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ORIGINAL RESEARCH PAPER

Variable fitness and reproductive effects of *Wolbachia* infection in populations of the two-spotted spider mite *Tetranychus urticae* Koch in China

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Abstract Maternally inherited *Wolbachia* bacteria are widely distributed among insects, and their presence usually causes modifications of the host. To understand the evolutionary history of diverse host-*Wolbachia* associations, we investigated the symbiosis between *Wolbachia* and the two-spotted spider mite *Tetranychus urticae* Koch in China. The cytoplasmic incompatibility (CI) level, fecundity, female ratio, host longevity and host development time were examined. Our results indicate that *Wolbachia* bacteria had variable effects on the reproduction and fitness of Chinese populations of *T. urticae*. Variability of CI expression within *T. urticae* ranged from no CI to a strong level of CI in spite of the low variability of the *wsp* gene. Relative to uninfected mites, infected females in one of the three populations showed enhanced fecundity associated with the infection of *Wolbachia*. This is the first report of a *Wolbachia* infection promoting the fecundity of infected females in *T. urticae*. Furthermore, we found both positive and negative effects of *Wolbachia* infection on longevity and the development time. The differences in ecological characters may be attributed to both *Wolbachia* and host genotype.

Keywords *Wolbachia* · *Tetranychus urticae* Koch · Cytoplasmic incompatibility · Fitness

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Introduction

Wolbachia are maternally inherited alpha-proteobacteria known to infect a wide range of arthropods (Werren et al. 1995; Jeyaprakash and Hoy 2000). Recent meta-analysis estimated that >65% of insect species harbour *Wolbachia*, making it among the most abundant intracellular bacteria genera so far discovered, infecting at least 10^6 insect species alone (Hilgenboecker et al. 2008). The success of *Wolbachia* can be attributed in large part to its ability to manipulate the reproduction of its host to promote the spread of infection into the host population. The effects of *Wolbachia* infection on reproduction include: feminization of genetic males (Rousset et al. 1992); parthenogenetic induction, which results in the development of unfertilized eggs (Stouthamer et al. 1993); the killing of male progeny from infected females (Hurst et al. 1999); and sperm-egg incompatibility, which is referred to as cytoplasmic incompatibility (CI) (Bourtzis et al. 1996). Although variable in strategy, the multiple mechanisms by which *Wolbachia* manipulates host reproduction are similar in that they provide infected female hosts with a reproductive advantage relative to uninfected females. In addition to the effect on reproduction, *Wolbachia* can also influence the fitness of the host. The *Wolbachia*-host interaction ranges along a continuum from mutualism (Bandi et al. 1999; Dedeine et al. 2001; Dobson et al. 2002, 2004) to commensalism (Hoffmann et al. 1996, Charlat et al. 2004) and parasitism (Hoffmann et al. 1990; Stevens and Wade 1990; Snook et al. 2000; Champion de Crespigny and Wedell 2006). Fitness costs or benefits conferred by the symbiont also hinder or promote the spread of infection (Hoffmann et al. 1990; Turelli and Hoffmann 1995; Dobson et al. 2002).

In previous studies, *Wolbachia* have been found to induce CI in the two-spotted spider mite *Tetranychus*

urticae Koch, with the variability of CI expression ranging from no CI to complete CI, including either female embryonic mortality or male conversion types of CI (Breeuwer 1997; Perrot-Minnot et al. 2002; Vala et al. 2002; Gotoh et al. 2003, 2007). A few studies also examined the effect of *Wolbachia* on the fitness of *T. urticae*. *Wolbachia* in mites collected from cucumber plants did not affect longevity, but infected females produced smaller clutch sizes, a more daughter-biased sex ratio and had decreased F1 mortality (Vala et al. 2003). In the L strain collected from Rose-bay, the fecundity of infected females in the first 7 days was 80–100% less than that in cured females (Perrot-Minnot et al. 2002).

Prior study in our laboratory showed that *Wolbachia* was widely distributed in Chinese populations of *T. urticae*. All populations were found to be infected with *Wolbachia*, and the infection rate was between 2.5 and 85% (Xie et al. 2006). How *Wolbachia* manipulate the reproduction of *T. urticae* in China, and whether their relationship is beneficial or harmful are still unclear. China has a widely varying topography and different climatic conditions. High genetic differentiation between Chinese populations of *T. urticae* has been revealed by microsatellite markers (Li et al. 2009). We chose three populations from the north to south of China to represent Chinese populations: Liaoning (LN), Jiangsu (JS) and Hunan (HN) populations. To understand the relationship between *Wolbachia* and *T. urticae* in China, we measured the strength of CI, female ratio, fecundity, survival and development time between infected and uninfected strains under laboratory conditions.

Materials and methods

Mite populations

Three populations, in which *Wolbachia* was previously detected, were used in this study (Xie et al. 2006). Their locations, host plants, collection dates and abbreviations are summarized in Table 1. Mites were reared on leaves of the common bean (*Phaseolus vulgaris* L.) placed on a water-saturated sponge mat in Petri dishes (diameter 9) at 25 ± 1°C, 60% relative humidity and with a L16–D8 photoperiod. All experiments were carried out under these conditions.

DNA extraction, PCR amplification and sequencing

DNA was extracted by homogenizing a single female adult in a 25-µl mixture of STE buffer (100 mM NaCl, 10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and proteinase K (10 mg/ml, 2 µl) in a 1.5-ml Eppendorf tube. The mixture was incubated at 37°C for 30 min and later at 95°C for 5 min. The samples were centrifuged briefly and were used immediately for PCR reactions or stored at −20°C for later use.

All PCR reactions were run in 25 µl buffer using the TAKARA Taq kit (no. R001B; Takara Co., Ltd.): 16.3 µl H_2O, 2.5 µl 10× buffer, 1.5 µl of 2.5 mM dNTP, 1.5 µl of 25 mM $MgCl_2$, 0.2 µl Taq (1 U), 2 µl sample and 1 µl primers (20 pmol each). The primers used in this study were for the *Wolbachia wsp* gene (Zhou et al. 1998): 5′-TGG TCC AAT AAG TGATGA AGA AAC-3′ and 5′-AAA AAT TAA ACG CTA CTC CA-3′. Reactions were cycled 35 times at 94°C for 30 s, 52°C for 45 s and 72°C for 1 min. Reagent negative and positive controls were included in the reactions. The PCR products were electrophoresed on a 1.0% agarose gel in TBE/EtBr for 40 min at 60 mA, and then photographed on a UV transilluminator. The techniques used here are a modified version of the methods reported by Johanowicz and Hoy (1996) and Gomi et al. (1997).

The PCR product was cloned into a pGEM-T vector (Promega). The template DNA was amplified by PCR using M13–20 and reverse primers. The sequence was determined by the dye terminator sequencing method with a DNA Sequencer (model 377 and 3700; PE Applied Biosystems).

Preparation of infected and uninfected lines

In order to cross infected and uninfected individuals, 100% infected and 100% uninfected lines were prepared for each population. One female from the teleiochrysalis stage was allowed to lay eggs without being crossed with males. The eggs were reared until adulthood (males). After the males reached sexual maturity they were backcrossed with the mother. After crossing, the female adults were transferred to new leaf discs and were allowed to lay eggs for 3–5 days. A female was checked for *Wolbachia* infection by PCR amplification. The eggs were separately reared on new leaf discs depending on the infection status of the

Table 1 Collection history, locality and host plant data of *Tetranychus urticae* Koch from China

Location	Abbreviation	Host plant	Collection date	Latitude	Longitude
Xingcheng, Liaoning	LN	Apple (*Malus pumila* Mill.)	August 2002	40.36N	120.42E
Xuzhou, Jiangsu	JS	Apple (*M. pumila* Mill.)	July 2003	34.41N	116.35E
Changsha, Hunan	HN	Soybean [*Glycine max* (L.) Merr.]	August 2003	28.11N	112.58E

mother. The above process was continued for three to four generations until a 100% infected population was obtained.

Uninfected lines were established by treating infected lines with tetracycline. Small leaf discs (ca. 3 cm^2) from the common bean were placed on a cotton bed soaked in tetracycline solution (0.1%, w/v) in Petri dishes (9 cm in diameter) and kept for 24 h before they were used for rearing newly hatched larvae. Distilled water was added daily to keep the cotton beds wet. The cotton and the leaf discs were replaced every 4 days. Four to eight generations later, mites were checked by PCR to confirm that the lines were free of *Wolbachia*. These lines were maintained in a mass-rearing environment without antibiotic for about four generations (2 months) before use to avoid potential side effects of the antibiotic treatment.

Cross experiments

In order to determine reproductive compatibility in intra-population crosses, four cross combinations were carried out: uninfected females were crossed with uninfected males, uninfected females were crossed with infected males, infected females were crossed with uninfected males, and infected females were crossed with infected males. Infected colonies were designated as 'W' and antibiotic-cured colonies as 'U'. Female teleiochrysalids, the last developmental stage before adult emergence, were placed with two males on the same leaf disk. We used 1-day-old virgin males produced as a cohort by groups of females isolated as teleiochrysalids. This procedure was designed to avoid the potential decrease of the *Wolbachia* effect due to male ageing or repeated consecutive matings. Males were discarded 2 days after the females reached adulthood, and mated females were allowed to oviposit for 5 days. Eggs on leaf discs were checked daily to determine the hatchability, survival rate in immature stages and sex ratio (% daughters). Fecundity was estimated as the total number of eggs laid in the first 5 days. Data were analyzed with one-way analysis of variance (ANOVA), and means were compared using the Tukey-HSD test (SPSS 13.0). To normalize the data, log transformation was used for the number of eggs laid per female, and arcsine square root transformation was used for egg hatchability, survival rate and female ratio.

Survival assessment

Differences in host longevity were observed in comparisons of the different *Wolbachia* infection types. We measured age-specific survival of the U and W lines by placing 8 virgin females and 8 virgin males of the same infection status of the same population on the same leaf. Three leaves were used for each *Wolbachia* infection status per population. The leaves were monitored every day, and dead females were removed and counted until all females had died. Survivor curves for individual hosts were compared using the Kaplan-Meier method and log-rank test (Dobson et al. 2004).

Development time assessment

The effect of *Wolbachia* on the development time of mites of the three populations was assessed. Thirty *Wolbachia*-infected or uninfected females were placed on a leaf disk and allowed to lay eggs for 8 h. The eggs were moved to new small leaf disks individually. The small disks were monitored every 8 h, and the stage of the mite was recorded until adulthood. The development time of every stage was calculated. The distributions of development times were non-normal, even after attempts to transform the data. We used non-parametric Mann-Whitney *U* tests to estimate the effects of infection.

Results

Sequences of *Wolbachia* strains

We amplified a 552 base pair (bp) fragment of the *wsp* gene from the three *Wolbachia* strains infecting *T. urticae*. *Wsp* sequences were submitted to the GenBank database (GenBank numbers GU014539, GU014541, GU014542). *Wolbachia* strains in the LN and HN populations of *T. urticae* had an identical *wsp* gene sequence. The *wsp* sequence of the *Wolbachia* strain in the JS population had 99.5% similarity (3 different nucleotides out of 552 nucleotides) to the sequence in the LN and HN populations.

Strength of cytoplasmic incompatibility

The results of intra-population crosses between infected and cured individuals of the three populations are presented in Table 2. The HN population showed strong unidirectional CI. The hatchability of eggs, survival rate in the immature stage and sex ratio in the cross between uninfected females and infected males (U/W) were significantly lower than those of other crosses (U/U, W/U and W/W).

Wolbachia showed an intermediate level of CI in the LN population. The LNU/LNW cross resulted in significantly reduced hatchability, survival rate at the immature stage and sex ratio among the four combinations, but the hatchability and sex ratio in this incompatible cross were higher than in the HN and JS populations.

The JS population showed no CI. No reduction in egg hatchability and survival rate at immature stages was observed among the four combinations. The sex ratios of

Table 2 Compatibility of crosses between *Wolbachia*-infected (W) and antibiotic-treated (U) colonies of Liaoning (LN), Jiangsu (JS) and Hunan (HN) populations of *Tetranychus urticae*

Cross Female × male	n[a]	No. of eggs/female	Hatchability (100%)	Survival rate in immature stage (%)	Female offspring (%)	No. of F1 females	No. of F1 males
LN(U) × LN(U)	22	26.18 ± 1.30	95.19 ± 0.98b	93.70 ± 0.86b	76.37 ± 1.44b	17.86 ± 1.02c	5.36 ± 0.34
LN(U) × LN(W)	60	24.45 ± 0.77	44.79 ± 1.74a	85.40 ± 1.30a	37.08 ± 2.85a	3.72 ± 0.35a	5.45 ± 0.27
LN(W) × LN(U)	31	24.74 ± 0.87	94.35 ± 1.16b	93.99 ± 1.04b	78.17 ± 0.94b	17.16 ± 0.73bc	4.68 ± 0.20
LN(W) × LN(W)	21	22.00 ± 1.55	93.76 ± 1.50b	93.98 ± 0.99b	72.35 ± 1.46b	14.29 ± 1.31b	5.38 ± 0.49
$F_{3,130}$[b]		2.263 NS	211.991***	7.706***	49.028***	114.543***	1.256 NS
JS(U) × JS(U)	23	29.22 ± 1.72	94.73 ± 1.24	94.07 ± 1.00	74.44 ± 1.18a	19.48 ± 1.29	6.43 ± 0.32b
JS(U) × JS(W)	22	27.27 ± 1.37	95.47 ± 1.25	93.63 ± 0.88	72.88 ± 1.43a	17.82 ± 1.04	6.59 ± 0.49b
JS(W) × JS(U)	23	28.48 ± 1.10	96.40 ± 0.90	94.09 ± 0.95	81.45 ± 1.11b	21.13 ± 0.97	4.65 ± 0.21a
JS(W) × JS(W)	38	26.16 ± 0.90	94.27 ± 0.70	93.66 ± 0.77	82.92 ± 1.02b	19.05 ± 0.65	3.92 ± 0.26a
$F_{3,102}$[b]		1.173 NS	1.472 NS	0.086 NS	17.603***	1.814 NS	17.874***
HN(U) × HN(U)	22	23.59 ± 0.91a	94.88 ± 0.73b	97.80 ± 0.70b	79.03 ± 0.88b	17.41 ± 0.86b	4.55 ± 0.22a
HN(U) × HN(W)	34	27.12 ± 1.22a	30.82 ± 2.56a	88.02 ± 2.01a	22.71 ± 4.09a	1.82 ± 0.33a	5.59 ± 0.68ab
HN(W) × HN(U)	21	36.10 ± 1.22b	96.30 ± 0.57b	96.22 ± 0.60b	77.71 ± 0.85b	25.95 ± 0.89c	7.48 ± 0.41bc
HN(W) × HN(W)	22	45.95 ± 1.44c	97.60 ± 0.47b	95.68 ± 0.71b	79.33 ± 0.61b	34.05 ± 1.12d	8.82 ± 0.32c
$F_{3,95}$[b]		51.874***	292.809***	5.392**	81.877***	364.839***	12.051***

Values in a column followed by different letters are significantly different at $P < 0.05$ (Tukey HSD test). The number of eggs per female was ln-transformed, and hatchability, survival rate and female ratio were arcsine-root transformed before ANOVA

NS, Not significant at the 5% level

[a] Number of pairs tested

[b] Means (±SE) differ significantly at $P < 0.01$ (**) and $P < 0.001$ (***) (ANOVA)

the cross JSW/JSU and the cross JSW/JSW were similar, but the values were significantly different from those in the JSU/JSU and JSU/JSW crosses; that is, infected females produced a higher daughter-biased sex ratio. Since *Wolbachia* are maternally transmitted and males are an evolutionary dead end for maternally inherited infections, more female offspring would promote the spread of infection.

Fecundity

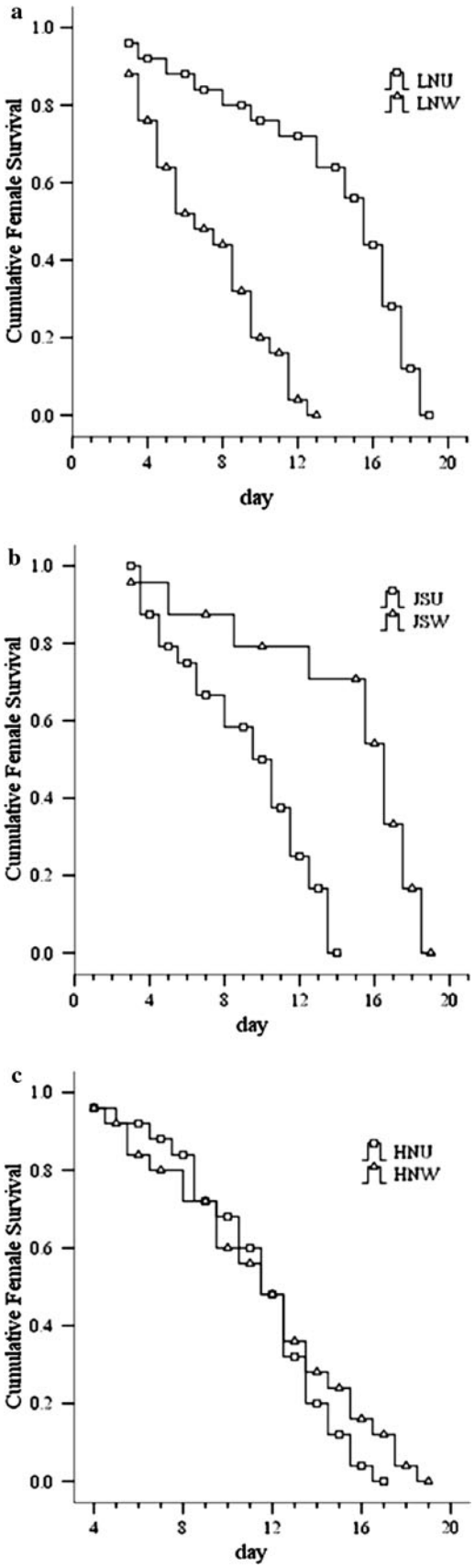
The effects of infection on female fecundity were tested by comparing the number of eggs laid in the first 5 days by infected and uninfected females in crosses involving infected and uninfected males. The results are shown in Table 2. No difference in fecundity was observed between infected and uninfected females in the LN and JS populations. In the HN population, a significant effect of female infection was found, with infected females laying more eggs on average than uninfected females, regardless of the infection status of males. Infected females often had the greatest fecundity when mated with infected males. The difference in fecundity between the cross HNW/HNW and cross HNW/HNU was significant. In summary, *Wolbachia* in the HN population was the only strain that could promote the fecundity of infected females.

Host longevity

The effects of *Wolbachia* on host longevity were tested by comparing the life span between infected and uninfected females. The results are presented in Fig. 1. In the LN population (Fig. 1a), uninfected females (14.16 ± 0.97) lived two times longer ($\chi^2 = 28.487$, $df = 1$, $P < 0.001$) than infected females (7.44 ± 0.65). *Wolbachia* shortened the longevity of infected females and provided a fitness advantage to uninfected females. As shown in Fig. 1b, infected females (15.17 ± 0.90) were significantly longer lived than uninfected females (9.63 ± 0.72) in the JS population ($\chi^2 = 27.954$, $df = 1$, $P < 0.001$); *Wolbachia* benefited infected females in the JS population. As shown in Fig. 1c, the HN population showed no difference in longevity between infected (11.88 ± 0.86) and uninfected females (11.72 ± 0.66) ($\chi^2 = 0.827$, $df = 1$, $P > 0.05$). Our results clearly show that *Wolbachia* prolonged the longevity of the JS population, shortened the longevity of the LN population and had no effect on the longevity of the HN population.

Development time

The effects of infection on the development time of females and males were tested by comparing the



◀ **Fig. 1** Comparison of *Wolbachia* effect on female longevity in LN (**a**), JS (**b**) and HN (**c**) populations. W, *Wolbachia* infected strains; U, uninfected strains. Survivor curves for individual hosts were compared using the Kaplan-Meier method and log-rank test

development time of each stage of mites with different infection statuses. The development times of females with different infection statuses are presented in Table 3.

In the LN population, *Wolbachia* shortened the time to adulthood of infected females. Infected females reached adulthood significantly earlier than uninfected females according to Mann-Whitney *U* tests. In the JS and HN populations, *Wolbachia* prolonged the time to adulthood of infected females. Time to adulthood was significantly shorter in uninfected than infected females. We noted that the effect of *Wolbachia* on the development time of each stage was different.

The development duration of males was shorter than females in the three populations. The development time of males of different infection status are presented in Table 4. In the LN population, infected males had a shorter development time than uninfected males. In the JS population, infected males had a longer development time than uninfected males. No difference in development duration between infected and uninfected males was observed in the HN population.

Discussion

Diversity of CI expression

We found diversity of CI expression in Chinese populations of *T. urticae*. CI was expressed as a reduction in egg hatchability and a male-biased sex ratio in crosses between uninfected females and infected males. This is concordant with the female mortality type of CI (Breeuwer 1997; Vavre et al. 2000). We found that CI manifested not only in embryonic development but also in nymphal development in the LN and HN populations. This is the first record of a *Wolbachia* infection that can affect the nymphal development of hosts.

Several factors have been identified that influence the expression of CI phenotype, such as environmental factors, including the age of the host and temperature, bacteria or host genes and bacterial density (Clancy and Hoffmann 1998; Poinsot et al. 1998; Reynolds and Hoffmann 2002; Sakamoto et al. 2005). In our experiments, we used 1-day-old virgin males and performed experiments at a constant temperature. Real-time quantitative PCR was performed to estimate the numbers of *Wolbachia*, and no difference in bacterial density was observed among the three populations

Table 3 Development times of infected (W) and uninfected (U) females in Liaoning (LN), Jiangsu (JS) and Hunan (HN) populations of *Tetranychus urticae*

Strain	n[a]	Egg (day)	Larval	Protochrysalis	Protonymph	Deutochrysalis	Deutonymph	Teliochrysalis	Total (days)
LNU	20	4.12 ± 0.04	1.13 ± 0.05	0.98 ± 0.04	1.03 ± 0.05	1.02 ± 0.04	1.22 ± 0.08	1.42 ± 0.05	10.92 ± 0.17
LNW	17	4.04 ± 0.05	1.14 ± 0.06	0.90 ± 0.04	0.92 ± 0.05	0.86 ± 0.04	1.14 ± 0.09	1.14 ± 0.06	10.14 ± 0.19
		$P = 0.317$	$P = 0.777$	$P = 0.152$	$P = 0.126$	$P = 0.012$	$P = 0.419$	$P = 0.002$	$P = 0.004$
JSU	36	4.00 ± 0.04	1.02 ± 0.03	0.82 ± 0.03	0.89 ± 0.03	0.81 ± 0.03	1.07 ± 0.04	1.19 ± 0.03	9.81 ± 0.09
JSW	36	4.03 ± 0.05	1.20 ± 0.04	0.72 ± 0.03	1.10 ± 0.05	0.81 ± 0.03	1.34 ± 0.06	1.19 ± 0.05	10.40 ± 0.11
		$P = 0.913$	$P < 0.001$	$P = 0.006$	$P = 0.001$	$P = 0.922$	$P < 0.001$	$P = 0.489$	$P < 0.001$
HNU	21	4.14 ± 0.05	0.95 ± 0.05	0.97 ± 0.04	0.81 ± 0.05	0.95 ± 0.05	0.75 ± 0.05	1.16 ± 0.05	9.73 ± 0.17
HNW	21	4.02 ± 0.06	1.32 ± 0.06	0.84 ± 0.04	0.97 ± 0.05	0.97 ± 0.05	1.03 ± 0.03	1.21 ± 0.04	10.35 ± 0.12
		$P = 0.146$	$P < 0.001$	$P = 0.029$	$P = 0.036$	$P = 0.930$	$P < 0.001$	$P = 0.480$	$P = 0.010$

Mann-Whitney U tests were performed to compare the development time of infected lines (W) with those from the uninfected lines (U) in each population

[a] Number of individuals tested

Table 4 Development times of infected (W) and uninfected (U) males in Liaoning (LN), Jiangsu (JS) and Hunan (HN) populations of *Tetranychus urticae*

Strain	n[a]	Egg (day)	Larval	Protochrysalis	Protonymph	Deutochrysalis	Deutonymph	Teliochrysalis	Total (days)
LNU	23	4.29 ± 0.07	0.94 ± 0.05	1.14 ± 0.05	0.75 ± 0.05	1.09 ± 0.06	0.81 ± 0.05	1.20 ± 0.05	10.23 ± 0.18
LNW	24	4.11 ± 0.04	0.89 ± 0.04	0.90 ± 0.03	0.75 ± 0.04	0.85 ± 0.03	0.76 ± 0.05	1.07 ± 0.03	9.33 ± 0.09
		$P = 0.049$	$P = 0.580$	$P < 0.001$	$P = 0.724$	$P = 0.001$	$P = 0.331$	$P = 0.033$	$P < 0.001$
JSU	45	4.35 ± 0.04	0.74 ± 0.02	0.96 ± 0.02	0.59 ± 0.02	0.93 ± 0.02	0.67 ± 0.02	1.06 ± 0.02	9.29 ± 0.05
JSW	45	4.50 ± 0.04	0.88 ± 0.03	0.87 ± 0.02	0.79 ± 0.02	0.89 ± 0.03	0.79 ± 0.03	1.08 ± 0.02	9.79 ± 0.08
		$P = 0.019$	$P = 0.001$	$P = 0.007$	$P < 0.001$	$P = 0.263$	$P = 0.001$	$P = 0.376$	$P < 0.001$
HNU	22	4.12 ± 0.05	0.91 ± 0.04	0.82 ± 0.04	0.79 ± 0.05	0.79 ± 0.03	0.81 ± 0.05	1.06 ± 0.03	9.30 ± 0.14
HNW	26	4.23 ± 0.04	0.97 ± 0.05	0.92 ± 0.04	0.67 ± 0.04	0.90 ± 0.03	0.72 ± 0.04	1.13 ± 0.04	9.54 ± 0.11
		$P = 0.083$	$P = 0.322$	$P = 0.094$	$P = 0.083$	$P = 0.024$	$P = 0.314$	$P = 0.189$	$P = 0.129$

Mann-Whitney U tests were performed to compare the development time of infected lines (W) to those from the uninfected lines (U) in each population

[a] Number of individuals tested

(unpublished data); therefore, it is unlikely that environmental factors and bacterial density are the reason for the variability of CI.

The CI-*Wolbachia* strains in the LN and HN populations of *T. urticae* had an identical *wsp* gene sequence, which was the same as the sequence of non-CI-*Wolbachia* in the R23 population of *T. urticae* in Japan (AB266837). The *wsp* sequence of non-CI-*Wolbachia* strain in the JS population had the same *wsp* sequence as the CI-*Wolbachia* in the G1 population (AB266804) and non-CI-*Wolbachia* in the G14 population (AB266804) of *T. urticae* in Japan. All these *Wolbachia* strains belong to the Ori subgroup; therefore, we estimated that the variability of the CI expression among the four Chinese populations of *T. urticae* was due to the interaction between *Wolbachia* and host genotypes.

Diversity of fitness effects

In addition to the heterogeneity of CI intensity, *Wolbachia* bacteria can have variable affects on *T. urticae* fitness. Although *Wolbachia* strains in the LN and HN populations shared identical *wsp* sequences, they apparently affected fitness in different ways. *Wolbachia* infection had a positive effect on fecundity in the HN population. By contrast, no fecundity change was observed in the other strains studied. The *Wolbachia*-associated effect of promoting fecundity may depend on the nuclear background of the host. This is the first report of a *Wolbachia* infection promoting the fecundity of infected females in *T. urticae*. This result is opposite to that found in *T. urticae* in France, in which *Wolbachia* infection decreased fecundity by 80–100% (Perrot-Minnot et al. 2002). Models can predict

the selection of *Wolbachia* variants that increase female fecundity (Stevens and Wade 1990). There may be an "attenuation" of *Wolbachia* effects, progressing toward a relationship less detrimental to the host. A recent study of a California population of *D. simulans* showed that *Wolbachia* has changed from a parasite to a mutualist over the last 20 years, so that infected females now exhibit an average 10% fecundity advantage over uninfected females in the laboratory. This change was accompanied by the rapid evolution of *Wolbachia* (Weeks et al. 2007). The underlying process leading to the increased fecundity of infected females is unknown, but may be due to *Wolbachia* or compensatory evolution in the host.

We found both positive and negative effects of *Wolbachia* infection on longevity. The effect of *Wolbachia* on the longevity of two-spotted spider mites was investigated in a strain collected from cucumber plants, and no difference in longevity between infected and uninfected females was observed (Vala et al. 2003). Our result is the first report of a *Wolbachia* infection affecting the longevity of infected female spider mites. Infected females in the JS population were significantly longer lived than uninfected females. Our data indicate that 21% infected females died 5 days after emergence, while only 4% infected females died 5 days after emergence. *Wolbachia* had no effect on fecundity in this population. This result was in a relatively lower number of offspring produced by uninfected females, and therefore it gradually reduced the number of uninfected individuals in the following generations. The fitness benefits to survival contributed to the maintenance of *Wolbachia* infection in the JS population in the absence of CI. *Wolbachia*-associated fitness, which benefits survival, was also found in mosquito and fruit fly (Dobson et al. 2002, 2004; Fry et al. 2004). The *Wolbachia*-associated fitness cost to survival was only reported in *D. melanogaster* (Min and Benzer 1997; Fry et al. 2004). The *popcorn Wolbachia* strain (*w*MelPop), which has been found to over-replicate within the cells of its host, caused a reduction in host longevity, and the effects strongly depended on the host nuclear background and temperature (Carrington et al. 2010). Since the *Wolbachia* strains in the LN and HN populations shared identical *wsp* sequences, we estimated that the *Wolbachia*-associated effect of shorting longevity in the LN population may depend on the nuclear background of the host. The *Wolbachia*-associated effect of prolonging longevity in the JS population may depend on the interaction between *Wolbachia* and host genotypes. Future experiments (introgression crosses between populations) are required to generate a homogeneous host genetic background for the characterization of *Wolbachia* effects.

We noticed that the development time of uninfected females in the LN population (10.92 days) was longer than in the JS (9.81 days) and HN (9.73 days) populations. Located in the northeastern part of China, Liaoning Province is much colder than Jiangsu and Hunan provinces. In the long process of evolution, the suitable temperature for development of the LN population may be lower than for the JS and HN populations, leading to the variation in development time among the populations. Both positive and negative effects of *Wolbachia* infection on the development time of hosts were found in this study. *Wolbachia* in the LN population shortened the development time of infected females, and *Wolbachia* in the JS and HN populations prolonged the development time. This is the second instance of *Wolbachia* infection affecting the development time of hosts. The other known instance is the virulent *w*Dm*popcorn Wolbachia* strain in *D. melanogaster*, which can delay the development time of the host (Reynolds et al. 2003). The *popcorn Wolbachia* strain in *Drosophila simulans* showed both positive and negative effects on the host depending on the nuclear background (Carrington et al. 2010). Our results also suggest that host background can influence the effect of *Wolbachia* on development. To clarify whether line divergence is due to *Wolbachia*, the host nuclear genome, or the interaction between these genomes, we should generate genetically homogeneous strains with different *Wolbachia* using microinjection methodology or introgression crosses.

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