

Full Length Research Paper

## Effect of some environmental and biological factors on reproductive characters of *Trichogramma* spp.

Haitao QIAN, Bin CONG\*, Zaolin ZHANG and Qihui DAI

Department of Plant Protection, Shenyang Agriculture University, Shenyang 110866, China.

Accepted 15 May, 2013

Many studies have demonstrated that many species of parasitoid wasps can control offspring sex ratio in response to environmental variables. This article tests the effects of temperature, relative humidity, photoperiod, foundress number, foundress age, host number, interval time of host supplying, species of food, species of host and symbiotic bacterium (*Wolbachia*) on sex ratio of *Trichogramma ostrinae*. Results showed that the sex ratio of *T. ostrinae* was affected significantly by temperature (35°C), number of foundress (>2 number), age of foundress (3-day-old) and *Wolbachia*. Through transmitted *Wolbachia* from *Trichogramma embryophagum* to *Trichogramma dendrolimi*, even the reproductive mode of *T. dendrolimi* was changed from gamogenesis to thelytoky. Our work verifies the sex ratio theory and implies that the potential effectiveness of *Trichogramma* spp. as a biological control agent can be further improved.

**Key words:** Environmental factors, biological factors, sex ratio, *Wolbachia*, *Trichogramma ostrinae*.

### INTRODUCTION

Most hymenopteran parasitoids are haplodiploid (White, 1973; Cook, 1993) and have the potential for controlling offspring sex ratio by regulating sperm access to eggs (Flanders, 1956). In fact, many studies have demonstrated that many species of parasitoid wasps can control offspring sex ratio in response to environmental variables. It has been reported that the sex ratio of parasitoid was affected by the superparasitism (Kanungo, 1955; Salt, 1936; Walker, 1967; Abram et al., 2012; Montoya et al., 2010), the host size (Ueno, 1998), the photoperiod (Bouletreau, 1976; Hoelscher and Vinson, 1971), the relative humidity (Legner, 1977), the number of wasp foundresses (Somjee et al., 2011) and the body size of male and female wasps (Kant et al., 2012) and so on.

Symbiotic microorganism has notable influence on the sex ratio of wasp. It was found that the thelytoky of *Muscidifurax uniraptor* and *Trichogramma deion* was

caused by the existence of rickettsia-like bacteria (*Wolbachia*), one endosymbiont of the wasps (Stouthamer and Luck, 1993). It is estimated that 16% of the insect species have this kind of microorganism (Werren, 1997). In arthropods, *Wolbachia* are generally facultative endosymbionts not involved in host survival and act as a key manipulator of host reproduction by inducing feminization, parthenogenesis, male killing, or cytoplasmic incompatibility (Duron and Gavotte, 2007). It can be vertically transmitted to progeny through eggs or be horizontally transmitted to other insects. Based on the amplification of part of *wsp* gene which encodes the outer membrane protein of *Wolbachia*, the presence of *Wolbachia* has been successfully detected in *A. gossypii* Glover. The parasitizing and bionomics behavior of *Trichogramma dendrolimi* have been studied in detail, but it lack the knowledge of *Wolbachia* affecting the sex ratio in this kind of parasitoid wasp. Through horizontal

\*Corresponding author. E-mail: bin1956@163.com, qianht1975@163.com.

**Table 1.** The relationship of saturated salt aqueous solution with the relative humidity.

Parameter	Saturated salt aqueous solutions						
	KOH	NaNO <sub>2</sub>	KOH	NaNO <sub>3</sub>	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	KCl	(Ba)Cl <sub>2</sub>
Relative humidity (%)	60	65	70	75	80	85	90

transmission of *Wolbachia* from *Trichogramma embryophagum* to *T. dendrolimi*, we study these biological factors on sex ratio *T. dendrolimi*.

The corn borner is a major pest of corn worldwide. Infestation rates have been reported to reach 30 to 50% when no adequate control methods were applied. *T. ostriniae* was the dominance parasite of corn borner eggs in field all over the world. At present, *T. ostriniae* can be mass produced and be used for biocontrol for the first and the second generation of *Ostrinia furnacalis* (Guenee) in China. So it is important for us to investigate the factors which affect the sex ratio of *T. ostriniae* in order to enhance the efficiency of mass production and biocontrol. In this study, the effects of several environmental and biological factors on sex ratio of *T. ostriniae* was examined.

## MATERIALS AND METHODS

### Study insects

*T. ostriniae*, *T. dendrolimi*, *Ostrinia furnacalis* (Guenée), *Galleria mellonella*, *Braconidae* spp, *Corcyra cephalonica* and *Antheraea pernyi* were kindly provided by Laboratory For Biological Control of Noxious Insects and Plants, Shenyang, China, *T. embryophagum* and *T. cacoeciae* were provided by Institute for Biological Control, BBA, Heinrichstr (243, D-64287, Darmstadt, Germany).

### The rearing of *O. furnacalis*

*O. furnacalis*, which were kept at 28 to 30°C, relative humidity (RH) 70 to 80% and a photoperiod of 16 light: 8 dark (L:D), were reared in the laboratory. Half of the eggs were used to maintain the population of *O. furnacalis* (come from Shenyang, Liaoning province, China) and the rest, which was sanitized in 2 to 5% formaldehyde for 15 to 20 min followed by three time distilled water washes, were used for experiment.

### Investigation of factors effect on reproductive characters of *T. ostriniae*

Mass mating was done an hour after eclosion. Then single female (The female had no access to host egg) was reared in a glass tube (13.3 mm in length × 10 mm in diameter) with a piece of cotton soaked with 10% honey for two hour at 23°C, RH 80% , 14:10 (L:D) (There no food were provided in experiment of the species of food at this two hour). After reared the female wasp was introduced into tube for experiments immediately. The factors affected on the reproductive characters of *T. ostriniae* were studied as follows: Temperature, relative humidity, photoperiod, foundress number, foundress age, host number, interval time of egg supplying, species of food and species of host. After the host was parasitized 24 h, the

female was removed out. The parasitized host eggs were reared at 23°C , RH 80% and 14:10 (L:D) in constant temperature incubator. When the hatched wasp died naturally, the male and female were counted under a stereomicroscope. All dead hosts were dissected immediately to determine whether or not they were parasitized. Each treatment was replicated three times.

### Temperature

The mated female wasp was provided by a egg-mass of *O. furnacalis* (about 80 eggs) and treated at five temperature conditions: 15, 20, 25, 30 and 35°C (14:10 (L:D), RH 60 to 80%). The temperature was controlled within±0.5°C of the set value.

### Relative humidity

Super saturation aqueous salt solution placing in desiccators was used to maintain the relative humidity (Rockland, 1960). The tube with the female put in the desiccators. In order to retain the same humidity with the desiccator, the nozzle of tube was covered with mesh gauze. Seven levels of relative humidity were carried out (Table 1) (14:10 (L:D), 23°C). The variation range of relative humidity (RH) was ±3%.

### Photoperiod

The offspring sex ratio of *T. ostriniae* affected by photoperiod was studied with thirteen treatments: 0:24, 2:22, 4:20, 6:18, 8:16, 10:14, 12:12, 14:10, 16:8, 18:6, 20:4, 22:2 and 24:0 (L:D). The photoperiod was controlled in diurnal growth incubator (23°C, RH80%).

### Foundress (Maternal wasp) number

The 1, 2, 4, 6 and 8 foundress was introduced into tube respectively. The number of eggs-mass for parasitizing was enhanced accordingly, so a ratio 1 female per 80 host eggs could be kept (14:10 (L:D), 23°C, RH80%).

### Foundress age

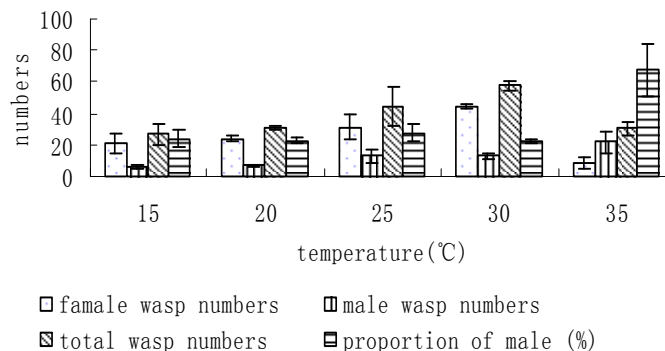
First day, second-third day and fourth day age of foundress were used for experimental respectively (14:10 (L:D), 23°C, RH80%).

### Host number

A mated foundress was placed in a glass tube provided by *O. furnacalis* egg-mass with 8 treatments: 10, 20, 30, 40, 50, 60, 70 and 80 eggs (14:10 (L:D), 23°C, RH80%).

### Interval time of host supplying

A mated foundress was placed in a glass tube provided by *O.*



**Figure 1.** Effect of temperature upon the sex ratio of *Trichogramma ostriniae*.

*furnacalis* egg-mass twice, the interval between them was: 0.5, 1, 2, 4, 6 and 8 h (14:10 (L:D), 23°C, RH 80%).

#### Species of food

The foundress was fed on water, 10% glucose, 10% honey and no food for experiment respectively. Sufficient food were provided until the end of experiment (14:10 (L:D), 23°C, RH 80%).

#### Species of host

Four forms of host egg were supplied for parasitizing, *O. furnacalis* only, *C. cephalonica* only, first *O. furnacalis* then *C. cephalonica* and first *C. cephalonica* then *O. furnacalis* (14:10 (L:D), 23°C, RH 80%).

#### Cytological analysis of *Wolbachia*

The Macchiavello method was used to dye the cell of *T. ostriniae* and *T. embryophagum*. The Rickettsia-like organism was stained with the modified Macchiavello's stain: Using a counterstain of 0.1% methylene blue in distilled water without phenol and by staining deparaffinized histological sections instead of air-dried tissue smears. Macchiavello stain-organisms were bright red against the blue background of the tissue.

#### Horizontal transmission of *Wolbachia*

##### Interspecific transfer between *Trichogramma*

*Wolbachia* was transferred between *T. dendrolim* and *T. embryophagum*, *T. ostriniae* and *T. cacoeciae*, *T. ostriniae* and *T. embryophagum*.

##### Interspecific transfer between order and family in insecta

**For the suspension of *Wolbachia*:** *T. embryophagum* infected by *Wolbachia* (check by PCR) was collected in an eppendorf tube and was placed in freezer for 5 min and then was added phosphate-buffered saline (PBS) and crushed with a pestle on ice. The suspension mixture was centrifuged for 15 min at 3100 rpm; then the supernatant was transferred to a clean eppendorf tube and centrifuged at 8300 rpm for 5 min. After discarding the supernatant,

the deposition was suspended in 100  $\mu$ l of PBS buffer, which was the suspension of *Wolbachia*. To prepare the capillary for injection, an injection needle was made by using glass capillary which was burnt and pulled on alcohol burner. For microinjection of *Braconidae*, *A. pernyi* pupa and *G. mellonella* larva, the *Wolbachia* suspension was injected with the glass-needles and microinjected in the pupa abdomen of *Braconidae*, in the third leg of *G. mellonella* old larva and in the first segment of *A. pernyi* pupa abdomen, and then developed to eclosion at 24°C and conserved at -20°C for test.

**Test for horizontal transfer of *Wolbachia* by *wsp* gen:** The presence of *Wolbachia* was verified using a *wsp* gene specific reaction by PCR. A total of 30 to 50 adult females were washed in double distilled water and then put into tube with 200  $\mu$ l extraction buffer (100 mmol/L Tris-HCl pH 7; 1.4 mol/L NaCl; 20 mmol/L EDTA; 2% hexadecyl trimethyl ammonium bromide (CTAB)). The tube was placed in -20°C icebox for five minutes and then taken out. The sample was crashed and incubated at 65°C for 1 h. Extracting with chloroform-isoarnylic alcohol (24:1), the DNA was washed with 70% ethanol, dried in air, resuspended in 50  $\mu$ l double distilled water and preserved at -20°C. The primers used to amplify the *wsp* gene were described by Zhou et al. (1998). 81FOR: 5'—TGG TCC AAT AAG TGA TGA AGA AAC; 691 REV: 5'—AAA AAT TAA ACG CTA CTC CA.

The PCR solution contained 2  $\mu$ l DNA template, 2  $\mu$ l 10 $\times$ buffer, 2  $\mu$ l, 25mmol/L MgCl<sub>2</sub>, 0.5  $\mu$ l 10mmol/L dNTPs, 0.5  $\mu$ l 20  $\mu$ mol/L of each primer, 1 Unit of Taq DNA polymerase and 12.3  $\mu$ l ddH<sub>2</sub>O. PCR reactions were performed in a GeneAmp PCR System PTC-200 thermal cycler. Cycle parameters were 30 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 1 min. After amplification, the product was analyzed on a 1.2% agarose gel to determine the presence and size of the amplified DNA.

#### Data analysis

All analysis were performed with statistical package SAS, version 8.2 (SAS Institute Inc.). The effect of factors on the sex ratio was compared using one-way analysis of variance (ANOVA) and means were separated using LSD test when the ANOVA was significant ( $P < 0.05$ ). The percentage data were transformed to arcsine square root before analysis.

## RESULTS

### Sex ratio distorters for *T. ostriniae*

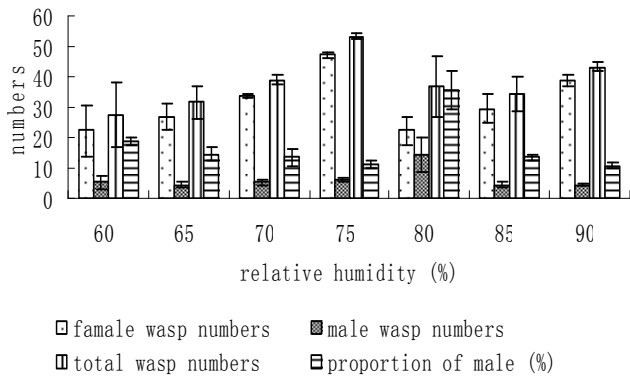
#### Temperature

Significant differences in sex ratio among the various temperatures were detected. From 15 to 30°C, the sex ratio had no significant change (about 20%). At 35°C, the sex ratio increased to 67.7% (Figure 1).

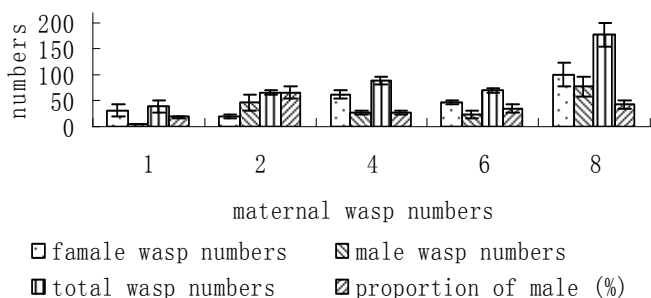
The number of female, male and the total *T. ostriniae* also could be remarkably influenced by temperature. The number increased linearly with temperature in the range of 15 $\leq$ T $\leq$ 30°C, but dropped at 35°C (30.33). The number was highest at 30°C (57.67).

#### Relative humidity

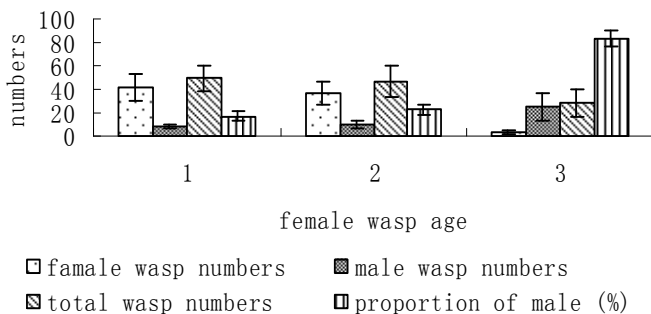
The sex ratio of did not differ significantly in all relative



**Figure 2.** Effect of relative humidity upon the sex ratio of *T. ostriniae*.



**Figure 3.** Effect of maternal wasp numbers upon the sex ratio of *T. ostriniae*.

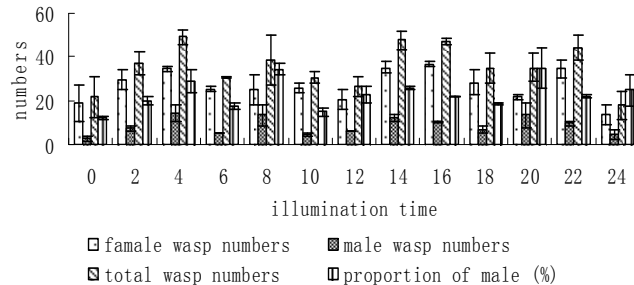


**Figure 4.** Effect of female wasp age upon the sex ratio of *T. ostriniae*.

humidity. At 80%, the sex ratio was highest (35.7%) (Figure 2). Humidity had a significant impact on the number of female, male and total of *T. ostriniae*. At 75% relative humidity, the number of offspring reached the highest values (53.33).

**Number of foundress**

Significant differences were found among the sex ratio of *T. ostriniae* at the foundress numbers studied (Figure 3).



**Figure 5.** Effect of photoperiod upon the sex ratio of *T. ostriniae*.

There was a significant difference between one and two foundresses. The sex ratio was 17.5 and 65.2% when only one foundress laid eggs and two foundresses laid eggs in the same environment, respectively. The proportion of female was also significantly lower than one foundress when four, six or eight foundresses laid eggs in the same environment. It was meant that the foundress could adjust the sex ratio of offspring according to the quantity of the foundress in the same environment.

The increasing of parasitism rate was not linear with the increasing of the quantity of foundress. The total quantity of offspring increased with the increasing of the quantity of foundress, but the average effective fecundity was about 20 when eight foundresses laid eggs in the same environment. It reduced double than only one foundress laid eggs.

**Age of foundress**

The sex ratio of *T. ostriniae* differed significantly in response to foundress age (Figure 4). The sex ratio of offspring increased with the increasing of foundress age, highest on 3-day-old foundress (83.6%).

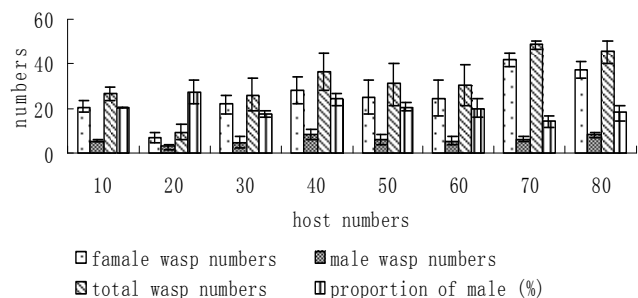
The fecundity was inversely to the foundress age. The fecundity of one-day-old foundress was at most (49), three-day-old at least (29).

**Photoperiod**

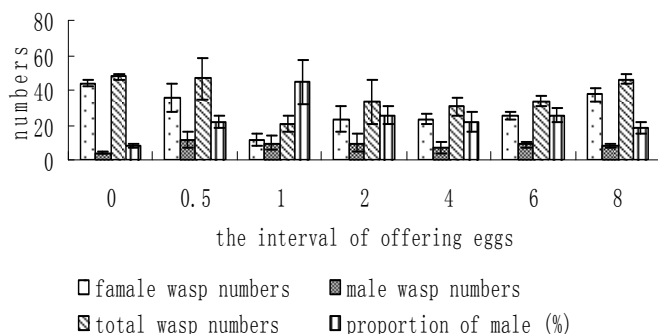
The sex ratio of *T. ostriniae* did not differ significantly at all photoperiod (Figure 5). The male proportion of offspring was lowest (12.1%) at treatment 0L:24D and highest (34.8%) at 20L:4D, ranged between 20 and 30% at other treatments. Photoperiod had not significant impact on fecundity. The fecundity was highest at treatments 14L:16D and lowest at 24L:0D .

**Quantity of host**

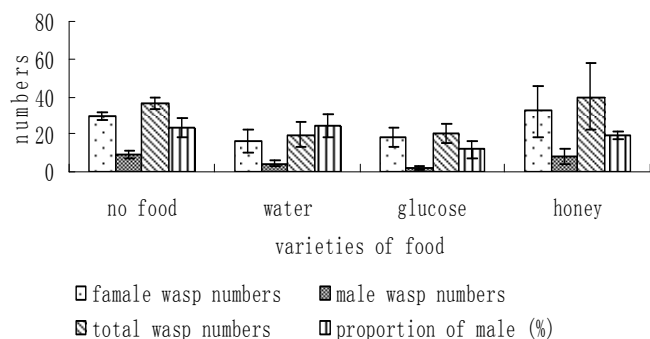
Quantity of host had not significant impact on the sex ratio of *T. ostriniae* (Figure 6). The sex ratio was about



**Figure 6.** Effect of host numbers upon the sex ratio of *T. ostrinia*.



**Figure 7.** Effect of the interval of offering eggs upon the sex ratio of *T. ostrinia*.

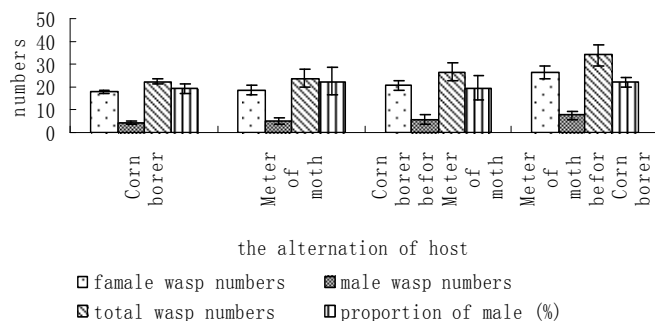


**Figure 8.** Effect of varieties of food upon the sex ratio of *T. ostrinia*.

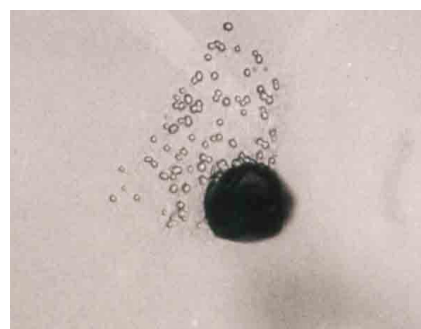
80% no matter whether 10 or 80 corn borer eggs were supplied.

**Interval of host supply**

The interval of offering eggs had not significant impact on the sex ratio of *T. ostrinia* (Figure 7). The change of sex ratio was “V” type. The sex ratio was lowest (8.4%) at the treatment of zero hour interval and highest (44.5%) at one hour interval. The fecundity could be influenced significantly by the interval of offering eggs. The fecundity



**Figure 9.** Effect of the alteration of host upon the sex ratio of *T. ostrinia*.



**Figure 10.** The dyeing result of *T. embryophagum* celling result of *T. embryophagum* cell.

reduced with the increasing of the interval.

**Species of food**

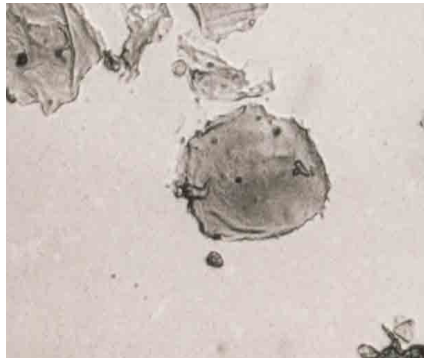
Food had no significant impact on the sex ratio of *T. ostrinia* (Figure 8). The sex ratio had no significant difference between feeding with 10% honey, 10% glucose, water or no food. The fecundity reached the highest values when the water of honey was fed.

**Species of host**

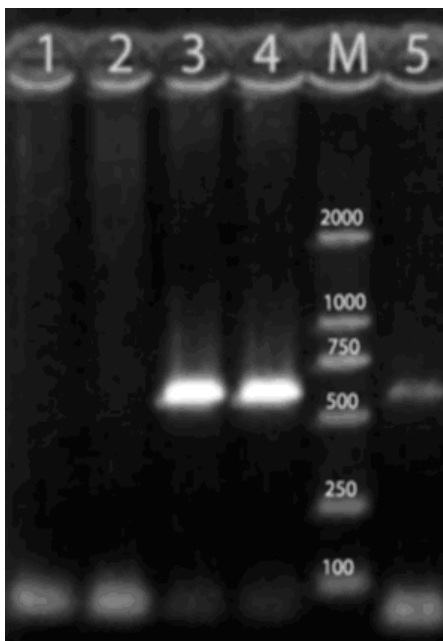
The species of host had no significant impact on *T. ostrinia* (Figure 9). The sex ratio and the fecundity showed no significant change whether the eggs of corn borer or the eggs of rice moth were supplied.

**Cytological analysis of Wolbachia**

Dyeing with the method of Macchiavello (Agricultural entomology research group, Department of plant protection, 1975), microorganisms (*Wolbachia*) could be found in the cell of *T. embryophagum* (Figure 10) and



**Figure 11.** The dyeing result of *T. ostrinae* cell.

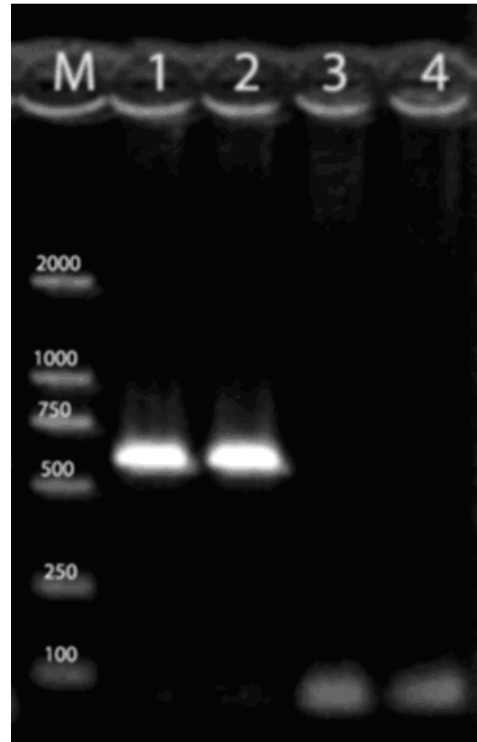


**Figure 12.** The *wsp* gene PCR result of *T. dendrolimi*. Lane 1, *T. ostrinae*; lane 2, *T. dendrolimi* (arrhenotokous line); lane 3, positive control *T. cacaoeciae*; lane 4, positive control *T. embryophagum*; lane M, DL2000 Marker; lane 5, *T. dendrolimi* (thelytokous line).

nothing in the cell of *T. ostrinae* (Figure 11).

### Horizontal transmission

Amplification of specific genes by the polymerase chain reaction has been the usual route taken to circumvent the problems that arise in identifying bacterial parasites of insects. By using the general primers (81F, 691R), a 500 to 750 bp fragment of the *wsp* gene was amplified from genomic DNA of *Wolbachia* harbored in *T. embryophagum* (Figure 12).



**Figure 13.** The *wsp* gene PCR result of *T. ostrinae*. Lane M, DL2000 marker; lane 1, positive control *T. cacaoeciae*; lane 2, positive control *T. embryophagum*; lane 3, *T. ostrinae*; lane 4, *T. ostrinae* (after sharing foods with the *Wolbachia* containing *Trichogramma*).

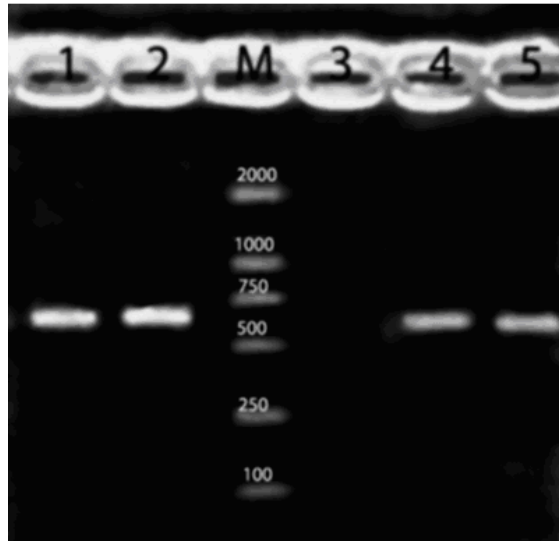
### Transmission between Trichogramma spp.

DNA fragments of the *wsp* gene were amplified from the treated *T. dendrolimi* (lane 5) but not in the treated *T. ostrinae* (lane 4) (Figure 13). This demonstrated that *T. dendrolimi* can be infected by *Wolbachia* (it had proven to become a stable thelytokous lines after multi-generation feeding), but *T. ostrinae* could not be infected by *Wolbachia* through the way of sharing food.

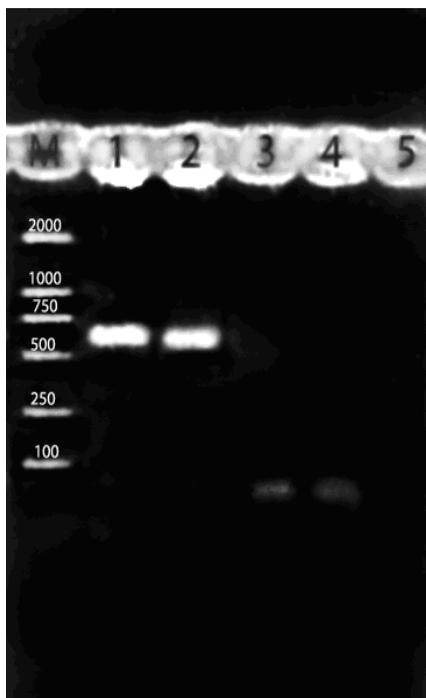
DNA fragments of the *wsp* gene were amplified from the treated *Braconidae* spp. (lane 4 and lane 5), but not in untreated *Braconidae* spp. (lane 3) (Figure 14). The result show that *Wolbachia* could transmit between families in *Hymenoptera*.

### Transmission between orders (from Hymenoptera to Lepidoptera)

DNA fragments of the *wsp* gene were not amplified from the treated *Galleria mellonella* (lane 4) and tussah ovarian tissue (lane 5). This demonstrated that *Wolbachia* could not transmit from *Hymenoptera* to *Lepidoptera* (Figure 15).



**Figure 14.** The *wsp* gene PCR result of microinjected *Braconidae* spp. lane 1, Positive control *T. cacoeciae*; lane 2, positive control *T. embryophagum*; lane M, DL2000 marker; lane 3, *Braconidae* spp.(CK); lane 4, *Braconidae* spp.(after microinjection); lane 5, *Braconidae* spp. (living on the *C. cephalonica* no injection).



**Figure 15.** The *wsp* gene PCR result of microinjected *G. mellonella* and *Antheraea pernyi*. Lane M, DL2000 marker; lane 1, positive control *T. cacoeciae*; lane 2, positive control *T. embryophagum*; lane 3, *G. mellonella* (before microinjection); lane 4, *G. mellonella* (after microinjection); lane 5, *Antheraea pernyi* (after microinjection).

## DISCUSSION

Temperature is one of the most important abiotic factors affecting the sex ratio of *Trichogramma* spp. According to Al-Ahmed and Kheir (2003), temperature is considered an important factor affecting the duration of the pre-oviposition period. Results that is similar to ours. We found that the sex ratio of *T. ostrinia* could be affected significantly by temperature. The proportion of male increased when the temperature was over high (>30°C) or over low (<20°C). As the temperature increased, the activity of female strengthened and the duration of development decreased. So the oviposition or parasitization was affected. Crozier (1977) suggested that lower temperatures could promote fusion of nuclei and increase the proportion of diploid offspring, which could explain the high sex ratio at 15°C in all strains studied. However, we found the sex ratio of *T. ostrinia* could not be affected when the temperature was over low. But the oviposition amount decreased, resulting in decreasing of reproduce efficiency. Between 15 and 30°C, the sex ratio had no significant change. But the fecundity increased significantly as temperature increased. The optimal temperature for development and survival was 25°C.

The development and reproductive of many species of parasitoids could be affected significantly by relative humidity. According to Legner (1977), the sex ratio of many species of *Braconidae* and *Pteromalidae* could be affected by relative humidity. Kajita (1979) noticed that the parasitic quantity of *Encarsia formosa* Gahan could not be significantly affect by relative humidity of 43 to 90%, but affected significantly in 30%. The relative humidity of 74% was favorable to the reproductive of *E. formosa* Gahan. The results that are similar to ours, we found that the progeny sex ratio of *T. ostrinia* could be affected by relative humidity, especially in low relative humidity (RH<55%). So the low relative humidity should be avoided for reproduction of *T. ostrinia*. The high relative humidity had no significant effect on the sex ratio, but decreased the quantity of oviposition. In addition, the host would be moldy and degenerative in high relative humidity, and the progeny could not enclose successfully. In conclusion, the female proportion and the oviposition of *T. ostrinia* were max at 75% RH.

The sex ratio of *T. ostrinia* could be affected by photoperiod. But the degree of effect by photoperiod was weak than by temperature or relative humidity. The proportion of male was higher on the 8 and 20 h illumination time than others. The reason that the sex ratio could not be affected by photoperiod significantly maybe was the short longevity of the *T. ostrinia* adult, so the modulation mechanism of sex ratio in response to the photoperiod did not form.

The local mate competition (LMC) theory (Hamilton, 1967) predicts that the foundresses should adjust their sex ratios in response to the variations in foundress numbers. The sex ratio is predicted to increase toward

50% with increasing numbers of foundresses. The theoretical predictions empirically supported by gregarious parasitoid wasps and fig wasps with a variable number of foundresses in local oviposition patches (Guo, 1992; Herre et al., 1997; King, 1996; Wang, 1990; Werren, 1983). We found that the female of *T. ostrinia* could adjust the sex ratio of the offspring themselves based on the foundress quantity of ovipositing in the same environment. The proportion of the male progeny increased and the parasitic efficiency of every foundress declined, as the increasing of the ovipositing foundress in the same environment.

The physical condition of foundress was directly related to their age. The female progeny proportion of young foundress was high; conversely, the proportion was low. For example, the female progeny proportion of *Pseudanastatus albitarsis* was more than 80% within 18 days after eclosion, below 70% after 20 days, and all drones after 27 days (Wang, 1990). It was found that the sex ratio of *T. ostrinia* was related to the age of foundress. The proportion of male increased with the increasing of foundress age. Because the longevity of *T. ostrinia* was short, the physical condition of foundress decreased rapidly with the increasing of foundress age. On the other hand, the sperm in the female were exhausted, so the ovum could not be fertilized. Thus, the male offspring proportion of three days foundress was very high.

The progeny sex ratios of gregarious parasitoids were affected by various environmental factors (Flanders, 1965). A most prevalent factor was the host/parasitoid ratio. The decrease of this ratio results in the increase of the progeny sex ratio (Kanungo, 1955; Salt, 1936; Walker, 1967). It was found that the progeny sex ratios of *T. ostrinia* could not be affected by host quantity, but the fecundity could be affected by it. The fecundity of every foundress increased with the increase of host.

It was that if the *T. ostrinia* was supplied for sufficient host at twice and there were some interval time between the two supplies. The sex ratio of *T. ostrinia* would be affected by the length of interval. In conclusion, with the prolonging of interval, the proportion of male increased. The sex ratio reached the highest values at 1h interval, then the male ratio trended to decrease.

The offspring sex ratio could be directly affected by food sort supplied to foundress. When *Telenomus enproctidis* was fed on 10% hydromel or water, the proportion of female offspring accounted for 69.1 and 56.9% respectively (Wang, 1991). For *Bracon hebetor*, the proportion of female offspring was increased to a great extent if foundress has been fed on host larvae's body fluids (Wang et al., 1992). We found that although the offspring sex ratio of *T. ostrinia* could not be affected significantly by food sort, the proportion of male was the lowest when glucose water was supplied. This reduction could be due to the fact that glucose could be quickly and effectively used.

*Trichogramma* had the ability to identify host. *T. dendrolimi* could change the sex ratio of offspring in one host according to the host species or the size of eggs (Guo and Zhang, 1993). Qiu et al. (1999) found that the host reception behavior of *T. dendrolimi* was flexibility and had adaptation process to new host. For example, when the *T. dendrolimi* from the tussah eggs was supplied by corn borer eggs as host, the contemporary foundress was not suited to the next host, so the parasite rate and fecundity reduced. The parasite rate and fecundity increased after continuous supplying corn borer eggs. However, we found that there were no differences in sex ratio and fecundity no matter corn borer eggs or rice moth eggs were supplied to *T. ostrinia* as host. This meant *T. ostrinia* had a strong adaptability to different host eggs.

Huigens and Luck (2000) noticed that *Wolbachia* could transmit from strains (infected) to others of the same species (not infected). In our study, *Wolbachia* transmitted from *T. embryophagum* (from Germany) to *T. dendrolimi* (from China) successfully, indicating that *Wolbachia* could transmit between species in *Trichogramma*. The possible transmission ways of *Wolbachia* between two *Trichogramma*: (a) The larvae of *T. Dendrolimi* were infected after eating dead larvae of *T. embryophagum*; (b) This two species of larvae fought in the host egg, then *Wolbachia* spread through blood contact; (c) *Wolbachia* was transmitted into host with the ovipositing of foundress, then the larvae of *Trichogramma* were infected by oral or wounds. It was found that the arrhenotoky *T. Dendrolimi* was successfully changed to the thelytoky *T. Dendrolimi* through the food sharing, but failed for *T. ostrinia* using the same method. This result indicating that many factors, such as the contents of *Wolbachia* in the host, the adaptation of *Wolbachia* to the host and the immune ability of host to *Wolbachia*, could affect the ability of *Wolbachia* horizontal transmission between species of *Trichogramma*.

Boyle et al. (1993) suggested that cytoplasmic incompatibility expression was affected by the density of *Wolbachia*. Our PCR results showed that *Braconidae* was successfully infected by *Wolbachia* through microinjection, but could not be change to arrhenotoky. It was the reason that the density of *Wolbachia* in *Braconidae* was too low to change the reproduction mode or *Wolbachia* was not adapting to the new host. The injection of *G. mellonella* and tussah defeated, due to there were many differences between orders, such as nutrition, immune system and so on, so *Wolbachia* could not adapt to new host. In short, the *Wolbachia* can transmit between species of *Trichogramma* and affect the sex ratio of hosts significantly, but the success ratio of transfection was decided by the similarity degree of two hosts. The higher the degree of similarity, the higher the success ratio of transfection and the change chance of the reproduction mode after transfection, and vice versa.

In conclusion, the sex ratio of *T. ostrinia* could be affected by many factors (such as temperature, relative



humidity, photoperiod, biological factors). The foundress of *T. ostrinae* could adjust offspring sex ratio responding to these factors through a complex mechanism. Which verifies the sex ratio theory and implies that the potential effectiveness of *T. ostrinae* as a biological control agent can be further improved. In addition. Only the independent effect of various factors to sex ratio was studied; the combined effects need to be confirmed further.

## ACKNOWLEDGMENT

This research was supported by financial assistance from National Natural Science Foundation of China (No.30971962).

## REFERENCES

- Abram PK, Haye T, Mason PG, Cappuccino N, Boivin G, Kuhlmann U (2012). Biology of *Synopeas myles*, a parasitoid of the swede midge, *Contarinia nasturtii*, in Europe. *Bio. Control* DOI 10.1007/s10526-012-9459-x. 57(6):789-800
- Agricultural entomology research group, Department of plant protection (1975). Study on the technology of agricultural entomology. Northwestern Agriculture University.
- Al-Ahmed AM, Kheir SM (2003). Life cycle and survival of *Hyalomma dromedarii* (Acari: Ixodidae) under laboratory conditions. *Agric. Mar. Sci.* 8:11–14
- Bouletreau M (1976). Effect of photoperiod on sex ratio of the progeny in a parasitoid wasp *Pteromalus puparum*. *Entomol. Exp. Appl.* 19:197-204.
- Boyle L, O'Neill SL, Robertson HM, Karr TL (1993). Interspecific and Intraspecific Horizontal Transfer of *Wolbachia* in *Drosophila*. *Science* 260:1796-1799.
- Cook JM (1993). Sex determination in the Hymenoptera: a review of models and evidence. *Heredity*, 71:421-435.
- Crozier RH (1977). Evolutionary genetics of the Hymenoptera. *Ann. Rev. Entomol.* 22:263–288.
- Flanders SE (1956). The mechanism of sex-ratio regulation in the parasitic Hymenoptera. *Insectes Soc.* 3:325-334.
- Flanders SE (1965). On the sexuality and sex ratios of hymenopterous populations. *American Naturalist.* 99:489–494.
- Duron O, Gavotte L (2007) Absence of *Wolbachia* in Nonfilarid Worms Parasitizing Arthropods. *Curr. Microbiol.* 55:193–197.
- Guo MF (1992). Study of parasitic behavior of *Trichogramma* (I) Affect of superparasitization on sex ratio of progeny. *Nat. Enemies Insects* 14:6-12.
- Guo MF, Zhang JY (1993). Study of parasitic behavior of *Trichogramma* (IV) Allocation of offspring number and sex ratio. *Nat. Enemies Insects* 15:51-59.
- Hamilton WD (1967). Extraordinary sex ratios. *Science* 156:477-488.
- Herre EA, West SA, Cook JM, Compton SG, Kjellberg F (1997). Fig-associated wasps: pollinators and parasites, sex-ratio adjustment and male polymorphism, population structure and its consequences. In: Choe JC, Crespi BJ (eds) *The evolution of mating systems in insects and arachnids*. Cambridge University Press, Cambridge, pp. 226-239.
- Hoelscher CE, Vinson SB (1971). The sex ratio of a hymenopterous parasitoid, *Campoletis perdinctus*, as affected by photoperiod, mating and temperature. *Ann. Entomol. Soc. Am.* 64:1373-1376.
- Huigens ME, Luck RF (2000). Infectious parthenogenesis. *Nature* 405:178-179.
- Kajita H (1979). The effectiveness of the introduced parasite *Encarsia formosa* Gahan in the control of the greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) in a plastic house. *Sci. Bull. Fac. Agric. Kyushu University* 33:119-125.
- Kant R, Minor MA, Trewick SA, Sandanayaka WRM (2012). Body size and fitness relation in male and female *Diaeretiella rapae*. *Bio. Control* DOI 10.1007/s10526-012-9452-4. 57:759–766.
- Kanungo K (1955). Effects of superparasitism on sex-ratio and mortality. *Curr. Sci.* 24:59-60.
- King BH (1996). Sex ratio responses to other parasitoid wasps: multiple adaptive explanations. *Behavioral Ecol. Sociobiol.* 39:367-374.
- Legner EF (1977). Temperature, humidity and depth of habitat influencing host destruction and fecundity of muscoid flies. *Entomophaga* 22:199-206.
- Montoya P, Cancino J, Pérez-Lachaud G, Liedo (2010). Host size, superparasitism and sex ratio in mass-reared *Diachasmimorpha longicaudata*, a fruit fly parasitoid. *Bio. Control* 10.1007/s10526-010-9307-9. 56(1):11-17
- Qiu HG, Qiu ZL, Shen BJ, Fu WJ (1999). Study of host preference plasticity of *Trichogramma dendrolimi*. *Nat. Enemies Insects* 21:49-53.
- Rockland LB (1960). Saturation salt solutions for static control of relative humidity between 5 and 40 degree celcius. *Analytical Chemistry*, 32:1375-1376.
- Salt G (1936). Experimental studies on insect parasitism. IV. The effect of superparasitism on populations of *Trichogramma evanescens*. *J. Exp. Biol.* 13:363-375.
- Somjee U, Ablard K, Crespi B, Schaefer PW, Gries G (2011). Local mate competition in the solitary parasitoid wasp *Ooencyrtus kuvanae*. *Behav. Ecol. Sociobiol.* 65:1071–1077.
- Stouthamer R, Luck RF (1993). Influence of microbe-associated parthenogenesis on the fecundity of *Trichogramma deion* and *T. pretiosum*. *Entomol. Exp. Appl.* 67:183-192.
- Ueno T (1998). Adaptiveness of sex ratio control by the pupal parasitoid *Itoplectis naranayae* (Hymenoptera: Ichneumonidae) in response to host size. *Evol. Ecol.* 12:643-654.
- Walker I (1967). Effect of population density on the viability and fecundity in *Nasonia vitripennis* Walker (Hym.:Pteromalidae). *Ecology* 48:294-301.
- Wang Q, Wang LP, Yan YH (1992). Enhancing host parasitization of *Encarsia formosa* by feeding the adults with honey solution. *Chinese J. Biol. Cont.* 8: 64-67.
- Wang WX (1990). Sex allocation by parasitic Hymenoptera. *Chinese J. Biol. Cont.* 6:173-178.
- Wang WX (1991). Studies on the relationship between host size and sex ratio of *Bracon hebetor* [Hym.:Broconidae]. *Chinese J. Biol. Cont.* 7: 16-18.
- Werren JH (1983). Sex ratio evolution under local mate competition in a parasitic wasp. *Evolution* 37:116-124.
- Werren JH (1997) Biology of *Wolbachia*. *Ann. Rev. Entomol.* 42:587-609.
- White MJD (1973). *Animal cytology and evolution*. Cambridge University Press. Cambridge.
- Zhou W, Rousset F, O'Neill SL (1998). Phylogeny and PCR-based classification of *Wolbachia* strain using wsp gene sequences. *Proceed. Royal Soci. London, Ser. B* 265:509-515.