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Ratio-dependent competitions between a *Wolbachia*-uninfected bisexual strain and *Wolbachia*-infected thelytokous strain of the egg parasitoid, *Trichogramma dendrolimi* Matsumura (Hymenoptera: Trichogrammatidae)

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Abstract

Background *Wolbachia*-infected thelytokous *Trichogramma* wasps have been considered as potential effective biocontrol agents against lepidopteran pests in agriculture and forests. However, intra-specific competition may arise when *Wolbachia*-infected thelytokous *Trichogramma* coexist with their uninfected bisexual counterparts in fields or during mass-rearing procedures. This competition could potentially impact the efficacy of *Trichogramma* females in biological control programs.

Results This study examined the parasitic capacities of bisexual (*TdB*) and *Wolbachia*-infected thelytokous (*TdT*) strains of *Trichogramma dendrolimi* as influenced by host density (ranging from 5 to 70 host eggs) and wasp density (two, four, and six females per arena). The experiments involved inter-strain competition between *TdB* and *TdT* strains (Inter-*Td*) as well as intra-strain competition within the *TdT* (Intra-*TdT*) or *TdB* (Intra-*TdB*) strains. Results revealed that females in the Inter-*Td* group exhibited a higher attack rate compared to those in the Intra-*TdT* and Intra-*TdB* groups, irrespective of wasp density. Specifically, when six females were present, the Inter-*Td* group showed significantly shorter host handling times than the Intra-*TdT* group. Within intra-strain cohorts, *TdT* females displayed a lower attack rate than *TdB* females when four or six females were present, and longer host handling times when six females were present. Mutual interference between individuals was not affected by competition types. Females of the Inter-*Td* group exhibited a higher probability of superparasitism than those of the Intra-*TdB* group or Inter-*Td* group. When both strains coexisted, the proportion of *TdT* offspring with six females was higher than that with two females or four females. Male ratios of bisexual offspring in Inter-*Td* groups were higher than those in Intra-*TdB* groups. The male ratio of bisexual offspring in the Inter-*Td* group increased with wasp density.

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Conclusions Our study highlights the negative impact of *Wolbachia* on parasitic capacity of *Trichogramma* females, increasing the likelihood of superparasitism. The coexistence of both strains enhances total parasitic efficacy, albeit resulting in a higher proportion of male offspring compared to scenarios with only uninfected females. The results will provide the reference for the usage of *Wolbachia*-infected thelytokous strain of *T. dendrolimi* in augmentative biological control programs.

Keywords *Trichogramma dendrolimi*, Thelytokous, *Wolbachia*, Intraspecific competition, Parasitic efficacy

Background

Wolbachia is a maternally transmitted, symbiotic bacterium harbored in numerous arthropod species (Werren et al. 2008; Liu et al. 2019). It has been known to manipulate the reproductive process of the host including: cytoplasmic incompatibility (CI), male-killing, parthenogenesis inducing (PI), and feminization (Werren et al. 1995, 2008; Liu et al. 2019; Katsuma et al. 2022). The parthenogenesis induced by PI-*Wolbachia* has been found in at least 33 species of parasitic Hymenoptera and 15 species comprising the *Trichogramma* genus (Stouthamer et al. 1993; Liu et al. 2018). The egg parasitoid wasps, *Trichogramma* spp. (Hymenoptera: Trichogrammatidae), are the most popular biological control agents for controlling lepidopteran pests (Li 1994; Smith 1996; Zhou et al. 2019a; Zang et al. 2021). Even without mating, *Wolbachia*-infected *Trichogramma* females can produce nearly 100% females. This trait is often termed as “thelytoky” (Stouthamer et al. 1990; Zang et al. 2021). Compared with bisexual-reproducing parasitoids, thelytokous parasitoids can be a superior biological control agent due to some of the advantages: (1) easier population establishment because released females can produce generations of female offspring without mating; (2) a potentially higher capacity of reproduction since all offsprings are female (Stouthamer and Luck 1993; Pannebakker et al. 2004; Liu et al. 2018). Many studies have documented the coexistence of *Wolbachia*-infected and uninfected *Trichogramma* females within the same habitat. For instance, *Wolbachia* infection has been observed in 4–26% of *T. kaykai* wasps and approximately 4.5% of *T. turkestanica* individuals in field conditions (Stouthamer 1993; Huigens et al. 2004; Liu et al. 2019; Zhou et al. 2019a). Moreover, the mass release of *Wolbachia*-infected female wasps may lead to intense competition between these infected wasps and local uninfected ones, potentially impacting the control efficacy of these wasps in biological pest control programs (Lindsey and Stouthamer 2017b; Zang et al. 2021).

Wolbachia can not only affect the reproductive phenotype but also influence other important biological characteristics of its host wasps (Zang et al. 2021; Zhou et al. 2022, 2023; Zhang et al. 2022). Thelytokous *Trichogramma* had been found to often perform lower emergence rate, smaller body size, lower fecundity, and shorter

adult longevity than the bisexual strain (Stouthamer and Luck 1993; Hohmann et al. 2001; Tagami et al. 2001; Miura and Tagami 2004; Zhou et al. 2022). Positive or neutral effects on the fitness of host *Trichogramma* have also been reported (Girin and Boulétreau 1995; Wade and Chang 1995). Although the effects of *Wolbachia* on wasps' fitness have been studied in a large body of researches, the behavior changes of host wasps induced by *Wolbachia* have been rarely investigated.

The optimal foraging theory (OFT) predicts that wasp individuals should be selected to exploit the resources more efficiently (Stephens and Krebs 1986; Godfray 1994). As the host egg is the only nutrition resource available to the immature parasitoid offspring, parasitoid wasps have to ensure the survival of their offspring and decide which host should be accepted or rejected by estimating the nutritional quality of their hosts. Females could discriminate between unparasitized and parasitized hosts and often avoid depositing their eggs on the parasitized hosts (Godfray 1994). However, following infection by *Wolbachia*, wasps often perform behavioral changes, and can result from adaptations of the parasites to enhance its vertical transmission from mother wasps to offspring or horizontal transmission among wasp individuals (Parratt et al. 2016; Zhou et al. 2019a) and negative side effects on host wasps (Huigens et al. 2004; Farahani et al. 2015; Parratt et al. 2016). These implied the foraging behavior of wasps might be largely manipulated by *Wolbachia* and therefore, the efficacy of attacking insect pests has been affected. The potential competition may occur especially when host resources are limited in certain seasons (Lindsey and Stouthamer 2017a, b). Firstly, once a female finds a host egg mass, leaving it would mean a potential risk of failing to find a new one (Van Alphen and Visser 1990). Thus, the parasitoids may aggregate in the host patches since the limited resource of host eggs was in a clumped distribution (Van Alphen and Visser 1990; Lindsey and Stouthamer 2017b). Secondly, in biological control programs, the augmentative biological control strategy is often applied by releasing large amounts of mass-reared *Trichogramma* females for a fast suppression of the pest population (Zhou et al. 2019b). Once *Wolbachia*-infected thelytokous or uninfected bisexual *Trichogramma* females are

mass released in the field with a limited density of pest eggs, the competition among released populations could be fierce (Lindsey and Stouthamer 2017b; Zang et al. 2021). Thirdly, during the mass-rearing procedure of *Trichogramma*, the intra-specific competition of females may also occur especially when host eggs are supplied to excess females, and may subsequently reduce the fitness of *Trichogramma* offsprings and increase the cost of rearing (Zhou et al. 2019a). Therefore, the investigation of the intraspecific competition and parasitic capacity of bisexual strain and *Wolbachia*-infected thelytokous strain of *Trichogramma* are particularly important for the improvement of mass rearing and field application of *Trichogramma*. In this study, experiments were conducted to focus primarily on the following two questions: (1) Is the *Wolbachia*-infected thelytokous strain of *Trichogramma* a better biological control agent than its uninfected bisexual conspecifics in the condition of inter- and intra-strain competitions? (2) Does *Wolbachia* infection affect the oviposition strategy of *Trichogramma* females when they encounter their same strain females or their uninfected bisexual conspecifics? Early studies have shown that *Wolbachia* would induce the retention of the ability to evaluate host quality (Liu et al. 2018; Godfray 1994). We hypothesize these behavior changes may consequently affect the parasitic efficacy of the wasps.

Among all *Trichogramma* species, *Trichogramma dendrolimi* Matsumura (Hymenoptera: Trichogrammatidae) is an important biological control agent against lepidopteran pests in China (Zhang et al. 2018; Zang et al. 2021). The massive production of *T. dendrolimi* has been inundatively released against multiple species of lepidopteran pest across millions of hectares annually in the crop area in China since the 1980s (Zhang et al. 2018; Li et al. 2019; Ma et al. 2006). To assess the parasitic capacity and competitive ability of a bisexual strain and *Wolbachia*-infected thelytokous strain of *T. dendrolimi* in inter- and intra-strain competitions, we investigate the parasitic capacity and oviposition strategies of the females as influenced by host density and wasp density in the cohorts of their inter- or intra-strain. The results will provide the reference for the usage of *Wolbachia*-infected thelytokous strain of *T. dendrolimi* in augmentative biological control programs and highlight possible parasitic behavior manipulation by *Wolbachia*.

Materials and methods

Insects

The insects used in this study, including the bisexual *Wolbachia*-uninfected strain (*TdB*) and the thelytokous *Wolbachia*-infected strain (*TdT*) of *T. dendrolimi* and *Corcyra cephalonica* (the host of *T. dendrolimi*), were maintained by the Biological Control Laboratory of

Shenyang Agricultural University. All insects were reared at $70 \pm 5\%$ RH, 25 ± 1 °C, and L16:D8.

Both *T. dendrolimi* strains were originally obtained from one bisexual isofemale line. The *TdT* strain of *T. dendrolimi* was first obtained by artificially transferring *Wolbachia* into *TdB* females. Thereafter, the females could produce nearly 100% female bias even without mating. The infection by *Wolbachia* in *T. dendrolimi* individuals was detected by specific primers (691R: 5'-AAAAATTAACGCTACTCCA-3' and 81F: 5'-TGG TCCAATAAGTGATGAAGAAAC-3') for the *wsp* gene of PI *Wolbachia* (Braig et al. 1998; Liu et al. 2019). Both *T. dendrolimi* strains were reared over 40 generations in the laboratory. The host larvae, *C. cephalonica*, were reared on an artificial diet (Yang et al. 1990).

Experimental procedure

Three factors were considered in this study: host density, wasp density (2, 4, and 6 females per arena), and competition group (intra-strain competition of *TdB* strain (Intra-*TdB*), intra-strain competition of *TdT* strain (Intra-*TdT*), and inter-strain competition between *TdB* and *TdT* strains (Inter-*Td*). To obtain different host densities, the host egg card (50 mm × 10 mm) with a random number (varied from 5 to 70) of host eggs was used in this study. To make the comparisons of the competition types, there were three treatments of exposure of hosts to different cohorts of females for 4 h: (1) "Intra-*TdB*" was set as the host eggs were supplied to two, four, or six females of *TdB* strain; (2) "Intra-*TdT*" was set as the host eggs were supplied to two, four, or six females of *TdT* strain; (3) "Inter-*Td*" was set as the hosts were supplied to two, four, or six females of the mixture of *TdB* and *TdT* strains with one *TdT* female and one *TdB* female, two *TdT* females and two *TdB* females, or three *TdT* females and three *TdB* females, respectively.

In this study, the host egg card with different host densities was originally transferred into a small glass tube (10 mm length, 8 mm diameter). Thereafter, a group of two, four, or six females of Intra-*TdB*, Intra-*TdT*, or Inter-*Td* group were introduced into the tube for parasitization. The duration for parasitization was set for 4 h, after which the wasps were removed. The parasitized *C. cephalonica* egg cards were reared until the eggs blackened, which indicated the pre-pupal stage; unsuccessful parasitism was indicated by the failure to blacken. The parasitized host eggs were cut off and transferred singly into new glass tubes. The number of parasitized host eggs on an egg card was recorded. After that, the blackened host egg was reared individually until the wasp(s) emerged. After the wasp(s) emerged, the blackened eggs were dissected under a dissection microscope (Olympus-SZX16, Japan) to confirm whether the egg contained the dead body(ies) of other *Trichogramma* offspring or not. The parasitism form of a host egg was classified into two types:

(1) single-parasitism and (2) superparasitism. The single-parasitism form was defined as a host egg containing only a single *T. dendrolimi* offspring, and the superparasitism form was determined as a host egg containing more than one *T. dendrolimi* offspring. The frequency of superparasitism in each egg card was recorded. The sexes of offspring individuals were detected based on the characteristics of tentacles,

time. DeLong and Vasseur reviewed different methods and found that the Ardit-Akçakaya model was unbiased and that intermediate values of m were likely to be more common (DeLong and Vasseur 2011). Thus, the Ardit-Akçakaya model was applied to account for interference behavior between female individuals in Intra-*TdB*, “Intra-*TdT*”, and Inter-*Td* groups in this study (Eq. 4).

$$Ne = \frac{P^{2m} + ahN^2 + PaTN - \sqrt{(P^{2m} + ahN^2 + PaTN)^2 - 4TPha^2N^3}}{2ahN} \quad (4)$$

and the male number in each egg card was recorded. The male ratio of the bisexual offspring in each egg card supplied to the mother females of Intra-*TdB* and Inter-*Td* groups were calculated, respectively. To distinguish between the thelytokous female offspring and bisexual female offspring emerging from the same egg card in the Inter-*Td* group, each emerged virginal female was supplied with an egg card containing approximately 100 eggs for a duration of 24 h. Thelytokous females were identified when all their offspring were females, while bisexual females were identified when their offspring consisted of a mix of males and females.

Data analysis

The modified Holling type 2 and Holling type 3 models based on Rogers’ random predator equation were applied to test the parasitic efficacy of wasp individuals as the function of wasp density and host density (Rogers 1972; Lang et al. 2011). The type of functional response was determined by the most parsimonious model with the lower value of Akaike’s Information Criterion (AIC) (Symonds and Mousalli 2011). The Rogers’ random foraging equation could be described as.

$$Ne = N(1 - \exp(\beta(Neh - PT))) \quad (1)$$

where Ne is the number of parasitized host eggs, at the wasp density, P , supplied by different host densities, N . The variable T is the time duration of host eggs supplied to wasps. The parameter h is the host handling time. The coefficient β could be described as.

$$\beta = a \quad (2)$$

Or.

$$\beta = aN \quad (3)$$

for the Holling type 2 (Eq. 2) and Holling type 3 (Eq. 3), respectively. The parameter a is used to describe the attacking rate of parasitoid wasps.

How to estimate the coefficients that describe the mutual interference among parasitoids has been debated for a long

The model estimated the coefficient, m , that could describe the mutual interference among parasitoids as the function of host density and wasp density. The coefficient m was tested by the *t*-test and differed from 0 (pure prey dependence) to 1 (pure wasp ratio dependence). Holling type 2, Holling type 3, and Ardit-Akçakaya models were fitted by nonlinear function through Newton’s iteration method using the “nlr” package in R software (<https://cran.r-project.org/web/packages/nlsr/vignettes/nlsr-dev-doc.pdf>) (R Developmental Core Team 2018).

Generalized linear models (GLMs) were applied to analyze the probability of superparasitism (Binomial distribution) as influenced by host density, wasp density, and competition group. To estimate the competitive capacity of *TdT* and *TdB* females in intra-strain competition, the ratio of *TdT* and *TdB* offspring (Binomial distribution) as influenced by host density and wasp density was analyzed by GLM as well. To investigate the sex allocation strategy of *TdB* females in Intra-*TdB* and Inter-*Td* groups, male ratios of *TdB* offspring as influenced by host density, wasp density, and two different competition groups were analyzed by GLM.

Homogeneities of GLMs were examined by using Shapiro tests. All analyses were conducted by using R version 3.6.2 (Koenker 1981; Hall 1992; R Developmental Core Team 2018). All data were expressed as Mean \pm SE unless otherwise specified.

Results

Determination of functional response models

The AIC values of Holling type 2 models were significantly lower than those of Holling type 3 models regardless of wasp density and competition group (Table 1). This suggested the type 2 functional response is the better model to describe the functional responses of female individuals in different competition types.

Table 1 Parameters estimated by Holling II and Holling III models

Model	Wasp density	Competition type	a ± SE	t	P	h ± SE	t	P	AIC
Holling II	2	Intra- <i>TdB</i>	0.10 ± 0.032 b	3.23	0.0032	0.21 ± 0.16 a	1.30	0.20	165.65
		Intra- <i>TdT</i>	0.070 ± 0.012 b	6.01	<0.001	0.23 ± 0.17 a	0.10	0.031	135.81
		Inter- <i>Td</i>	0.16 ± 0.029 a	5.59	<0.001	0.052 ± 0.059 a	0.88	0.39	138.20
	4	Intra- <i>TdB</i>	0.062 ± 0.015 a	4.18	<0.001	0.29 ± 0.20 a	1.42	0.17	156.04
		Intra- <i>TdT</i>	0.039 ± 0.0073 b	5.31	<0.001	0.37 ± 0.20 a	0.16	0.026	160.56
		Inter- <i>Td</i>	0.055 ± 0.011 a	5.09	<0.001	0.30 ± 0.18 a	1.68	0.10	145.05
	6	Intra- <i>TdB</i>	0.056 ± 0.013 a	4.19	<0.001	0.010 ± 0.19 b	0.056	0.96	162.39
		Intra- <i>TdT</i>	0.010 ± 0.0030 b	3.53	<0.001	1.92 ± 0.81 a	2.37	0.025	178.60
		Inter- <i>Td</i>	0.041 ± 0.0092 a	4.42	<0.001	0.39 ± 0.29 b	1.34	0.19	156.14
Holling III	2	Intra- <i>TdB</i>	0.0095 ± 0.0022 b	4.35	<0.001	0.15 ± 0.032 a	6.73	<.001	183.87
		Intra- <i>TdT</i>	0.0041 ± 0.00061 c	6.65	<0.001	0.12 ± 0.033 a	3.56	0.0014	165.27
		Inter- <i>Td</i>	0.011 ± 0.0014 a	7.92	<0.001	0.16 ± 0.010 a	15.21	<.001	148.54
	4	Intra- <i>TdB</i>	0.0042 ± 0.00072 a	5.83	<0.001	0.25 ± 0.37 a	0.058	0.88	173.70
		Intra- <i>TdT</i>	0.0021 ± 0.00034 b	6.05	<0.001	0.18 ± 0.037 a	4.93	<0.001	181.88
		Inter- <i>Td</i>	0.0031 ± 0.00048 a	6.31	<0.001	0.20 ± 0.50 a	0.0010	0.99	169.75
	6	Intra- <i>TdB</i>	0.0031 ± 0.00062 a	4.96	<0.001	0.45 ± 0.52 a	0.062	0.86	181.86
		Intra- <i>TdT</i>	0.00073 ± 0.00015 b	4.82	<0.001	0.15 ± 0.13 a	1.17	0.25	200.74
		Inter- <i>Td</i>	0.0025 ± 0.00042 a	5.89	<0.001	0.34 ± 0.69 a	0.0011	0.99	176.49

Different letters indicate significant differences ($P < 0.05$) between different competition types within the same wasp density

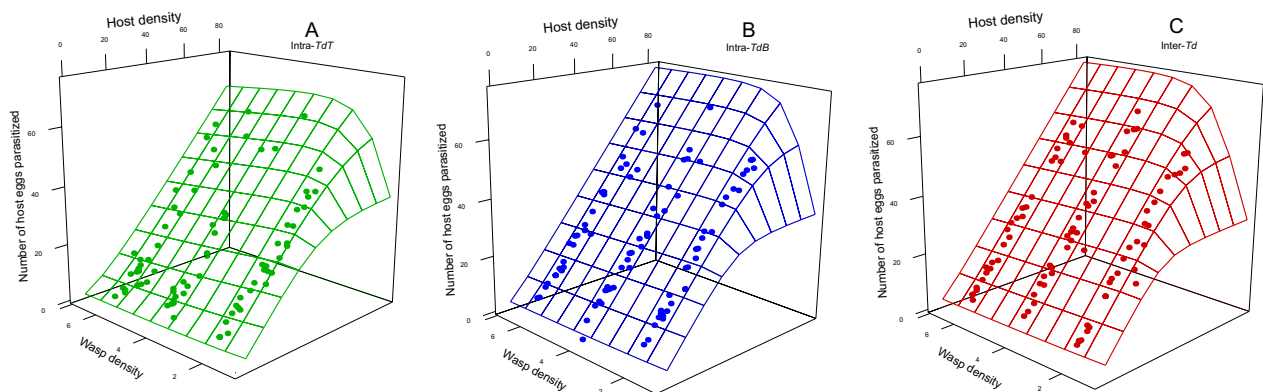


Fig. 1 Parasitized host number as a function of wasp density and host density in intra-*TdT* (A), intra-*TdB* (B), and inter-*Td* (C) competitions fitted by Holling II functional responses. The grid surfaces are predicted values from Holling II models. The jitter method is applied to avoid overlaps of points in the figures

Functional response and mutual interference among parasitoids

The females in the Inter-*Td* group exhibited a significantly higher attack rate compared to those in the Intra-*TdT* and Intra-*TdB* groups, regardless of wasp density. When two or four females were provided, those in the Inter-*Td* group showed no difference in host handling time compared to those in the Intra-*TdT* and Intra-*TdB* groups. However, with six females, those in the Inter-*Td* group had a significantly shorter host

handling time than those in the Intra-*TdT* group, but showed no difference compared to those in the Intra-*TdB* group (Table 1; Fig. 1).

Within the intra-strain cohorts, when six females were provided, those in the Intra-*TdT* group displayed a significantly lower attack rate and longer host handling time than those in the Intra-*TdB* group. With four females, the Intra-*TdT* group showed a lower attack rate than the Intra-*TdB* group but had no difference in host handling time. With only two females, there were

no significant differences in attack rate and host handling time between the Intra-*TdT* and Intra-*TdB* groups (Table 1; Fig. 1).

The Arditi-Akçakaya model showed a significant mutual interference among parasitoids in all competition types, but the value of coefficient m , which indicates the level of mutual interference, remained similar among three competition types (Intra-*TdT*: $m = 0.94 \pm 0.12$, $t = 7.95$, $P < 0.001$;

Intra-*TdB*: $m = 0.93 \pm 0.19$, $t = 4.95$, $P < 0.001$; Inter-*Td*: $m = 0.89 \pm 0.15$, $t = 5.96$, $P < 0.001$).

Probability of superparasitism

The probability of superparasitism was significantly affected by competition types ($\chi^2 = 47.54$, $df = 2$, $P < 0.001$), and the interaction of host density and wasp density ($\chi^2 = 6.06$, $df = 1$, $P = 0.014$). The average probability of superparasitism in Intra-*TdT* group ($18.00 \pm 1.59\%$) was 2.92 times higher than that in Intra-*TdB* group

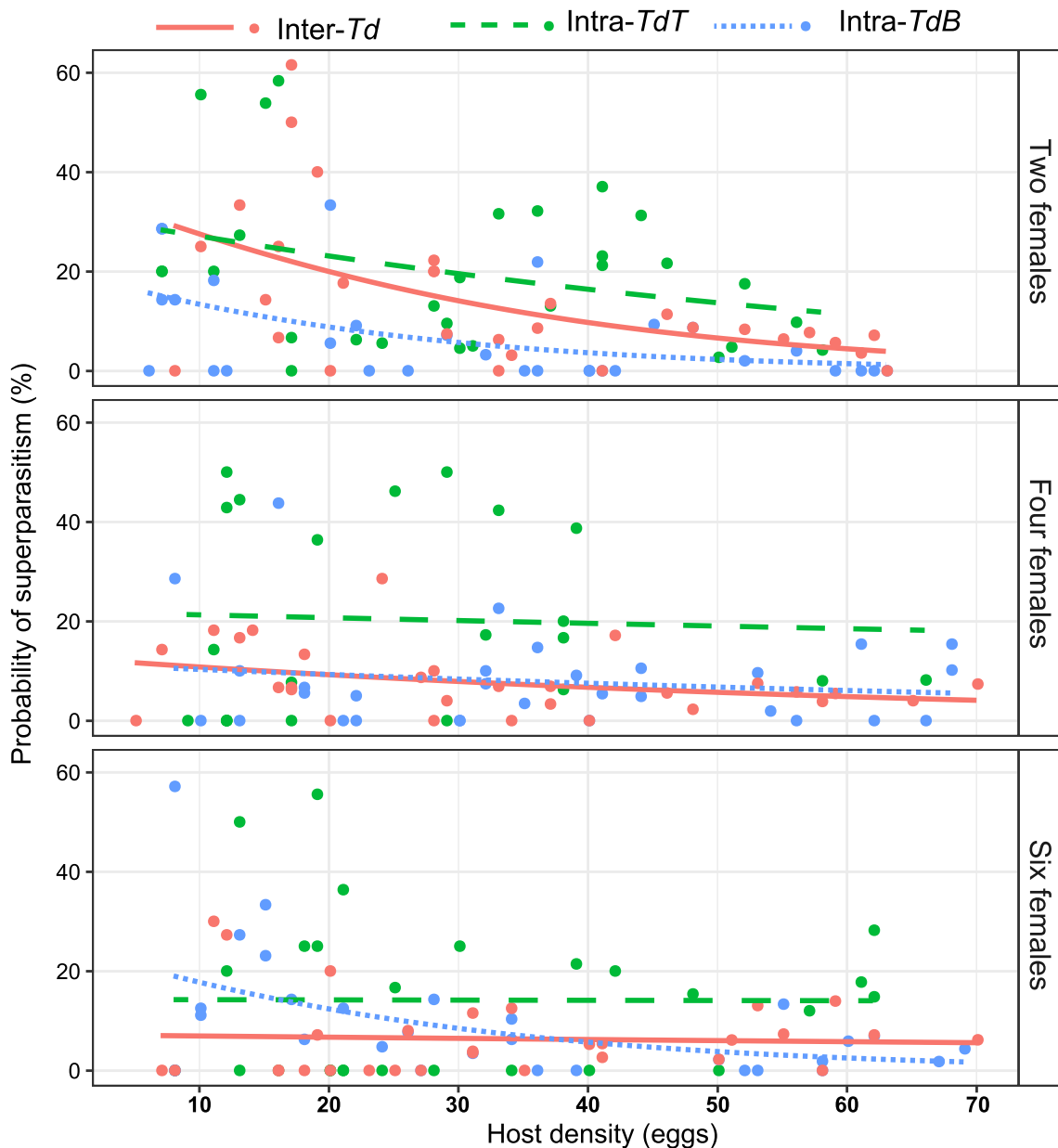


Fig. 2 Probability of superparasitism as influenced by wasp density, competition types, and host density. The lines are fitted by predicted probability from logistic models

($7.00 \pm 0.85\%$; $z=6.49$, $P<0.001$), and 2.27 times higher than that in Inter-*Td* group ($8.81 \pm 0.95\%$; $z=5.26$, $P<0.001$). When only two females were supplied, the probability of superparasitism significantly decreased with increasing host density in Intra-*TdT* group (Coefficient \pm SE: -0.021 ± 0.0073 ; $z=2.91$, $P=0.0036$), Intra-*TdB* group (-0.050 ± 0.016 ; $z=2.99$, $P=0.0058$), and Inter-*Td* group (-0.040 ± 0.0091 ; $z=4.35$, $P<0.001$). When four females were supplied, host density did not affect the probability of superparasitism in the Intra-*TdT* group (-0.0077 ± 0.012 ; $z=0.66$, $P=0.52$), Intra-*TdB* group (0.0066 ± 0.016 ; $z=0.41$, $P=0.68$), and Inter-*Td* group (-0.013 ± 0.0091 ; $z=1.42$, $P=0.17$). However, with six females, the likelihood of superparasitism significantly decreased with increasing host density in the Intra-*TdB* group (-0.034 ± 0.011 ; $z=3.09$, $P=0.0047$), but it was not true in Intra-*TdT* group (0.013 ± 0.012 ; $z=1.05$, $P=0.30$) and Inter-*Td* group (-0.0028 ± 0.012 ; $z=0.24$, $P=0.81$)(Fig. 2).

The competitive capacity of *TdT* and *TdB* females in inter-strain competition

The ratio of *TdT* and *TdB* offspring was not influenced by host density ($\chi^2=0.14$, $df=1$, $P=0.71$), and the interaction of host density and wasp density ($\chi^2=3.67$, $df=2$, $P=0.16$) (Fig. 3), but was significantly affected by wasp density ($\chi^2=23.88$, $df=2$, $P<0.001$). When six females were supplied, the proportion of *TdT* offspring (Mean \pm SE: $60.08 \pm 2.78\%$) was significantly higher than that when two females were supplied (Mean \pm SE: $42.03 \pm 2.90\%$; $z=3.61$, $P<0.001$) or when four females were supplied (Mean \pm SE: $46.45 \pm 2.71\%$; $z=4.61$, $P<0.001$). However, there was no significant difference ($z=1.16$, $P=0.48$) in the proportion of *TdT* offspring when four females were supplied compared to when only two females were supplied (Fig. 3D).

Male ratios of *TdB* offspring in Intra-*TdB* and Inter-*Td* groups

The male ratio of *TdB* offspring was significantly affected by host density ($\chi^2=18.80$, $df=1$, $P<0.001$), competition groups ($\chi^2=88.65$, $df=1$, $P<0.001$), and the interaction of wasp density and two competition

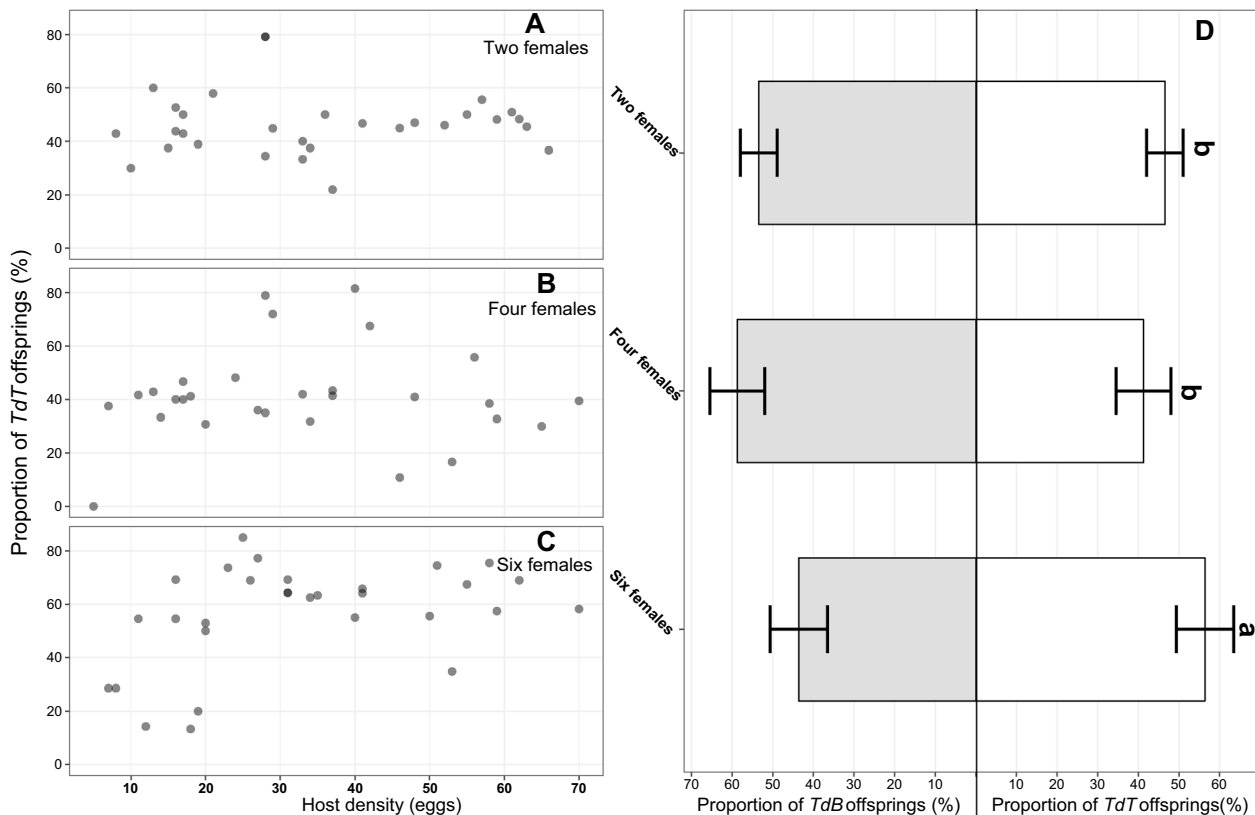


Fig. 3 Proportion of *TdT* offspring (A–C) and *TdB* offspring (D) as influenced by wasp density and host density. Error bars indicate the 95% confidential intervals. Different lowercase letters indicate the significant differences at $P<0.05$

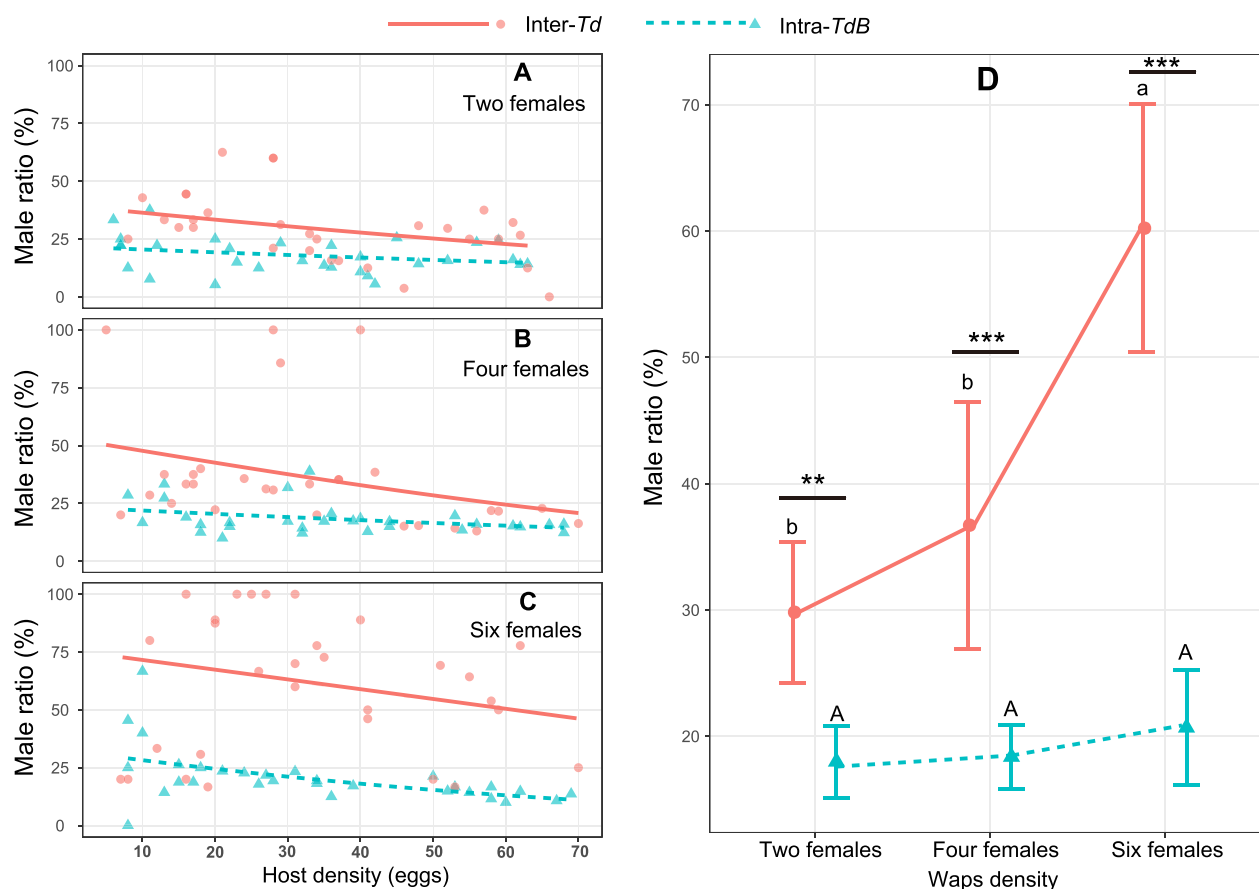


Fig. 4 The male ratio of *TdB* offspring was influenced by host density (A–C) and wasp density (A–D) in the Inter-*Td* or Intra-*TdB* group. The lines are fitted by predicted ratio from logistic models. Different upper letters indicate significant differences ($P < 0.05$) between different wasp densities in the Intra-*TdB* group. Different lower letters indicate significant differences ($P < 0.05$) between different wasp densities in the Inter-*Td* group. The “***” and “****” indicate significant differences between the Intra-*TdB* group and Inter-*Td* group as $P < 0.01$ and 0.001 , respectively

types ($\chi^2 = 28.78$, $df = 2$, $P < 0.001$). The male ratio of *TdB* offspring significantly decreased with the increase in host density (Slope \pm SE: -0.012 ± 0.0027 , $z = 4.34$, $P < 0.001$). The average male ratios of *TdB* offspring in Inter-*Td* groups were significantly higher ($t = 3.23$, $P < 0.001$) than those in Intra-*TdB* groups. Interestingly, the male ratio of bisexual offspring in the Inter-*Td* group with six females ($60.15 \pm 3.29\%$) was significantly higher than that with two females ($29.47 \pm 2.49\%$; $z = 6.51$, $P < 0.001$) or with four females ($36.97 \pm 2.64\%$; $z = 6.05$, $P < 0.001$). However, when hosts were supplied to females of the Intra-*TdB* group, the male ratio of offspring was not affected by wasp density ($\chi^2 = 2.25$, $df = 2$, $P = 0.32$) (Fig. 4).

Discussion

Based on functional response analysis within intra-strain cohorts, *TdT* females exhibited a lower attacking rate than *TdB* females when four or six females were supplied, and they also showed longer host handling time

when six females were supplied. These findings suggest that *TdT* females may experience decreased parasitic capacity under higher wasp density conditions. Previous studies reported infected females showed a lower host discrimination ability and spent more time than their counterparts in accepting or rejecting hosts, which may consequently affect the parasitic capacity of infected females (Farrokhi et al. 2010; Farahani et al. 2015; Liu et al. 2018).

The declined parasitic capacity of infected females under high wasp density can be illustrated by two potential explanations: (1) PI *Wolbachia* may negatively affect its hosts’ fitness, including lower fecundity (Stouthamer and Luck 1993), shorter longevity (Hohmann et al. 2001; Miura and Tagami 2004), and lower emergence rates (Hohmann et al. 2001; Miura and Tagami 2004), and then consequently negatively affect host searching ability and parasitic function. Farahani et al. (2015) observed infected females spent more time feeding instead of oviposition and inferred that infected females had less

energy available than uninfected ones and that they needed to refuel more regularly (Farahani et al. 2015). (2) Infected females seemed to be unable to evaluate host quality properly. Previous studies reported infected females behaved similarly toward both fresh eggs (low quality) and old eggs (high quality), and spent more time than their counterparts in accepting or rejecting hosts, while uninfected displayed the optimal foraging behavior, and could visit more host eggs and parasitized more in fresh eggs than old eggs (Farahani et al. 2015; Liu et al. 2018). Thus uninfected females can discriminate between healthy eggs and parasitized eggs (Van Dijken and Waage 1987) and avoid superparasitism. Our findings indicate that infected females exhibited a higher likelihood of superparasitism, suggesting that infected females do not exercise host discrimination. When more wasps were introduced, a greater number of host eggs were immediately parasitized by these females. Consequently, infected females had to spend more time discriminating between parasitized and healthy eggs or re-parasitizing already parasitized eggs, which likely led to reduced attacking rates and prolonged host handling times.

Furthermore, our study revealed that *Wolbachia*-infected females had a significantly higher probability of superparasitism compared to *Wolbachia*-uninfected bisexual females. Notably, when only two females were introduced, the likelihood of superparasitism decreased with increasing host density. Conversely, when six females were introduced, bisexual females exhibited a relatively high probability of superparasitism under low host density conditions, while this probability decreased as host density increased. As the fitness of parasitoid offspring declined in superparasitism condition, superparasitism is often viewed as maladaptive for wasps (Stouthamer and Luck 1993; Hohmann et al. 2001; Tagami et al. 2001). To avoid superparasitism, females can evaluate host quality before depositing their eggs and can label the host eggs with host marking pheromone (HMP) after oviposition (Van Dijken and Waage 1987; Van Alphen and Visser 1990). However, a higher rate of superparasitism by infected wasps may be induced by *Wolbachia*. It is early known that *Wolbachia* could transmit horizontally from infected offspring to uninfected offspring when the offspring coexist and share the same host (Huigens et al. 2004; Farahani et al. 2015). As both strains coexist in the same niches, *Wolbachia* could gain an opportunity to enhance its spread in *Trichogramma* populations by inducing superparasitism of their host wasps (Farahani et al. 2015; Zhou et al. 2019a). Thus, some authors suspected *Wolbachia* may manipulate host behavior to increase the probability of superparasitism (Parratt et al. 2016; Zhou et al. 2019a). A potential explanation is that the higher rate of superparasitism is

possibly due to the memory retention of females induced by *Wolbachia*. Infected females may “forget” information on host quality or previously parasitized eggs after a time, and then lose the ability to estimate host quality (Farahani et al. 2015, 2017; Zhou et al. 2019a). Some studies also revealed *Wolbachia* can invade and replicate rapidly in the central nervous system of its host insects (Strunov et al. 2013, 2016). On the other hand, superparasitism may be an adaptive strategy for wasps in certain conditions. For example, even though the fitness of individual progeny declines in the super-parasitized host, total fitness may still increase as the number of offspring reaches a maximum (Van Dijken and Waage 1987; Van Alphen and Visser 1990). In this study, when both strains coexisted in the same egg patch and excess wasps were supplied (six females), infected females could produce more offspring than their counterparts (Fig. 4). In general, when healthy host eggs are rare (egg-limited model) or short-lived females have many mature eggs (time-limited model), remaining the host patch and re-parasitizing the hosts may be a better strategy (Van Dijken and Waage 1987; Van Alphen and Visser 1990; Godfray 1994). Hence these infected females may gain an edge by superparasitism, and consequently produce more offspring. Considering host eggs are limited in certain seasons in field conditions, females have to search for hosts quickly during their short life span. Thus, infected females would produce more offspring through superparasitism to minimize the “wastage” of hosts and offspring eggs (Lindsey and Stouthamer 2017b). As more infected offspring were produced, *Wolbachia* could vertically transmit to more individual progenies from infected mother females. However, superparasitism may be not a favorable factor for the application of egg parasitoids against pests in biological control programs, as unparasitized host eggs will be left to hatch.

Notably, females of the Inter-*Td* group performed a higher attacking rate than the Intra-*TdT* or Intra-*TdB* group, regardless of host density. This could be explained by our previous statements by the following: When host eggs were sufficient, uninfected females would display the optimal foraging behavior, visit more hosts, and prefer to parasitize high-quality host eggs than low-quality ones (Farahani et al. 2015; Liu et al. 2018), and some low-quality host eggs would be abandoned. However, infected females may have lost the ability to estimate host quality and could parasitize at least some low-quality host eggs. According to the present results, the mixed release of both strains may bring positive effects on the control of pests, as more host eggs would be parasitized due to the different parasitic behavior modes of these two strains.

Interestingly, when both strains coexisted, the male ratios of the bisexual offspring were significantly higher

than those when only the bisexual strain was supplied. Nevertheless, the male ratio of the bisexual offspring increased with wasp density in the Inter-*Td* group. Generally, parasitoid females could adjust the sex ratio of offspring under the local male competition (LMC) scenario. *Trichogramma* males often mate with their sibling females before dispersal in or on the same egg mass (Waage and Lane 1984; Luck et al. 2001; Martel et al. 2010). Because of this mating pattern, bisexual females prefer to produce more females and the least number of males necessary to inseminate their female siblings. Previous studies showed, that during each oviposition bout, *Trichogramma* females would produce the first male offspring at the second oviposition, and thereafter allocate one male offspring when the female deposit every eight offspring (Suzuki et al. 1984). When both strains coexisted, a portion of host eggs would be occupied by infected females, while uninfected bisexual females would produce fewer offspring on the limited host eggs. As each single female would produce the least number of males, the total male ratio would increase with an increase in wasp density.

In conclusion, our findings suggest that *Wolbachia* has negative effects on parasitic capacity and increases the likelihood of superparasitism. The coexistence of both strains enhances total parasitic efficacy, particularly at low wasp densities, and results in a higher proportion of male offspring compared to scenarios with only uninfected females present. These results imply that releasing a mixture of *Wolbachia*-infected thelytokous and bisexual strains of *Trichogramma* females may improve the pest control efficacy against pests in field conditions. Future studies should focus on optimizing pest control effectiveness and mass-rearing efficiency by adjusting the density and ratio of *Wolbachia*-infected and uninfected *Trichogramma* populations in biological control programs.

Acknowledgements

Not applicable.

Author contributions

HD conceptualized the idea. JCZ, WNC, and YH designed the experiments. QJD conducted sample collection and all experiments along with YH. JCZ analyzed the data. All graphical illustrations were prepared by JCZ and YZD. QJD drafted the manuscript initially. JCZ and HD revised the draft finally.

Funding

This research was funded by the National Key R&D Program of China (2023YFE0123000), Projects of the National Natural Science Foundation of China (32172476, 32102275), and Central Funds Guiding the Local Science and Technology Development (XZ202301YD0042C).

Availability of data and materials

The datasets are available from the corresponding author upon request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors consented to the publication of this manuscript.

Competing interests

All authors declare that they have no competing interests.

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Received: 18 January 2024 Accepted: 29 April 2024

Published online: 14 May 2024

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