CrossMark

Bulletin of Entomological Research (2016) **106**, 685–694
doi:10.1017/S0007485316000444
© Cambridge University Press 2016

Geography has a greater effect than *Wolbachia* infection on population genetic structure in the spider mite, *Tetranychus puerariicola*

Y.-T. Chen†, Y.-K. Zhang†, W.-X. Du, P.-Y. Jin and X.-Y. Hong*

Department of Entomology, Nanjing Agricultural University, Nanjing, Jiangsu, China

Abstract

Wolbachia is an intracellular symbiotic bacterium that infects various spider mite species and is associated with alterations in host reproduction, which indicates the potential role in mite evolution. However, studies of *Wolbachia* infections in the spider mite *Tetranychus puerariicola*, a major agricultural pest, are limited. Here, we used multilocus sequence typing to determine *Wolbachia* infection status and examined the relationship between *Wolbachia* infection status and mitochondrial diversity in *T. puerariicola* from 12 populations in China. The prevalence of *Wolbachia* ranged from 2.8 to 50%, and three strains (*w*Tpue1, *w*Tpue2, and *w*Tpue3) were identified. We also found double infections (*w*Tpue1 + *w*Tpue3) within the same individuals. Furthermore, the *w*Tpue1 strain caused weak cytoplasmic incompatibility (CI) (egg hatchability ∼55%), whereas another widespread strain, *w*Tpue3, did not induce CI. There was no reduction in mitochondrial DNA (mtDNA) or nuclear DNA diversity among infected individuals, and mtDNA haplotypes did not correspond to specific *Wolbachia* strains. Phylogenetic analysis and analysis of molecular variance revealed that the distribution of mtDNA and nuclear DNA haplotypes were significantly associated with geography. These findings indicate that *Wolbachia* infection in *T. puerariicola* is complex, but *T. puerariicola* genetic differentiation likely resulted from substantial geographic isolation.

Keywords: *Wolbachia*, *Tetranychus puerariicola*, mtDNA, 6-phosphofructokinase, population structure

(Accepted 22 May 2016; First published online 14 June 2016)

Introduction

Wolbachia is one of the most widespread maternally transmitted bacteria in invertebrates (Werren, 1997). In addition to insects, *Wolbachia* also frequently infects mites (Breeuwer & Jacobs, 1996; Tsagkarakou *et al.*, 1996; Gotoh *et al.*, 2003), spiders (Rowley *et al.*, 2004; Goodacre *et al.*, 2006) and nematodes (Bandi *et al.*, 1998; Fenn *et al.*, 2006). Especially, many nematodes infected with these bacteria are either the vectors or the causative agents of serious human diseases. There are various statuses of *Wolbachia* in their hosts. A single insect species or population may be infected with more than one symbiotic microorganism and different populations may have different infection statuses (Perrot-Minnot *et al.*, 1996; Werren *et al.*, 2008). Moreover, in many hosts, *Wolbachia* trigger a phenomenon known as reproduction regulation by cytoplasmic incompatibility (CI), parthenogenesis, feminization, and male-killing (Hoffmann & Turelli, 1997). Several reports noted that CI, the most common of the reproductive abnormalities, facilitated the ability of *Wolbachia* to invade host populations (Turelli, 1994; Sinkins, 2004). Because various

*Author for correspondence
Fax: +86 25 84395339
Phone: +86 25 84395339
E-mail: xyhong@njau.edu.cn
†These authors contributed equally to this work.

https://doi.org/10.1017/S0007485316000444 Published online by Cambridge University Press

Wolbachia strains play different roles in manipulating their host reproduction, they may have different effects on their host's ecology and evolution.

In addition to reproduction regulation, *Wolbachia* also play an important role in their hosts' fitness and population genetic variability. There is increasing evidence that *Wolbachia* may influence a host's evolutionary history, social organization, and population structure (Werren, 1997; Jiggins, 2003; Hurst & Jiggins, 2005). In the birdnest blowfly, *Protocalliphora sialia*, a particular *Wolbachia* strain was found to be associated with a particular mtDNA haplotype (Baudry *et al*., 2003). Moreover, Dean *et al*. (2003) revealed that selective sweeps significantly reduced mtDNA nucleotide diversity in infected vs. uninfected populations. Similarly, most other CI-associated *Wolbachia* sweeps were associated with low mtDNA variation in many hosts (Rasgon *et al*., 2006; Nunes *et al*., 2008; Raychoudhury *et al*., 2010; Graham & Wilson, 2012; Xiao *et al*., 2012). However, other studies suggested that symbionts may either reduce or increase mtDNA diversity (Shoemaker *et al*., 2003; Keller *et al*., 2004; Yu *et al*., 2011). Studies have shown that the relationships between *Wolbachia* and host mtDNA can be divided into four categories: (a) *Wolbachia* induce the decrease of mtDNA diversity; (b) *Wolbachia* do not cause changes in mtDNA diversity; (c) *Wolbachia* induce mtDNA mutations; and (d) *Wolbachia* induce the paraphyly of a single species based on mtDNA. Therefore, when using mtDNA as a maker of population genetic analyses, we should take into account the influence of *Wolbachia*.

Given the influence of *Wolbachia* on host biology and the potential impact of *Wolbachia* on host population genetic structure, studies on effects of the *Wolbachia* infections on their hosts are necessary. Earlier studies have indicated that many species of spider mites are infected with *Wolbachia* (Gotoh *et al*., 2003; Grbić *et al*., 2011; Xie *et al*., 2011; Zhu *et al*., 2012). Tetranychidae is one of the most important families of subclass Acari in terms of economic impact, because it contains several agricultural pest species of major relevance (Helle & Sabelis, 1985; Baker & Tuttle, 1994; Bolland *et al*., 1998; Zhang, 2003; Migeon *et al*., 2011). Tetranychidae species are wingless and usually rely on crawling for dispersal (Mitchell, 1973), but many of them can also be carried long distances by wind and by human activities (Grafton-Cardwell *et al*., 1991). Several *Wolbachia* strains can induce CI in *Tetranychus urticae*, *Tetranychus piercei*, and *Tetranychus turkestani* (Breeuwer & Jacobs, 1996; Breeuwer, 1997; Zhu *et al*., 2012). To better control Tetranychidae pests, many studies have inferred the genetic structure (Navajas *et al*., 2002; Bailly *et al*., 2004; Sun *et al*., 2012). However, the studies on *Tetranychus puerariicola* were limited.

Since *Wolbachia* is one of the most widespread bacterium in insects, many studies increasingly focused on the transmission mode of *Wolbachia* in its host. Expect vertical transmission, *Wolbachia* is susceptible to horizontal transmission, although direct evidences are limited. A lack of evolutionary congruence between mtDNA haplotypes and *Wolbachia* indicated multiple infections and horizontal transmission have occurred between unrelated hosts (Ahmed *et al*., 2013; Zhang *et al*., 2013a). Furthermore, extensive horizontal transmissions of *Wolbachia* can explain its temporal and spatial heterogeneity in infection prevalence (Kraaijeveld *et al*., 2011). One approach to explore if horizontal transmission is occurring is to compare the evolutionary relationships of the endosymbionts and their host.

T. puerariicola was first found infesting kudzu vine and was described as a new species by Ehara & Gotoh (1996). Previous studies indicated that *T. puerariicola* is highly polyphagous, but especially prefers leguminous plants. For example, they showed higher developmental rates and fecundity on soybean, kidney bean, and cowpea (Gotoh *et al*., 2004). In addition, this species is similar to another mite, *T. urticae* Koch (red form) (Suwa & Gotoh, 2006). Recently, *T. puerariicola* was determined to be widely distributed on various plants and in many regions of China. However, the distribution of *Wolbachia* in *T. puerariicola*, and the effect on its host have not yet been tested.

The aim of this study was to address the following questions: (i) what is the distribution of *Wolbachia* in natural populations of *T. puerariicola* (ii) do *Wolbachia* induce CI in *T. puerariicola* (iii) whether there is a horizontal transmission of *Wolbachia* in *T. puerariicola*, and (iv) what is the main factor that influences the genetic differentiation of *T. puerariicola*? We examined *Wolbachia* diversity in 12 natural populations of *T. puerariicola* in China based on *Wolbachia* surface protein (*wsp*) sequences and multiple locus sequence typing (MLST) data. Additionally, we established laboratory cultures of *w*Tpue1- and *w*Tpue3-infected and uninfected lines to examine if these strains induce CI in *T. puerariicola*. To further understand the potential factors that influence genetic differentiation of *T. puerariicola*, we compared the effects of *Wolbachia* infection patterns and geographical distribution on host mtDNA and nuclear DNA diversity.

Materials and methods

Sample collection and DNA extraction

From 2013 to 2014, *T. puerariicola* adults were collected from 12 populations over a large area of China (table 1). At each locality, individuals were randomly collected in a 5×5 m^2 square. Samples were preserved in absolute alcohol until extraction.

Total genomic DNA was extracted from the entire spider mite with a DNeasy Blood and Tissue kit (Qiagen, Valencia, CA, USA) following the manufacturer's protocol. All specimens were placed in a 1.5-ml plastic tube with 180 µl lysis buffer and proteinase K, incubated at 56°C for 2 h or longer, and subjected to DNA purification with a DNeasy Tissue kit (Qiagen, Valencia, CA, USA). Total DNA was eluted with 100 µl of elution buffer.

Polymerase chain reaction (PCR) and sequencing

For each population, 28–78 adult mites were screened for the presence of *Wolbachia* strains. *Wolbachia* detection and identification were performed with the primers 81F (5′-TGGT CCAATAAGTGATGAAGAAAC-3′) and 691R (5′-AAAA ATTAAACGCTACTCCA-3′) for *wsp* (Braig *et al*., 1998). PCRs were performed on a Veriti machine (ABI Biosystems, Foster City, CA, USA) in a 25 µl reaction volume containing 0.2 µl Maxima Hot Start Taq polymerase (5 U µl^{-1}, Fermentas), 2.5 µl 10 × Hot Start Taq Buffer (Fermentas), 2.5 µl $MgCl_2$ (2 mM, Fermentas), 2.0 µl dNTPs (2.5 mM each, Takara), 1 µl of DNA (concentration not estimated), and 0.5 µl primer (20 µM each). Cycling conditions were 95°C for 3 min followed by 35 cycles of 95°C for 30 s, 54°C for 45 s, and 72°C for 1 min, with a final extension at 72°C for 10 min. Positive and negative controls were included in PCRs. After verification via gel electrophoresis, DNA from all *Wolbachia*-positive individuals were subjected to cloning and

https://doi.org/10.1017/S0007485316000444 Published online by Cambridge University Press

Table 1. *Tetranychus pueraricola* sample and Wolbachia infection status details.

No.	Location	Geographic coordinates	Population code	Host plant	Collection date	Infection rate (%)	Sequence type
1	Yanshou, Heilongjiang	45.45°N, 128.34°E	HY	*Vigna unguiculata* Linn	August 2014	42.9 (28[1]/12[2])	287, 220
2	Harbin, Heilongjiang	45.74°N, 126.63°E	HH	*Lycopersicon esculentum* Mill	August 2014	14.3 (42/6)	220
3	Nujiang, Yunan	25.82°N, 98.86°E	YL	*Solanum melongena* L	August 2013	20.4 (49/10)	287, 220
4	Haba, Yunan	27.38°N, 99.96°E	YH	*Cucumis sativus* Linn	July 2013	8.1 (74/6)	287, 220
5	Yuxi, Yunnan	24.35°N,102.55°E	YY	*V.unguiculata*	July 2014	50.0 (28/14)	287, 220, D[3]
6	Hutiaoxia, Yunan	27.18°N,100.11°E	YC	*V.unguiculata*	July 2013	29.0 (31/9)	287
7	Weixi, Yunan	27.49°N, 99.57°E	YW	*V.unguiculata*	July 2013	8.1 (37/3)	220
8	Batang, Sichuan	30.01°N, 99.12°E	SB	*V.unguiculata*	August 2014	25.6 (78/20)	220
9	Chengdu, Sichuan	30.66°N, 104.07°E	SC	*V.unguiculata*	June 2014	26.0 (50/13)	287,220,290
10	Yongfu, Guangxi	25.01°N, 110.00°E	GY	*C.sativus*	July 2013	2.8 (36/1)	287
11	Zhangjiajie, Hunan	29.12°N, 110.48°E	HZ	*V.unguiculata*	July 2013	23.7 (38/9)	287
12	Changchun, Jilin	43.82°N, 125.33°E	JC	*V.unguiculata*	July 2014	12.5 (48/6)	220, D

[1]Total number of individuals.
[2]Number of infected individuals.
[3]Double-infected (287, 220).

sequencing. For cloning, an AxyPrep DNA Gel Extraction kit (AxyGEN, USA) was used to purify amplified fragments and a Peasy-T3 Cloning kit (TransGen Biotech, Beijing, China) was used to ligate PCR products into the cloning vector. Three positive clones per individual were finally confirmed by direct sequencing.

Single-infection status was confirmed by analyzing the *wsp* sequences. Then, *Wolbachia* MLST analysis was undertaken using standard primers and PCR protocols for amplification of the five reported *Wolbachia* MLST genes (*ftsZ, coxA, fbpA, hcpA, and gatB*) (Baldo *et al*., 2006). All MLST sequences were compared with entries in the PubMLST *Wolbachia* MLST database. Sequence type (ST) of *Wolbachia* strains was characterized by the allele number at five MLST loci. Strains with five identical alleles were considered the same ST.

The mitochondrial *COI* gene, which encodes cytochrome oxidase subunit I was amplified using the primers PCOI1F (5′-TTTTTATAGTGATACCAGCAA-3′) and PCOI1R (5′-CTCTAAAGATAGCAAATACGG-3′), PCOI2F (5′-GTTGATTTATGTTTTCCACGA-3′), and PCOI2R (5′-CTCTTAAAGATAGCAAATACGG-3′) by nested PCR. Due to the low amplification efficiency of one-step PCR, we choose another better amplification method—nested PCR, which can improve the experimental accuracy and sensitiveness. All PCRs were performed under the following cycling conditions: one cycle of 95°C for 3 min; 35 cycles of 95°C for 30 s, 47°C for 45 s, and 72°C for 1 min, and a final extension 72°C for 10 min.

The nuclear gene, which encodes 6-phosphofructokinase (*PFK-6*), was amplified using the primers Ngene1F (5′-ACAAGACACGAGAATCACCGTA-3′) and Ngene1R (5′-GAACGGCAGCATTCATACCAC-3′). The PCR cycles were as follows: one cycle of 95°C for 3 min; 35 cycles of 95°C for 30 s, 52°C for 30 s, and 72°C for 1 min, and a final extension at 72°C for 10 min. PCR products were electrophoresed on 1.5% agarose gels stained with ethidium bromide and visualized under UV transillumination. Amplified fragments were purified using a Gel Extraction Mini kit (Watson, Shanghai, China). Then, the distinct single-band amplicons were cloned into the pEasy-T3 vector (TransGen Biotech, Beijing, China), and the positive clones were screened and finally confirmed by direct sequencing.

DNA sequence and phylogenetic analysis

Sequences were aligned using Clustal W implemented in Mega 6 software (Tamura *et al*., 2013), and the alignment was manually edited with Bioedit software (Hall, 1999). The *COI* sequences were collapsed into haplotypes using Collapse 1.2 (Posada, 2004). Haplotype number, haplotype diversity (Hd), and nucleotide diversity (Pi) were calculated in DnaSP5.10 (Librado & Rozas, 2009). An intraspecific phylogeny of *COI* and nuclear gene haplotypes was inferred using the network algorithm median-joining in Network (Bandelt *et al*., 1999). All sequences have been deposited in the GenBank database. Phylogenetic analyses were estimation for a concatenated data set of MLST genes. Maximum Likelihood tree was constructed using MEGA6.0, with gamma-distributed rates with 1000 bootstrap replications, and the method of Jukes and Cantor as genetic distance model.

Analysis of genetic differentiation

Analysis of molecular variance (AMOVA) was used to formally assess and test the association among *Wolbachia* and the mtDNA sequences. Dataset 1 included all sequences from *T. pueraricola* and was subdivided into north populations (HH, HY, and JC) and south populations (YL, YH, YY, YC, YW, SB, SC, GY, and HZ). Dataset 2 was subdivided into sequences isolated from infected and uninfected *T. pueraricola*, infected sets included any kind of *Wolbachia* infection (*w*Tpue1, *w*Tpue2, *w*Tpue3, and *w*Tpue1 + *w*Tpue3). The two structured datasets were then used to test whether mtDNA from *T. pueraricola* showed significant genetic differentiation relative to bacterial infection or geographic origin. Dataset 3 included all single-copy nuclear DNA sequences from *T. pueraricola* and was subdivided into north populations (HH, HY, and JC) and south populations (YL, YH, YY, YC, YW, SB, SC, GY, and HZ). Genetic differentiation was investigated by AMOVA and the related fixation index (Fst) as implemented in Arlequin3.1 (Excoffier & Lischer, 2010).

Preparation of spider mite lines

T. pueraricola infected with *w*Tpue1 were collected from Anhui Province in 2014, and *T. pueraricola* infected with

https://doi.org/10.1017/S0007485316000444 Published online by Cambridge University Press

*w*Tpue3 were collected from Jilin Province in 2014. Mites were reared on leaves of the common bean (*Phaseolus vulgaris* L.) placed on a water-saturated sponge mat in Petri dishes (dia. 9) at 25 ± 1°C, 70% relative humidity, and under L16–D8 conditions. Subsequently, we obtained 100% infected and 100% uninfected lines (Zhao *et al*., 2013).

Cross experiments

To reveal if *w*Tpue1 and *w*Tpue3 induce CI in *T. puearicola*, four crosses were conducted (♀U × ♂U, ♀U × ♂I, ♀I × ♂U, and ♀I × ♂I). Each female at the last developmental stage before adult emergence was placed with two males. We used 1-day-old virgin males produced as a cohort by groups of females isolated as teliochrysalids. This procedure was designed to avoid the potential decrease of the *Wolbachia* effects due to male aging or other reasons. Males were removed 2 days after female eclosion, and mated females were allowed to oviposit for 5 days. Data were analyzed with one-way analysis of variance (ANOVA), and the means were compared using the Tukey-HSD test (SPSS 17.0).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.

Results

Wolbachia infection rate and diversity

Examination of the 539 *T. puearicola* female adults that were collected from 12 natural populations revealed that all populations were infected with *Wolbachia*. The infection rates of the populations varied from 2.8 to 50% (table 1).

wsp sequence analysis revealed three strains: *w*Tpue1, *w*Tpue2 and *w*Tpue3. Double infections were found only in YY and JC populations (table 1, fig. 1). Three sequence types (STs, corresponding to *wsp* sequence types) were revealed by MLST analyses (table 2) and were unambiguously assigned to supergroup B (Supplementary fig. S1).

Additionally, more remarkably, in the SC population (n = 50), individuals were single-infected with strain *w*Tpue1, *w*Tpue2, or *w*Tpue3, but no individuals were double- infected (fig. 1).

mtDNA and nuclear DNA

The *COI* sequences of 396 individuals were sequenced and revealed 16 haplotypes (GenBank: KU516055–KU516070). The SC population had the most number of haplotypes (H1, H11, H12, and H13), and the remaining populations had 1–3 haplotypes. Haplotype H1 was the most common and most widespread, and was found in four out of 12 populations studied. However, H4 was the only haplotype shared between southern and northern China (fig. 2A). Alternatively, single-copy nuclear DNA sequences (6-phosphofructokinase) revealed 14 haplotypes (GenBank: KU516071–KU516084). Haplotypes I and V were the most widespread haplotypes, and the HH population had the most haplotypes (I, II, III, IV, V, VI, VII, and VIII); haplotypes IV, VI, VII and VIII were only found in the HH population (fig. 2B).

For these samples, haplotype and nucleotide diversity estimates calculated from the mtDNA and single-copy nuclear DNA sequence data for different populations, and infected and uninfected groups of *T. puearicola* are presented in table 3. The average *COI* nucleotide and haplotype diversity values were estimated to be 0.00638 and 0.863, respectively. The molecular diversity of infected individuals was higher than the molecular diversity of the uninfected groups (infected Hd: 0.872, Pi: 0.00696; uninfected Hd: 0.857, Pi: 0.00625). Similarly, in nuclear DNA sequence analysis, haplotype and nucleotide diversity were higher in the infected group compared with the uninfected group (infected Hd: 0.591, Pi: 0.00307; uninfected Hd: 0.541, Pi: 0.00234).

In the *T. puearicola* populations sampled, mtDNA haplotype relationships were estimated as a network based on *Wolbachia* status (fig. 3A). There was a high level of *COI* variation among sequences. Moreover, there were 13 haplotypes in infected individuals and 14 haplotypes in uninfected individuals, and no clear relationship between *Wolbachia* status and mitochondrial haplotypes. Usually, the same *Wolbachia* strains existed in *T. puearicola* individuals with divergent *COI* haplotypes. Moreover, based on the nuclear DNA haplotype results, there were eight haplotypes in infected individuals and 13 haplotypes in uninfected individuals (fig. 3B).

Population genetic differentiation

Analysis of mtDNA haplotype variation using AMOVA was performed (i) among all individuals, divided into geographic regions(south; north) and (ii) among all individuals, divided based on infection status (infected; uninfected). From dataset1, an AMOVA clearly showed significant differentiation among haplotypes from different geographical regions (south and north) ($P < 0.0001$) for all individuals (table 4), which indicates that there is a strong natural barrier to gene flow among these populations. However, when haplotype variation was partitioned between infected and uninfected individuals in dataset 2, the AMOVA results clearly showed absence of significant differentiation.

Furthermore, similar to the mtDNA for the nuclear DNA, AMOVA clearly showed significant differentiation among haplotypes from different geographical regions (Fst = 0.12186; $P < 0.001$) (table 4).

CI crossing experiments

We found that *w*Tpue1 induce a certain degree of CI, and the predicted incompatible cross (♀U × ♂I) showed a reduction in egg hatchability (fig. 4A). However, individuals infected with *w*Tpue3 yielded different results. No significant differences were found between the predicted incompatible cross (♀U × ♂I) and the other compatible crosses (fig. 4B). Therefore, *w*Tpue3 existed as a non-CI strain in this spider mite. Our results indicate that the two widespread *Wolbachia* strains have different effects on host reproduction.

Discussion

In the current study, we investigated the infection rates of *Wolbachia* in 12 natural populations of *T. puearicola*, and our results indicated that the rates of *Wolbachia* infection were lower than those in *T. truncates* (2.8–50% vs. 55.6–90%, respectively) (Zhang *et al*., 2013a). However, in different geographic populations of *T. puearicola, Wolbachia* infection rates also differed. We inferred that this may be associated with different invasion events, *Wolbachia* strains, region topography, climate, host genetic background, and *Wolbachia* transmission efficiency. For example, the infection prevalence in 2013 was

https://doi.org/10.1017/S0007485316000444 Published online by Cambridge University Press

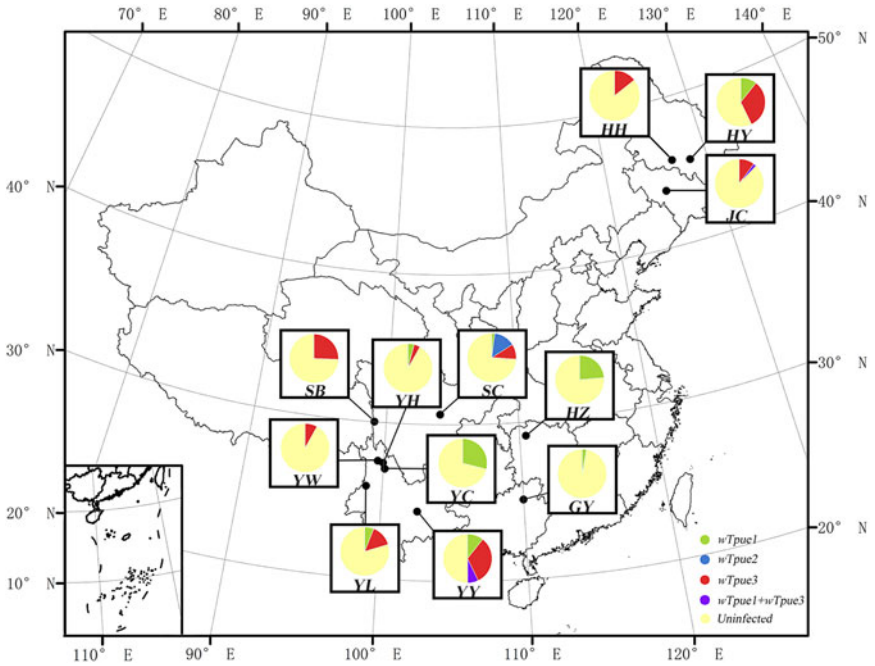

Fig. 1. Distribution and prevalence of *Wolbachia* variants in the examined populations of *T. pueraricola*. Each pie diagram shows the proportions of *Wolbachia*-infected and uninfected individuals in each geographic population.

Table 2. MLST allelic profiles of *Wolbachia* detected from *T. pueraricola*.

Strains	Sequence type (ST)	*gatB*	*coxA*	*hcpA*	*ftsZ*	*fbpA*
*w*Tpue1	287	126	122	13	106	237
*w*Tpue2	290	183	167	192	7	238
*w*Tpue3	220	9	121	143	23	4

lower than in 2014 (table 1). Similarly, *Wolbachia* prevalence was also found to differ among populations of the beetle *Chelymorpha alternans* (Keller *et al*., 2004) and the fire ant *Solenopsis invicta* (Ahrens & Shoemaker, 2005).

We then used MLST analysis to discover the diversity of *Wolbachia* strains in our samples. Our results revealed that three *Wolbachia* strains (*w*Tpue1, *w*Tpue2, and *w*Tpue3) infected *T. pueraricola*, of these strains, *w*Tpue1 and *w*Tpue3 were widespread in China. However, *w*Tpue2 was only present in the SC population. Four infection statuses were found in the examined mites, in addition to single infections for each strain, we also found double infection of strains of *w*Tpue1 and *w*Tpue3, which indicates that *Wolbachia* infection in *T. pueraricola* is complex. Moreover, we found several populations of *T. pueraricola* that contained more than one *Wolbachia* strain, which indicates that *Wolbachia* was introduced to *T. pueraricola* on multiple occasions. Similar phenomena have also observed in other arthropods (Kikuchi & Fukatsu, 2003; Ros *et al*., 2012; Symula *et al*., 2013; Zhang *et al*., 2013*b*). However, another potential cause of these results could be related to different effects of *Wolbachia* strains on their hosts.

Among insects, CI is a frequent reproductive effect of *Wolbachia*. Many studies showed that the CI levels induced by *Wolbachia* had great variability. Some of *Wolbachia* strains have neutral effects (they cannot induce CI); but several *Wolbachia* strains can induce extreme CI (Breeuwer, 1997; Perrot-Minnot *et al*., 2002; Vala *et al*., 2002; Gotoh *et al*., 2003). In our analysis, we found that *w*Tpue1 induced weak CI. However, *w*Tpue3 did not induce CI. The three *Wolbachia* strains found in *T. pueraricola* all belong to supergroup B, but *w*Tpue1 and *w*Tpue3 were not closely related (Supplementary fig. S1), therefore we considered the CI level induced by *Wolbachia* to be strain-specific. In addition, host background can also influence CI level. For example, *Wolbachia popcorn* cannot induce CI in *Drosophila melanogaster*. However, after the same strain was transinfected into a novel host, *Drosophila simulans*, the strain induced strong CI expression (McGraw *et al*., 2001).

The existence of CI was believed to be a driver that induces a decrease of mtDNA genetic diversity. However, the mtDNA Hd and Pi of the infected groups were higher than those of the uninfected groups in our study (infected: Hd: 0.872, Pi: 0.00696; uninfected: Hd: 0.857, Pi: 0.00625). Similar results were obtained based on single-copy nuclear DNA. These results are contrast with the previous findings in *T. urticae* and *T. truncatus*, in which *Wolbachia* reduced the mtDNA variation of infected mites (Yu *et al*., 2011; Zhang *et al*., 2013*a*). We formed some possibilities to explain this difference, different genetic background of mite host. On the other hand, the different abilities of various *Wolbachia* strains to manipulate host reproduction in different mites have an impact on the DNA diversity of the mitochondria. However, the lack of reduced diversity in *T. pueraricola* still needs to be elucidated. There are three potential reasons we hypothesized may lead to these results: (i) Firstly, the invasion time of *Wolbachia*, (a) *Wolbachia* have invaded in *T. pueraricola* for a long time. In our study, *w*Tpue1 induced CI in *T. pueraricola*, CI would decrease gene flow between adjacent areas if these areas were

https://doi.org/10.1017/S0007485316000444 Published online by Cambridge University Press

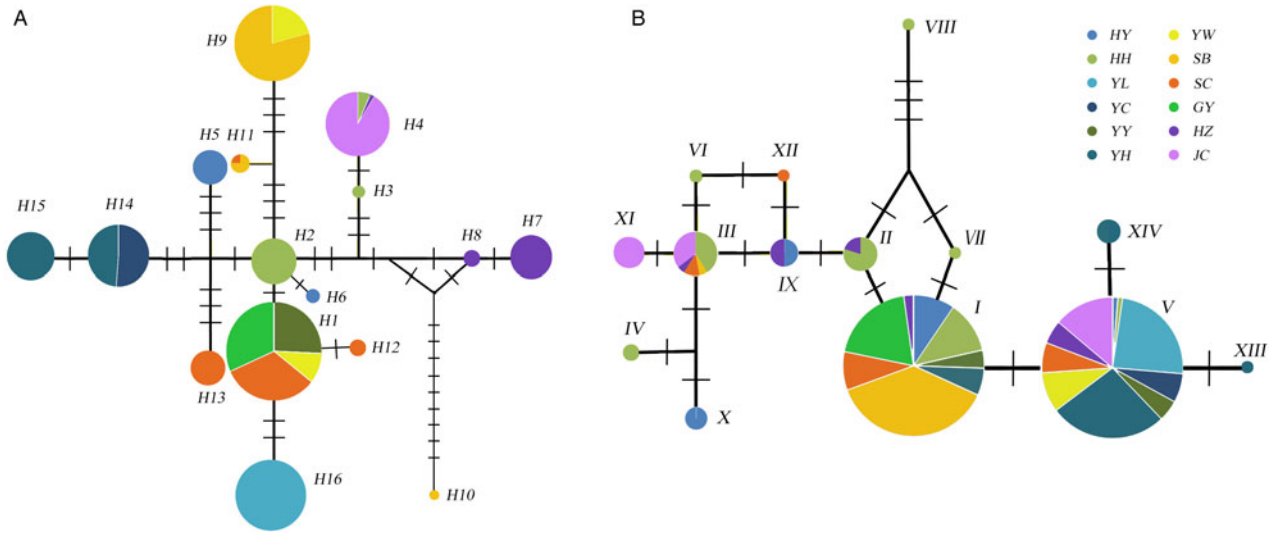

Fig. 2. Haplotype networks for populations based on (A) mitochondrial and (B) a single-copy nuclear gene of *T. pueraricola*. Different colors represented different geographic populations. Circles represent different haplotypes, and the size of each circle reflects the number of individuals with each haplotype. Bars on the branches refer to the number of mutations.

Table 3. Genetic variation in *T. pueraricola* populations from China.

Population	*COI*		*PFK-6*	
	Hd[1]	Pi[2]	Hd	Pi
HY	0.248	0.00155	0.431	0.00262
HH	0.280	0.00162	0.532	0.00390
YL	0	0	0	0
YH	0.511	0.00064	0.283	0.00088
YY	0	0	0.595	0.00210
YC	0	0	0	0
YW	0.514	0.00451	0	0
SB	0.142	0.00131	0.017	0.00015
SC	0.445	0.00253	0.558	0.00251
GY	0	0	0	0
HZ	0.255	0.00088	0.532	0.00306
JC	0	0	0.353	0.00476
Infected	0.872	0.00696	0.591	0.00307
Uninfected	0.857	0.00625	0.541	0.00234
ALL	0.863	0.00638	0.556	0.00256

COI (mtDNA), *PFK-6* (nuclear gene). For full site names and other details, see table 1.
[1]Haplotype diversity.
[2]Nucleotide diversity.

infected with incompatible strains of bacteria. Thus, one scenario is the infections could be long-standing rather than recent. Another possibility is that although *Wolbachia* may have induced reduction of mtDNA diversity when it initially infected *T. pueraricola*, as time goes on, new mutations accumulated (Avise, 2000; Marshall, 2004). This would mean that these *Wolbachia* infections have been maintained stably in the mites for a long period of time. (b) The lack of decreased diversity reflects the fact that these infections are recent, and there has thus not been sufficient time for selection against uninfected individuals to reduced mtDNA diversity. (ii) Secondly, the distribution of *Wolbachia* strains was found to be very complex in our study. We found three different *Wolbachia* strains and four infection statuses. In many populations, there were two or three *Wolbachia* infection statuses. Although a selective sweep associated with one strain may reduce the diversity of mtDNA, high levels of diversity may be maintained within the population as a whole across the different infection statuses and strains the population harbors (Hurst & Jiggins, 2005). (iii) Finally, in our study, because *w*Tpue1 induced CI but *w*Tpue3 did not, the low levels of reproduction regulation caused by the two widespread *Wolbachia* strains have different effects on the selection of their hosts' mtDNA haplotypes. However, given the low levels of CI, the association between *Wolbachia* and mtDNA may be a consequence of apparent co-evolution and not of a selective sweep or there was a weak selective sweep. Meanwhile, the presence of non-CI *w*Tpue3 indicated that this strain may have other effects or the infection could be recent. The exact reasons should be evaluated in future studies.

Our study found that mtDNA haplotype data were highly polymorphic, in total, 16 different haplotypes were detected. However, the haplotype data were not concordant with *Wolbachia* infection status. Hurst & Jiggins (2005) revealed that the association between *Wolbachia* and its host would be lost if horizontal transmission of *Wolbachia* occurs. We discovered the phenomenon of double infection within the same individual, and many mtDNA haplotypes shared between the infected and uninfected populations, suggesting horizontal transmission of *Wolbachia* or *Wolbachia* undergone an incomplete vertical transmission in *T. pueraricola*. In addition, both infected group and uninfected groups shared the same haplotypes; therefore, we inferred that correlation between haplotype and infection type may have degraded with the accumulation of mtDNA mutations and strain loss (Solignac *et al.*, 1994; Turelli, 1994; James *et al.*, 2002). However, another scenario is that there was incomplete transmission of *Wolbachia*.

AMOVAs of mtDNA and the single-copy nuclear gene showed significant genetic differentiation between southern and northern populations. Moreover, pairwise estimates of

https://doi.org/10.1017/S0007485316000444 Published online by Cambridge University Press

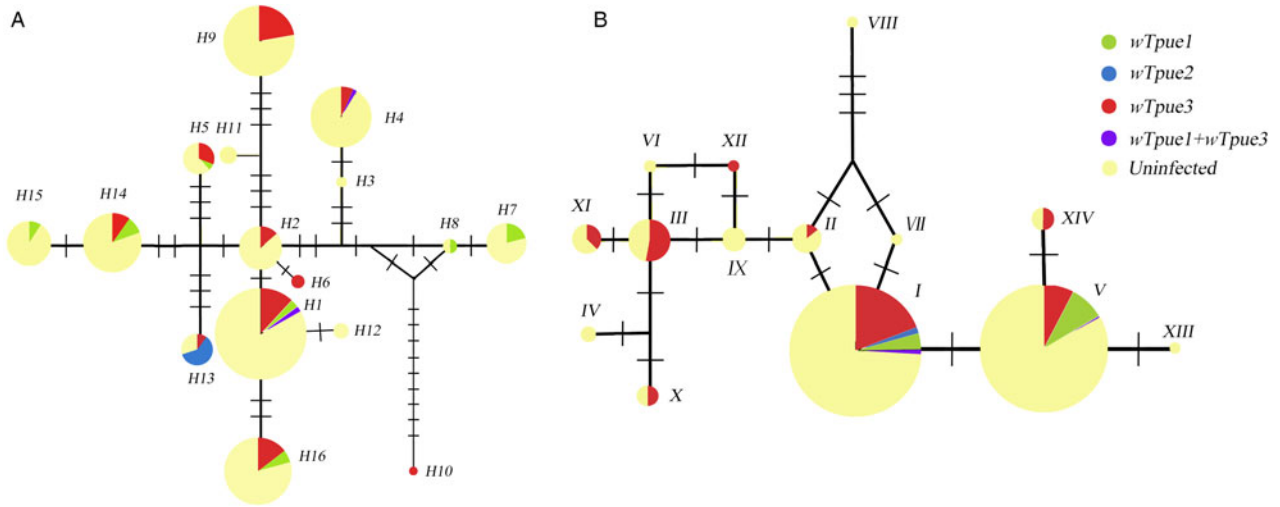

Fig. 3. Haplotype networks for *Wolbachia* infection based on (A) mitochondrial and (B) a single-copy nuclear gene of *T. pueraricola*. Each haplotype is colored based on the proportion of individuals with each *Wolbachia* infection status. Circles represent different haplotypes, and the size of each circle reflects the number of individuals with each haplotype. Bars on the branches refer to number of mutations.

Table 4. Datasets and AMOVA results for *T. pueraricola*.

Dataset	Subsets	Source of variation	df	Sum of squares	Variance components	Percentage of variation	Fixation indices	*P*-value
1	Mitochondrial DNA $South_{all}$ $North_{all}$	Dataset 1 (all, based on geographic regions)						
		Among subsets	1	131.138	0.98190 Va	30.67		
		Within subsets	395	876.810	2.21977 Vb	69.33		
		Total	396	1007.947	3.20167		0.30668	$P < 0.0001$
2	$Infected_{all}$ $Uninfected_{all}$	Dataset 2 (all, divided based on infection status)						
		Among subsets	3	9.342	0.02231 Va	0.88		
		Within subsets	360	904.029	2.51119 Vb	99.12		
		Total	363	913.371	2.53350		0.00881	0.21505
3	Nuclear DNA $South_{all}$ $North_{all}$	Dataset 3 (all, divided based on geographic regions)						
		Among subsets	1	14.565	0.05469 Va	12.19		
		Within subsets	754	297.176	0.39413 Vb	87.81		
		Total	755	311.741	0.44882		0.12186	$P < 0.0001$

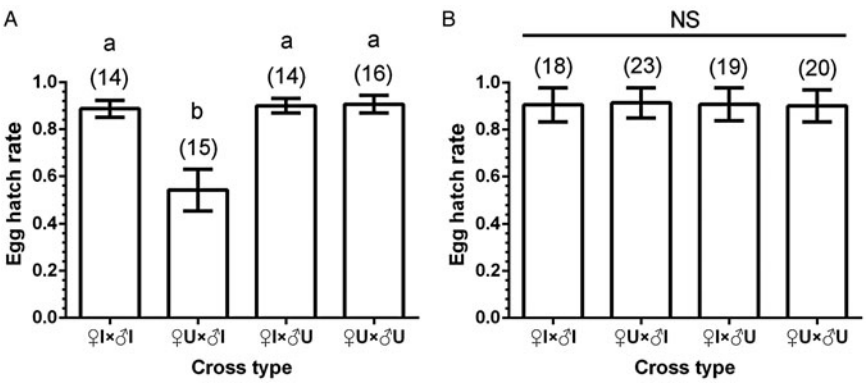

Fig. 4. CI resulting from crosses of two *Wolbachia* strains. Results are depicted as mean percent egg hatchability ± SE. Number of replicates for each of the nine cross types are shown in parentheses; a and b represent statistically different groups (Tukey-HSD test, $P < 0.05$); NS, not significant at the 5% level. A: *w*Tpue1 strain; B: *w*Tpue3 strain.

https://doi.org/10.1017/S0007485316000444 Published online by Cambridge University Press

Fst calculated between pairs of populations showed that most tests for population differentiation were significant (Supplementary table S1). Geographic barriers may play an important role in genetic differentiation. We observed high Fst values among *T. puearicola* population pairs, even among populations within very close distances, which indicated strong genetic structure that corresponds to small-scale geographic breaks. For example, samples were collected from different regions with variable mountain landscapes and climatic conditions, which influence population expansion and contraction, which have resulted in the relative lack of sharing of mitochondrial haplotypes among regions. We also found single haplotypes in populations, especially for mtDNA; this may have occurred because the mitochondrial genome has a smaller effective population size than that of an average nuclear locus, and the rate of genetic drift was therefore increased in mtDNA (Fay & Wu, 1999; Sakamoto *et al.*, 2015).

In conclusion, we provided a comprehensive overview of the infection status and reproductive effect of *Wolbachia* relative to the genetic diversity of *T. puearicola* in China. Our study revealed that *Wolbachia* infection statuses varied in *T. puearicola*. Additionally, the effects of *Wolbachia* are strain-dependent. The *w*Tpue1 strain can induce CI; however, *w*Tpue3 cannot. Moreover, *Wolbachia* did not dramatically reduce the mtDNA haplotype and nucleotide diversity. The *T. puearicola* population differentiation based on mitochondrial and nuclear markers can be best explained by differing demographic histories rather than a *Wolbachia*-associated selective sweep.

SUPPLEMENTARY MATERIAL

The supplementary material for this article can be found at http://dx.doi.org/10.1017/S0007485316000444.

Acknowledgements

We thank Jia Zhang and Xu Zhang of the Department of Entomology, Nanjing Agricultural University (NJAU) for help with the collection of spider mites. We are grateful to Dr Jin-Tao Sun of NJAU for his generous help with data analyses. This work was supported by a grant-in-aid from the Science and Technology Program of the National Public Welfare Professional Fund (201103020) from the Ministry of Agriculture of China, and a grant-in-aid for scientific research (31371944) from the National Natural Science Foundation of China.

References

Ahmed, M.Z., De Barro, P.J., Ren, S.X., Greeff, J.M. & Qiu, B.L. (2013) Evidence for horizontal transmission of secondary endosymbionts in the *Bemisia tabaci* cryptic species complex. *PLoS ONE* **8**, e53084.

Ahrens, M.E. & Shoemaker, D. (2005) Evolutionary history of *Wolbachia* infections in the fire ant *Solenopsis invicta*. *BMC Evolutionary Biology* **5**, 1.

Avise, J.C. (2000) *Phylogeography: the History and Formation of Species*. Cambridge, MA, Harvard University Press.

Bailly, X., Migeon, A. & Navajas, M. (2004) Analysis of microsatellite variation in the spider mite pest *Tetranychus turkestani* (Acari: Tetranychidae) reveals population genetic structure and raises questions about related ecological factors. *Biological Journal of the Linnean Society* **82**, 69–78.

Baker, E.W. & Tuttle, D.M. (1994) *A Guide to the Spider Mites (Tetranychidae) of the United States*. West Bloomfield, USA, Indira Publishing House.

Baldo, L., Hotopp, J.C.D., Jolley, K.A., Bordenstein, S.R., Biber, S.A., Choudhury, R.R., Hayashi, C., Maiden, M.C.J., Tettelin, H. & Werren, J.H. (2006) Multilocus sequence typing system for the endosymbiont *Wolbachia pipientis*. *Applied and Environmental Microbiology* **72**, 7098–7110.

Bandelt, H.J., Forster, P. & Röhl, A. (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* **16**, 37–48.

Bandi, C., Anderson, T.J., Genchi, C. & Blaxter, M.L. (1998) Phylogeny of *Wolbachia* in filarial nematodes. *Proceedings of the Royal Society of London B: Biological Sciences* **265**, 2407–2413.

Baudry, E., Bartos, J., Emerson, K., Whitworth, T. & Werren, J.H. (2003) *Wolbachia* and genetic variability in the birdnest blowfly *Protocalliphora sialia*. *Molecular Ecology* **12**, 1843–1854.

Bolland, H.R., Gutierrez, J. & Flechtmann, C.H. (1998) *World Catalogue of the Spider Mite Family (Acari: Tetranychidae)*. Leiden, Netherlands, Brill.

Braig, H.R., Zhou, W., Dobson, S.L. & O'Neill, S.L. (1998) Cloning and characterization of a gene encoding the major surface protein of the bacterial endosymbiont *Wolbachia pipientis*. *Journal of Bacteriology* **180**, 2373–2378.

Breeuwer, J.A. (1997) *Wolbachia* and cytoplasmic incompatibility in the spider mites *Tetranychus urticae* and *T.* turkestani. *Heredity* **79**, 41–47.

Breeuwer, J.A.J. & Jacobs, G. (1996) *Wolbachia*: intracellular manipulators of mite reproduction. *Experimental & Applied Acarology* **20**, 421–434.

Dean, M.D., Ballard, K.J., Glass, A., William, J. & Ballard, O. (2003) Influence of two *Wolbachia* strains on population structure of East African *Drosophila simulans*. *Genetics* **165**, 1959–1969.

Ehara, S. & Gotoh, T. (1996) Two new species of spider mites occurring in Japan (Acari, Tetranychidae). *Journal of the Acarological Society of Japan* **5**, 17–25.

Excoffier, L. & Lischer, H.E. (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* **10**, 564–567.

Fay, J.C. & Wu, C.I. (1999) A human population bottleneck can account for the discordance between patterns of mitochondrial versus nuclear DNA variation. *Molecular Biology and Evolution* **16**, 1003–1005.

Fenn, K., Conlon, C., Jones, M., Quail, M.A., Holroyd, N.E., Parkhill, J. & Blaxter, M. (2006) Phylogenetic relationships of the *Wolbachia* of nematodes and arthropods. *PLoS Pathogens* **2**, e94.

Goodacre, S.L., Martin, O.Y., Thomas, C.G. & Hewitt, G.M. (2006) *Wolbachia* and other endosymbiont infections in spiders. *Molecular Ecology* **15**, 517–527.

Gotoh, T., Noda, H. & Hong, X.Y. (2003) *Wolbachia* distribution and cytoplasmic incompatibility based on a survey of 42 spider mite species (Acari: Tetranychidae) in Japan. *Heredity* **91**, 208–216.

Gotoh, T., Suwa, A., Kitashima, Y. & Rezk, H.A. (2004) Developmental and reproductive performance of *Tetranychus puearicola* Ehara and Gotoh (Acari: Tetranychidae) at four constant temperatures. *Applied Entomology and Zoology* **39**, 675–682.

Grafton-Cardwell, E.E., Granett, J. & Normington, S.M. (1991) Influence of dispersal from almonds on the population dynamics and acaricide resistance frequencies of spider mites

https://doi.org/10.1017/S0007485316000444 Published online by Cambridge University Press

infesting neighboring cotton. *Experimental & Applied Acarology* **10**, 187–212.

Graham, R.I. & Wilson, K. (2012) Male-killing *Wolbachia* and mitochondrial selective sweep in a migratory African insect. *BMC Evolutionary Biology* **12**, 204.

Grbić, M., Van Leeuwen, T., Clark, R.M., Rombauts, S., Rouzé, P., Grbić, V. & Hernández-Crespo, P. (2011) The genome of *Tetranychus urticae* reveals herbivorous pest adaptations. *Nature* **479**, 487–492.

Hall, T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *In Nucleic Acids Symposium Series* **41**, 95–98.

Helle, W. & Sabelis, M.W. (Eds). (1985) *Spider Mites: Their Biology, Natural Enemies and Control*. Vol. **1**, p. 458. Amsterdam: Elsevier.

Hoffmann, A. & Turelli, M. (1997) Cytoplasmic incompatibility in insects. pp. 42–80 *in* O'Neill, S., Hoffman, A. & Werren, J. (Eds) *Influential Passengers: Inherited Microorganisms and Arthropod Reproduction*. Oxford: Oxford University Press.

Hurst, G.D. & Jiggins, F.M. (2005) Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: the effects of inherited symbionts. *Proceedings of the Royal Society of London B: Biological Sciences* **272**, 1525–1534.

James, A.C., Dean, M.D., McMahon, M.E. & Ballard, J.W.O. (2002) Dynamics of double and single *Wolbachia* infections in *Drosophila simulans* from New Caledonia. *Heredity* **88**, 182–189.

Jiggins, F.M. (2003) Male-killing *Wolbachia* and mitochondrial DNA: selective sweeps, hybrid introgression and parasite population dynamics. *Genetics* **164**, 5–12.

Keller, G.P., Windsor, D.M., Saucedo, J.M. & Werren, J.H. (2004) Reproductive effects and geographical distributions of two *Wolbachia* strains infecting the Neotropical beetle, *Chelymorpha alternans* Boh. (Chrysomelidae, Cassidinae). *Molecular Ecology* **13**, 2405–2420.

Kikuchi, Y. & Fukatsu, T. (2003) Diversity of *Wolbachia* endosymbionts in heteropteran bugs. *Applied and Environmental Microbiology* **69**, 6082–6090.

Kraaijeveld, K., Franco, P., Knijff, P.D., Stouthamer, R. & Alphen, J.J.M.V. (2011) Clonal genetic variation in a wolbachia-infected asexual wasp: horizontal transmission or historical sex? *Molecular Ecology* **20**, 3644–3652.

Librado, P. & Rozas, J. (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25**, 1451–1452.

Marshall, J.L. (2004) The allonemobius-*Wolbachia* host-endosymbiont system: evidence for rapid speciation and against reproductive isolation driven by cytoplasmic incompatibility. *Evolution* **58**, 2409–2425.

McGraw, E.A., Merritt, D.J., Droller, J.N. & O'Neill, S.L. (2001) *Wolbachia*-mediated sperm modification is dependent on the host genotype in *Drosophila*. *Proceedings of the Royal Society of London B: Biological Sciences* **268**, 2565–2570.

Migeon, A., Nouguier, E. & Dorkeld, F. (2011) Spider mites web: a comprehensive database for the Tetranychidae. pp. 557–560 *in* Sabelis, N.W. & Bruin, J. (*Eds*) *Trends in Acarology*. Netherlands, Springer.

Mitchell, R. (1973) Growth and population dynamics of a spider mite (*Tetranychus urticae* K., Acarina: Tetranychidae). *Ecology* **54**, 1349–1355.

Navajas, M., Perrot-Minnot, M.J., Lagnel, J., Migeon, A., Bourse, T. & Cornuet, J.M. (2002) Genetic structure of a greenhouse population of the spider mite *Tetranychus urticae*: spatio-temporal analysis with microsatellite markers. *Insect Molecular Biology* **11**, 157–165.

Nunes, M.D., Nolte, V. & Schlötterer, C. (2008) Nonrandom *Wolbachia* infection status of *Drosophila melanogaster* strains with different mtDNA haplotypes. *Molecular Biology and Evolution* **25**, 2493–2498.

Perrot-Minnot, M.J., Guo, L.R. & Werren, J.H. (1996) Single and double infections with *Wolbachia* in the parasitic wasp *Nasonia vitripennis* effects on compatibility. *Genetics* **143**, 961–972.

Perrot-Minnot, M.J., Cheval, B., Migeon, A. & Navajas, M. (2002) Contrasting effects of *Wolbachia* on cytoplasmic incompatibility and fecundity in the haplodiploid mite *Tetranychus urticae*. *Journal of Evolutionary Biology* **15**, 808–817.

Posada, D. (2004) Collapse 1.2. Program free. Available online at http://darwin.uvigo.es/

Rasgon, J.L., Cornel, A.J. & Scott, T.W. (2006) Evolutionary history of a mosquito endosymbiont revealed through mitochondrial hitchhiking. *Proceedings of the Royal Society of London B: Biological Sciences* **273**, 1603–1611.

Raychoudhury, R., Grillenberger, B.K., Gadau, J., Bijlsma, R., van de Zande, L., Werren, J.H. & Beukeboom, L.W. (2010) Phylogeography of *Nasonia vitripennis* (Hymenoptera) indicates a mitochondrial–*Wolbachia* sweep in North America. *Heredity* **104**, 318–326.

Ros, V.I., Fleming, V.M., Feil, E.J. & Breeuwer, J.A. (2012) Diversity and recombination in *Wolbachia* and *Cardinium* from Bryobia spider mites. *BMC Microbiology* **12**, 1.

Rowley, S.M., Raven, R.J. & McGraw, E.A. (2004) Wolbachia pipientis in Australian spiders. Current Microbiology **49**, 208–214.

Sakamoto, Y., Hirai, N., Tanikawa, T., Yago, M. & Ishii, M. (2015) Population genetic structure and *Wolbachia* infection in an endangered butterfly, *Zizina emelina* (Lepidoptera, Lycaenidae), in Japan. *Bulletin of Entomological Research* **105**, 152–165.

Shoemaker, D., Keller, G. & Ross, K.G. (2003) Effects of *Wolbachia* on mtDNA variation in two fire ant species. *Molecular Ecology* **12**, 1757–1771.

Sinkins, S.P. (2004) *Wolbachia* and cytoplasmic incompatibility in mosquitoes. *Insect Biochemistry and Molecular Biology* **34**, 723–729.

Solignac, M., Vautrin, D. & Rousset, F. (1994) Widespread occurence of the proteobacteria *Wolbachia* and partial cytoplasmic incompatibility in *Drosophila melanogaster*. Comptes rendus de l'Académie des sciences. Série *3*, Sciences de la vie **317**, 461–470.

Sun, J.T., Lian, C., Navajas, M. & Hong, X.Y. (2012) Microsatellites reveal a strong subdivision of genetic structure in Chinese populations of the mite *Tetranychus urticae* Koch (Acari: Tetranychidae). *BMC Genetics* **13**, 8.

Suwa, A. & Gotoh, T. (2006) Geographic variation in diapause induction and mode of diapause inheritance in *Tetranychus pueraricola*. *Journal of Applied Entomology* **130**, 329–335.

Symula, R.E., Alam, U., Brelsfoard, C., Wu, Y., Echodu, R., Okedi, L.M., Aksoy, S. & Caccone, A. (2013) *Wolbachia* association with the tsetse fly, *Glossina fuscipes*, reveals high levels of genetic diversity and complex evolutionary dynamics. *BMC Evolutionary Biology* **13**, 31.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, doi: 10.1093/molbev/mst197.

https://doi.org/10.1017/S0007485316000444 Published online by Cambridge University Press

Tsagkarakou, A., Gulllemaud, T., Rousset, F. & Navajas, M. (1996) Molecular identification of a *Wolbachia* endosymbiont in a *Tetranychus urticae* strain (Acari: Tetranychidae). *Insect Molecular Biology* **5**, 217–221.

Turelli, M. (1994) Evolution of incompatibility-inducing microbes and their hosts. *Evolution* **48**, 1500–1513.

Vala, F., Weeks, A., Claessen, D., Breeuwer, J.A.J. & Sabelis, M.W. (2002) Within-and between-population variation for *Wolbachia*-induced reproductive incompatibility in a haplo-diploid mite. *Evolution* **56**, 1331–1339.

Werren, J.H. (1997) Biology of *Wolbachia*. *Annual Review of Entomology* **42**, 587–609.

Werren, J.H., Baldo, L. & Clark, M.E. (2008) *Wolbachia*: master manipulators of invertebrate biology. *Nature Reviews Microbiology* **6**, 741–751.

Xiao, J.H., Wang, N.X., Murphy, R.W., Cook, J., Jia, L.Y. & Huang, D.W. (2012) *Wolbachia* infection and dramatic intraspecific mitochondrial DNA divergence in a fig wasp. *Evolution* **66**, 1907–1916.

Xie, R.R., Chen, X.L. & Hong, X.Y. (2011) Variable fitness and reproductive effects of *Wolbachia* infection in populations of the two-spotted spider mite *Tetranychus urticae* Koch in China. *Applied Entomology and Zoology* **46**, 95–102.

Yu, M.Z., Zhang, K.J., Xue, X.F. & Hong, X.Y. (2011) Effects of *Wolbachia* on mtDNA variation and evolution in natural populations of *Tetranychus urticae* Koch. *Insect Molecular Biology* **20**, 311–321.

Zhang, Z.Q. (2003) *Mites of Greenhouses: Identification, Biology and Control*. UK, CABI Publishing.

Zhang, Y.K., Ding, X.L., Zhang, K.J. & Hong, X.Y. (2013*a*) *Wolbachia* play an important role in affecting mtDNA variation of *Tetranychus truncatus* (Trombidiformes: Tetranychidae). *Environmental Entomology* **42**, 1240–1245.

Zhang, Y.K., Zhang, K.J., Sun, J.T., Yang, X.M., Ge, C. & Hong, X. Y. (2013*b*) Diversity of *Wolbachia* in natural populations of spider mites (genus Tetranychus): evidence for complex infection history and disequilibrium distribution. *Microbial Ecology* **65**, 731–739.

Zhao, D.X., Chen, D.S., Ge, C., Gotoh, T. & Hong, X.Y. (2013) Multiple infections with *Cardinium* and two strains of *Wolbachia* in the spider mite *Tetranychus phaselus* Ehara: revealing new forces driving the spread of *Wolbachia*. *PLoS ONE* **8**, e54964.

Zhu, L.Y., Zhang, K.J., Zhang, Y.K., Ge, C., Gotoh, T. & Hong, X. Y. (2012) *Wolbachia* strengthens *Cardinium*-induced cytoplasmic incompatibility in the spider mite *Tetranychus piercei* McGregor. *Current Microbiology* **65**, 516–523.

https://doi.org/10.1017/S0007485316000444 Published online by Cambridge University Press