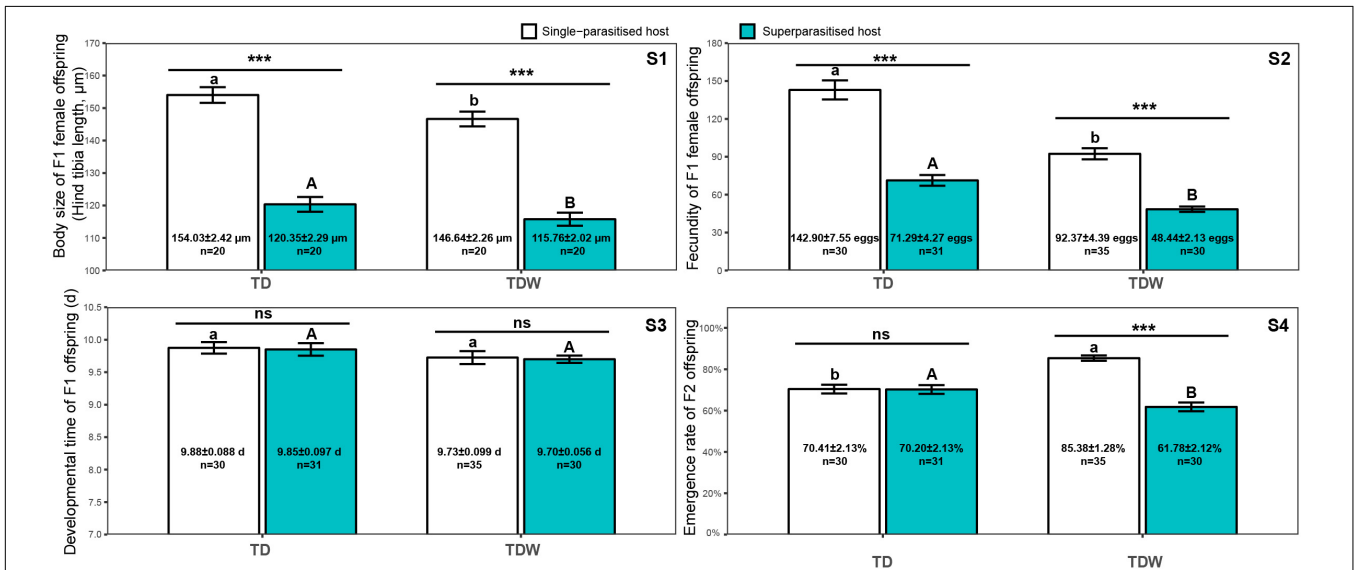


FIGURE 2 | Body size of F1 female offspring (S1), fecundity of F1 female offspring (S2), developmental time of F1 offspring (S3), and emergence rate of F2 offspring (S4) of TDW or TD line in superparasitised (cyan bars) or single-parasitised (white bars) hosts. Bars indicate Means. Error bars indicate 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.

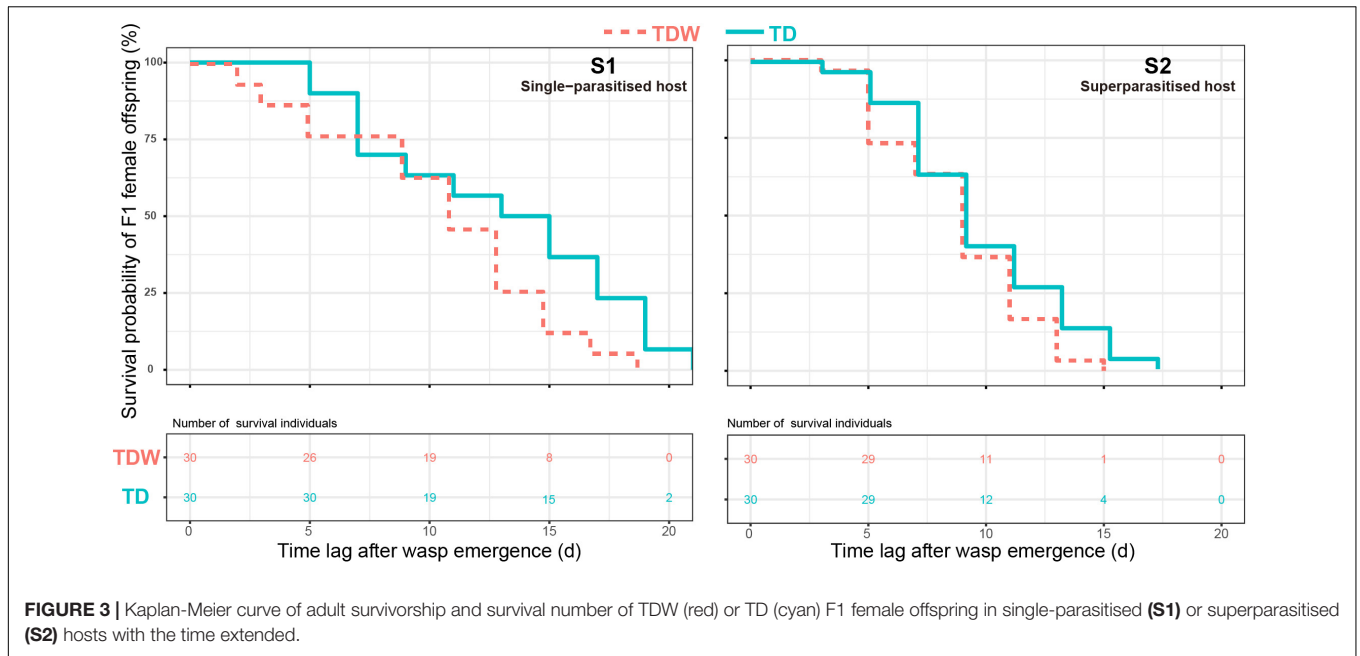


FIGURE 3 | Kaplan-Meier curve of adult survivorship and survival number of TDW (red) or TD (cyan) F1 female offspring in single-parasitised (S1) or superparasitised (S2) hosts with the time extended.

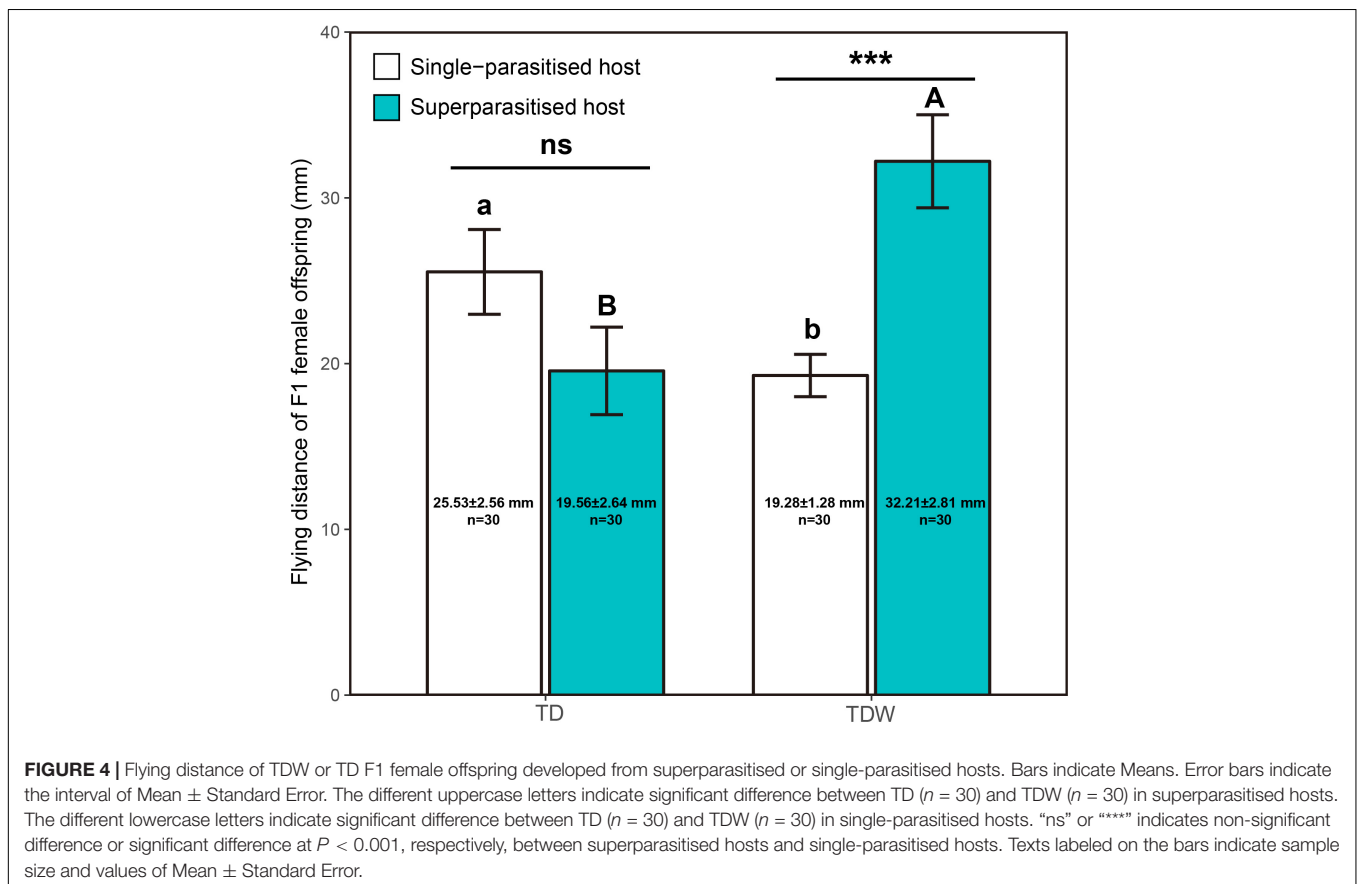


FIGURE 4 | Flying distance of TDW or TD F1 female offspring developed from superparasitised or single-parasitised hosts. Bars indicate Means. Error bars indicate the interval of Mean ± Standard Error. The different uppercase letters indicate significant difference between TD (n = 30) and TDW (n = 30) in superparasitised hosts. The different lowercase letters indicate significant difference between TD (n = 30) and TDW (n = 30) 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 adaptation 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 kaykai* 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 thelytokous *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), *Coccotrypes 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, XZ, 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.

FUNDING

This research was funded by the Projects of Guizhou Tobacco Corporation (201936, 201937, and 201941), the Major Projects of China National Tobacco Corporation [110202001032 (LS-01)], the National Natural Science Foundation of China (31972339), the Agricultural Science and Technology Innovation Program (CAAS-ZDRW202108), the Natural Science Foundation of

Liaoning Province (2020-BS-137), and the Fundamental Research Funds for the Universities of Liaoning Province (LR2019061).

ACKNOWLEDGMENTS

We should thank ELSEVIER Language Editing Services for improving the language.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fevo.2021.730664/full#supplementary-material>

REFERENCES

- Berk, R., and MacDonald, J. (2008). Overdispersion and poisson regression. *J. Quant. Criminol.* 24, 269–284. doi: 10.1007/s10940-008-9048-9044
- Beserra, E. B., Querino, R. B., and Parra, J. R. P. (2003). Occurrence of gynandromorphism in *Trichogramma pretiosum riley* (Hymenoptera: Trichogrammatidae). *Neotrop. Entomol.* 32, 507–509. doi: 10.1590/S1519-566X2003000300021
- Bowen, W. R., and Stern, V. M. (1966). Effect of temperature on the production of males and sexual mosaics in a uniparental race of *Trichogramma semifumatum* (Hymenoptera: Trichogrammatidae). *Ann. Entomol. Soc. Am.* 59, 823–834. doi: 10.1093/aesa/59.4.823
- Braig, H. R., Zhou, W., Dobson, S. L., and O'Neill, S. L. (1998). Cloning and characterization of a gene encoding the major surface protein of the bacterial endosymbiont *Wolbachia pipientis*. *J. Bacteriol.* 180, 2373–2378. doi: 10.1128/JB.180.9.2373-2378.1998
- Braquart-Varnier, C., Altinli, M., Pigeault, R., Chevalier, F. D., Greve, P., Bouchon, D., et al. (2015). The mutualistic side of *Wolbachia*–isopod interactions: *wolbachia* mediated protection against pathogenic intracellular bacteria. *Front. Microbiol.* 6:1388. doi: 10.3389/fmicb.2015.01388
- Brownlie, J. C., and Johnson, K. N. (2009). Symbiont-mediated protection in insect hosts. *Trends Microbiol.* 17, 348–354. doi: 10.1016/j.tim.2009.05.005
- Caragata, E. P., Real, K. M., Zalucki, M. P., and McGraw, E. A. (2011). *Wolbachia* infection increases recapture rate of field-released *Drosophila melanogaster*. *Symbiosis* 54, 55–60. doi: 10.1007/s13199-011-0124-4
- Charnov, E. L., and Skinner, S. W. (1984). Evolution of host selection and clutch size in parasitoid wasps. *Florida Entomol.* 67, 5–21. doi: 10.2307/3494101
- Cook, J. M. (1993). Sex determination in the Hymenoptera: a review of models and evidence. *Heredity* 71, 421–435. doi: 10.1038/hdy.1993.157
- Cox, D. R. (1972). Regression models and life-tables. *J. R. Stat. Soc.* 74, 187–120. doi: 10.1111/j.2517-6161.1972.tb00899.x
- Devescovi, F., Bachmann, G. E., Nussenbaum, A. L., Viscarret, M. M., Cladera, J. L., and Segura, D. F. (2017). Effects of superparasitism on immature and adult stages of *Diachasmimorpha longicaudata* Ashmead (Hymenoptera: Braconidae) reared on *Ceratitidis capitata* Wiedemann (Diptera: Tephritidae). *Bull. Entomol. Res.* 107, 756–767. doi: 10.1017/S000748531700027X
- Du, W. M., Xu, J., Hou, Y. Y., Lin, Y., Zang, L. S., Yang, X. B., et al. (2018). *Trichogramma* parasitoids can distinguish between fertilized and unfertilized host eggs. *J. Pest Sci.* 91, 771–780. doi: 10.1007/s10340-017-0919-z
- Duval, J., Brodeur, J., Doyon, J., and Boivin, G. (2018). Impact of superparasitism time intervals on progeny survival and fitness of an egg parasitoid. *Ecol. Entomol.* 43, 310–317. doi: 10.1111/een.12502
- Ebrahimi, V., Ashouri, A., Rugman-Jones, P. F., Lindsey, A. R. I., Javan-Nikkhah, M., and Stouthamer, R. (2019). Using parthenogenesis-inducing *Wolbachia* for the selection of optimal lines of the egg parasitoid *Trichogramma pretiosum* for use in biocontrol. *Entomol. Exp. Appl.* 167, 241–251. doi: 10.1111/eea.12755
- Liaoning Province (2020-BS-137), and the Fundamental Research Funds for the Universities of Liaoning Province (LR2019061).
- Evans, O., Caragata, E. P., McMeniman, C. J., Woolfit, M., Green, D. C., McGraw, E. A., et al. (2009). Increased locomotor activity and metabolism of *Aedes aegypti* infected with a life-shortening strain of *Wolbachia pipientis*. *J. Exp. Biol.* 212, 1436–1441. doi: 10.1242/jeb.028951
- Farahani, K. H., Ashouri, A., Goldansaz, S. H., Farrokhi, S., Ainouche, A., and Baaren, J. (2015). Does *Wolbachia* infection affect decision-making in a parasitic wasp? *Entomol. Exp. Appl.* 155, 102–116. doi: 10.1111/eea.12293
- Flanders, S. E. (1937). Notes on the life history and anatomy of *Trichogramma*. *Ann. Entomol. Soc. Am.* 30, 304–308. doi: 10.1093/aesa/30.2.304
- Grote, A., Voronin, D., Ding, T., Twaddle, A., and Ghedin, E. (2017). Defining *Brugia malayi* and *Wolbachia* symbiosis by stage-specific dual RNA-seq. *PLoS Neglected Trop. Dis.* 11:e0005357. doi: 10.1371/journal.pntd.0005357
- Guo, M. (1993). Study of parasitizing behaviour of *Trichogramma* (IV)—progeny number allocation and sex allocation. *Nat. Enemies Insects* 15, 51–59.
- Harvey, J. A., Poelman, E. H., and Tanaka, T. (2013). Intrinsic inter and intraspecific competition in parasitoid wasps. *Annu. Rev. Entomol.* 58, 333–351. doi: 10.1146/annurev-ento-120811-153622
- Herre, E. A. (1993). Population structure and the evolution of virulence in nematode parasites of fig wasps. *Science* 259, 1442–1445. doi: 10.1126/science.259.5100.1442
- Heslin, L. M., and Merritt, D. J. (2005). Cannibalistic feeding of larval *Trichogramma carverae* parasitoids in moth eggs. *Naturwissenschaften* 92, 435–439. doi: 10.1007/s00114-005-0015-8
- Hoffmann, A. A., Ratna, E., Sgro, C. M., Barton, M., Blacket, M., Hallas, R., et al. (2007). Antagonistic selection between adult thorax and wing size in field released *Drosophila melanogaster* independent of thermal conditions. *J. Evol. Biol.* 6, 2219–2227. doi: 10.1111/j.1420-9101.2007.01422.x
- Hohmann, C. L., and Luck, R. F. (2000). Effect of temperature on the development and thermal requirements of *Wolbachia*-infected and antibiotically cured *Trichogramma kaykai* Pinto and Stouthamer (Hymenoptera: Trichogrammatidae). *Anais Soc. Entomol. Do Brasil* 25, 3888–3898. doi: 10.1590/s0301-80592000000300012
- Hohmann, C. L., Luck, R. F., and Stouthamer, R. (2001). Effect of *Wolbachia* on the survival and reproduction of *Trichogramma kaykai* Pinto & Stouthamer (Hymenoptera: Trichogrammatidae). *Neotrop. Entomol.* 30, 607–612. doi: 10.1590/S1519-566X2001000400015
- Hosokawa, T., Koga, R., Kikuchi, Y., Meng, X. Y., and Fukatsu, T. (2010). *Wolbachia* as a bacteriocyte-associated nutritional mutualist. *Proc. Natl. Acad. Sci. U S A.* 107, 769–774. doi: 10.1073/pnas.0911476107
- Huang, J., Hua, H. Q., Wang, L. Y., Zhang, F., and Li, Y. X. (2017a). Number of attacks by *Trichogramma dendrolimi* (Hymenoptera: Trichogrammatidae) affects the successful parasitism of *Ostrinia furnacalis* (Lepidoptera: Crambidae) eggs. *Bull. Entomol. Res.* 107, 1–8. doi: 10.1017/S0007485317000335
- Huang, J., Hua, H. Q., Zhang, F., and Li, Y. X. (2017b). Suitability assessment of three *Trichogramma* species in the control of *Mythimna separata* (Lepidoptera: Noctuidae). *J. Appl. Entomol.* 142, 131–140. doi: 10.1111/jen.12414

- Huigens, M. E., de Almeida, R. P., Boons, P. A. H., Luck, R. F., and Stouthamer, R. (2004a). Natural interspecific and intraspecific horizontal transfer of parthenogenesis-inducing Wolbachia in *Trichogramma* wasps. *Proc. R. Soc. B-Biol. Sci.* 271, 509–515. doi: 10.1098/rspb.2003.2640
- Huigens, M. E., Hohmann, C. L., Luck, R. F., Gort, G., and Stouthamer, R. (2004b). Reduced competitive ability due to Wolbachia infection in the parasitoid wasp *Trichogramma kaykai*. *Entomol. Exp. Appl.* 110, 115–123. doi: 10.1111/j.0013-8703.2004.00126.x
- Iqbal, A., Chen, Y. M., Hou, Y. Y., Zhang, L. S., Desneux, N., and Zang, L. S. (2019). Factitious host species impact on the outcome of multiparasitism between egg parasitoids. *J. Pest Sci.* 92, 1261–1269. doi: 10.1007/s10340-019-01122-8
- Iqbal, A., Hou, Y. Y., Chen, Y. M., Ali, A., Monticelli, L. S., Desneux, N., et al. (2020). Impact of *Trichogramma* parasitoid age on the outcome of multiparasitism in the factitious host eggs of Chinese oak silkworm. *Antheraea pernyi*. *J. Pest Sci.* 93, 1347–1357. doi: 10.1007/s10340-020-01239-1
- Ju, J. F., Bing, X. L., Zhao, D. S., Guo, Y., Xi, Z. Y., Hong, X. Y., et al. (2020). Wolbachia supplement biotin and riboflavin to enhance reproduction in planthoppers. *ISME J.* 14, 676–687. doi: 10.1038/s41396-019-0559-9
- Kong, J., Peng, H., Chen, H. Y., and Bao, J. Z. (1988). Unusual mating behaviour of *Trichogramma dendrolimi* (Hym. Trichogrammatidae) reared on oak silkworm eggs. *Chinese J. Biol. Control* 4, 50–54. doi: 10.16409/j.cnki.2095-039x.1988.02.001
- Lack, D. (1947). The significance of clutch-size. *IBIS* 89, 309–352. doi: 10.1111/j.1474-919X.1947.tb04155.x
- Landmann, F., Foster, J. M., Slatko, B., and Sullivan, W. (2010). Asymmetric Wolbachia segregation during early *Brugia malayi* embryogenesis determines its distribution in adult host tissues. *PLoS Neglected Trop. Dis.* 4:e758. doi: 10.1371/journal.pntd.0000758
- Li, L. Y. (1994). “Worldwide use of *Trichogramma* for biological control on different crops: a survey,” in *Biological Control with Egg Parasitoids*, eds E. Wajnberg and S. A. Hassan (Wallingford: CABI).
- Li, Y. X., Dai, G. H., and Fu, W. J. (2008). Suitability of *Corcyra cephalonica* to three *Trichogramma* species and change of the content of free amino acids in its eggs parasitized. *Acta Entomol. Sinica* 51, 628–634. doi: 10.16380/j.kcxb.2008.06.012
- Liu, Q. Q., Zhang, T. S., Li, C. X., Gu, J. W., Hou, J. B., and Dong, H. (2018). Decision-making in a bisexual line and a thelytokous Wolbachia-infected line of *Trichogramma dendrolimi* Matsumura (Hymenoptera: Trichogrammatidae) toward their hosts. *Pest. Manag. Sci.* 74, 1720–1727. doi: 10.1002/ps.4867
- Ma, W. J., and Schwander, T. (2017). Patterns and mechanisms in instances of endosymbiont-induced parthenogenesis. *J. Evol. Biol.* 30, 868–888. doi: 10.1111/jeb.13069
- Martel, V., and Boivin, G. (2010). Unequal distribution of local mating opportunities in an egg parasitoid. *Ecol. Entomol.* 32, 393–398. doi: 10.1111/j.1365-2311.2007.00895.x
- Martinez, J., Duploux, A., Woolfit, M., Vavre, F., O'Neill, S. L., and Varaldi, J. (2012). Influence of the virus LbFV and of Wolbachia in a host-parasitoid interaction. *PLoS One* 7:e35081. doi: 10.1371/journal.pone.0035081
- Miura, K., and Tagami, Y. (2004). Comparison of life history characters of arrhenotokous and Wolbachia-associated thelytokous *Trichogramma kaykai* Pinto and Stouthamer (Hymenoptera: Trichogrammatidae). *Ann. Entomol. Soc. Am.* 97, 765–769.
- Ning, S. F., Zhou, J. C., Liu, Q. Q., Zhao, Q., and Dong, H. (2019). Gradual, temperature-induced change of secondary sexual characteristics in *Trichogramma pretiosum* infected with parthenogenesis-inducing Wolbachia. *PeerJ* 7:e7567. doi: 10.7717/peerj.7567
- Parratt, S. R., Frost, C. L., Schenkel, M. A., Rice, A., Hurst, G. D., and King, K. C. (2016). Superparasitism drives heritable symbiont epidemiology and host sex ratio in a wasp. *PLoS Pathogens* 12:e1005629. doi: 10.1371/journal.ppat.1005629
- Pu, Z. (1983). Studies on three potential pathogens, *Staphylococcus*, *Rickettsialike* and *Microplasma-like* organisms of *Trichogramma* spp and their causative diseases of the hosts. *Acta Sci. Nat. Universitatis Sunyatseni* 4, 118–122.
- Qin, Y. L. (2015). Morphological and molecular characterization of *Nosema pernyi*, a microsporidian parasite in *Antheraea pernyi*. *Parasitol. Res.* 114, 3327–3336. doi: 10.1007/s00436-015-4558-0
- R Core Team (2020). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.
- Rahimi Kaldeh, S., Ashouri, A., Bandani, A., and Modarres Hasani, S. A. (2017). The maternal effect of temperature on diapause induction in Wolbachia-infected and uninfected *Trichogramma brassicae*. *Plant Pest Res.* 7, 25–35. doi: 10.22124/IPRJ.2017.2437
- Rahimi-Kaldehy, S., Ashouri, A., Bandani, A., and Tomioka, K. (2017). The effect of Wolbachia on diapause, fecundity, and clock gene expression in *Trichogramma brassicae* (Hymenoptera: Trichogrammatidae). *Dev. Genes Evol.* 227, 401–410. doi: 10.1007/s00427-017-0597-0
- Ruan, C., Zhu, X., Meng, Z., and Su, R. (2000). Influences of *Antheraea pernyi* diseases on eggs host of *Trichogramma*. *J. Jilin Agricul. University* 3, 22–26.
- Russell, J. E., and Stouthamer, R. (2011). The genetics and evolution of obligate reproductive parasitism in *Trichogramma pretiosum* infected with parthenogenesis-inducing Wolbachia. *Heredity* 106, 58–67. doi: 10.1038/hdy.2010.48
- Schilthuisen, M., and Stouthamer, R. (1997). Horizontal transmission of parthenogenesis-inducing microbes in *Trichogramma* wasps. *Proc. R. Soc. B-Biol. Sci.* 264, 361–366. doi: 10.1098/rspb.1997.0052
- Sheng, S., Feng, S., Meng, L., and Li, B. P. (2014). Departure mechanisms for host search on high density patches in the parasitoid *Meteorus pulchricornis* (Wesmael) (Hymenoptera: Braconidae). *J. Insect. Sci.* 14, 1–5. doi: 10.1093/jisesa/ieu067
- Smith, S. M. (1996). Biological control with *Trichogramma*: advances, successes, and potential of their use. *Annu. Rev. Entomol.* 41, 375–406. doi: 10.1146/annurev.en.41.010196.002111
- Stouthamer, R. (1993). The use of sexual versus asexual wasps in biological control. *Entomophaga* 38, 3–6. doi: 10.1007/BF02373133
- Stouthamer, R. (1997). “Wolbachia-induced parthenogenesis,” in *Influential Passengers: Inherited Microorganisms and Arthropod Reproduction*, eds S. L. O'Neill, A. A. Hoffmann, and J. H. Werren (Oxford: Oxford University Press), 102–122.
- Stouthamer, R., and Luck, R. F. (1993). Influence of microbe-associated parthenogenesis on the fecundity of *Trichogramma deion* and *T. pretiosum*. *Entomol. Exp. Appl.* 67, 183–192. doi: 10.1111/j.1570-7458.1993.tb01667.x
- Stouthamer, R., Russell, J. E., Vavre, F., and Nunney, L. (2010). Intra-genomic conflict in populations infected by parthenogenesis inducing Wolbachia ends with irreversible loss of sexual reproduction. *BMC Evol. Biol.* 10:229. doi: 10.1186/1471-2148-10-229
- Strunov, A., Kiseleva, E., and Gottlieb, Y. (2013). Spatial and temporal distribution of pathogenic Wolbachia strain wmel pop in *Drosophila melanogaster* central nervous system under different temperature conditions. *J. Invertebrate Pathol.* 114, 22–30. doi: 10.1016/j.jip.2013.05.001
- Sun, J. T., Wang, M. M., Zhang, Y. K., Chapuis, M. P., Jiang, X. Y., Hu, et al. (2015). Evidence for high dispersal ability and mito-nuclear discordance in the small brown planthopper. *Laodelphax striatellus*. *Sci. Rep.* 5:8045.
- Tagami, Y., Miura, K., and Stouthamer, R. (2001). How does infection with parthenogenesis-inducing Wolbachia reduce the fitness of *Trichogramma*? *J. Invertebrate Pathol.* 78, 267–271. doi: 10.1006/jipa.2002.5080
- Takada, Y., Kawamura, S., and Tanaka, T. (2001). Host preference of *Trichogramma dendrolimi* (Hymenoptera: Trichogrammatidae) on its native host, *Mamestra brassicae* (Lepidoptera: Noctuidae) after 12 continuous generations on a factitious host. *Appl. Entomol. Zool.* 36, 213–218. doi: 10.1303/aer.2001.213
- Tremmel, M., Steinitz, H., Kliot, A., Harari, A., and Lubin, Y. D. (2020). Dispersal, endosymbiont abundance and fitness-related consequences of inbreeding and outbreeding in a social beetle. *Biol. J. Linnean Soc.* 129, 717–727. doi: 10.1093/biolinnean/blz204
- Tulgetskne, G. M., and Stouthamer, R. (2012). Characterization of intersex production in *Trichogramma kaykai* infected with parthenogenesis-inducing Wolbachia. *Naturwissenschaften* 99, 143–152. doi: 10.1007/s00114-011-0880-2
- Tunca, H., Colombel, E. A., Venard, M., and Tabone, E. (2017). Incidence of superparasitism in the egg parasitoid, *Ooencyrtus kuvanae* Howard (Hymenoptera: Encyrtidae). *Biocontrol Sci. Technol.* 27, 796–808. doi: 10.1080/09583157.2017.1342765
- van Alphen, J. J. M., and Visser, M. E. (1990). Superparasitism as an adaptive strategy for insect parasitoids. *Annu. Rev. Entomol.* 35, 59–79. doi: 10.1146/annurev.en.35.010190.000423
- van Dijken, M. J., and Waage, J. K. (1987). Self and conspecific superparasitism by the egg parasitoid *Trichogramma evanescens*. *Entomol. Exp. Appl.* 43, 183–192.

- Vavre, F., de Jong, J. H., and Stouthamer, R. (2004). Cytogenetic mechanism and genetic consequences of thelytoky in the wasp *Trichogramma cacoeciae*. *Heredity* 93, 592–596. doi: 10.1038/sj.hdy.6800565
- Vavre, F., Girin, C., and Boulétreau, M. (1999). Phylogenetic status of a fecundity-enhancing Wolbachia that does not include thelytoky in *Trichogramma*. *Insect Mol. Biol.* 8, 67–67. doi: 10.1046/j.1365-2583.1999.810067.x
- Walker, S. H., and Duncan, D. B. (1967). Estimation of the probability of an event as a function of several independent variables. *Biometrika* 54, 167–178. doi: 10.1093/biomet/54.1-2.167
- Wang, L., Huang, J., Dong, X., Zhang, F., and Li, Y. X. (2015). Superparasitism and ontogeny of two *Trichogramma* species on *Corcyra cephalonica* (Stainton). *Chinese J. Biol. Control* 31, 481–486. doi: 10.16409/j.cnki.2095-039x.2015.04.006
- Wang, Y., Xiang, M., Hou, Y. Y., Yang, X. B., Dai, H. J., Zang, L. S., et al. (2019). Impact of egg deposition period on the timing of adult emergence in *Trichogramma* parasitoids. *Entomol. Generalis* 39, 339–346. doi: 10.1127/entomologia/2019/0896
- Wang, Y., Zou, Z. P., Hou, Y. Y., Yang, X., and Zang, L. S. (2020). Manually-extracted unfertilized eggs of Chinese oak silkworm, *Antheraea pernyi*, enhance mass production of *Trichogramma* parasitoids. *Entomologia Generalis* 40, 397–406. doi: 10.1127/entomologia/2020/1060
- Wang, Z. Y., He, K. L., Zhang, F., Lu, X., and Babendreier, D. (2014). Mass rearing and release of *Trichogramma* for biological control of insect pests of corn in China. *Biol. Control* 68, 136–144. doi: 10.1016/j.biocontrol.2013.06.015
- Werren, J. H., Baldo, L., and Clark, M. E. (2008). Wolbachia: master manipulators of invertebrate biology. *Nat. Rev. Microbiol.* 6, 741–751. doi: 10.1038/nrmicro1969
- Yang, C. C., Wang, J. L., and Zhang, J. (1990). Screening the diet prescription for rice moth rearing. *J. Shenyang Agricultural University* 21, 48–52.
- Zang, L. S., Wang, S., Zhang, F., and Desneux, N. (2020). Biological control with trichogramma in china: history, present status, and perspectives. *Annu. Rev. Entomol.* 66, 463–484. doi: 10.1146/annurev-ento-060120-091620
- Zboralski, A., Vilaralle, M., Colombel, E., Tabone, E., and Vercken, E. (2016). Density-dependent dispersal in biological control agents: a reflexion on the side-effects of mass-rearing conditions. *BioControl* 61, 13–22. doi: 10.1007/s10526-015-9696-x
- Zhang, D., Wang, Y., He, K., Yang, Q., and Chen, L. (2020). Wolbachia limits pathogen infections through induction of host innate immune responses. *PLoS One* 15:e0226736. doi: 10.1371/journal.pone.0226736
- Zhang, H. Y. (2009). *Wolbachia Transfection and Biological Control Capability of Trichogramma spp.* thesis, China: Shenyang Agricultural University. PhD Dissertation.
- Zhang, J. J., Zhang, X., Zang, L. S., Du, W. M., Hou, Y. Y., Ruan, C. C., et al. (2018). Advantages of diapause in *Trichogramma dendrolimi* mass production on eggs of the Chinese silkworm. *Antheraea pernyi*. *Pest. Manag. Sci.* 74, 959–965. doi: 10.1002/ps.4795
- Zhao, L. N., Ma, Y., Yang, X., Iqbal, A., and Zang, L. S. (2019). Identification of *Serratia marcescens* isolated from *Antheraea pernyi* eggs and determination of bacterial pathogenicity and transmission pathway. *J. Invertebr. Pathol.* 169:107297. doi: 10.1016/j.jip.2019.107297
- Zhou, J. C., Dong, Q. J., Zhang, T. S., Duan, L. J., Ning, S. F., Liu, Q. Q., et al. (2019a). Effect of wind time on the dispersal capacity of *Trichogramma dendrolimi* Matsumura (Hymenoptera Trichogrammatidae). *J. Asia-Pacific Entomol.* 3, 742–749. doi: 10.1016/j.aspen.2019.06.001
- Zhou, J. C., Li, Y. Y., Liu, Q. Q., Ning, S. F., Che, W. N., Cong, B., et al. (2019b). Effects of temperature and superparasitism on quality and characteristics of thelytokous Wolbachia-infected *Trichogramma dendrolimi* Matsumura (Hymenoptera: Trichogrammatidae) during mass rearing. *Sci. Rep.* 9:18114. doi: 10.1038/s41598-019-54719-5
- Zhou, J. C., Liu, Q. Q., Wang, Q. R., Ning, S. F., Che, W. N., and Dong, H. (2020). Optimal clutch size for quality control of bisexual and Wolbachia-infected thelytokous lines of *Trichogramma dendrolimi* Matsumura (Hymenoptera: Trichogrammatidae) mass reared on eggs of a substitutive host, *Antheraea pernyi* Guerin-Meneville (Lepidoptera: Saturniidae). *Pest Manag. Sci.* 76, 2635–2644. doi: 10.1002/ps.5805

Conflict of Interest: The authors declare that this study received funding from Projects of Guizhou Tobacco Corporation. The funder was not involved in the study design, collection, analysis, interpretation of data, the writing of this article or the decision to submit it for publication.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2021 Zhou, Zhao, Liu, Shang, Zhao, Huo, Dong and Zhang. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.