

Further insights into the strange role of bacterial endosymbionts in whitefly, *Bemisia tabaci*: Comparison of secondary symbionts from biotypes B and Q in China

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Abstract

The percentage infection of secondary symbionts (SS) (*Wolbachia*, *Arsenophonus*, *Rickettsia*, *Hamiltonella*, *Fritschea* and *Cardinium*) in the exotic *Bemisia tabaci* (Genn.) invaders, commonly known as biotypes B and Q from China, were determined by PCR. In total, 373 biotype B and 1830 biotype Q individuals were screened for the presence of SS. Biotype B was more abundant than biotype Q from 2005 to 2006, and biotype Q was more abundant from 2007 to 2009. Each of the SS, with the exception of *Fritschea*, was detected in both biotypes B and Q; *Fritschea* was found in none of the samples examined. For biotype B, the percentage infection of *Hamiltonella* was the highest (92.0%) followed by *Rickettsia* (70.2%). For biotype Q, the percentage infection of *Hamiltonella* was again the highest (73.3%). *Arsenophonus* was the least common of the SS observed in both biotypes B and Q. The percentage infection of *Wolbachia*, *Rickettsia* and *Hamiltonella* in biotype B was each significantly higher than in biotype Q, whereas the percentage infection of *Cardinium* in biotype B was significantly lower than in biotype Q. The percentage infection of SS in biotypes B and Q varied from year to year over the period 2005–2009. Furthermore, within biotype Q, two distinct subgroups were identified which differ from each other in terms of their SS complement. We discuss these results in the light of the potentially influential factors and roles of the SS.

Keywords: *Bemisia tabaci* biotypes B and Q, *Wolbachia*, *Hamiltonella*, *Rickettsia*, *Arsenophonus*, *Cardinium*, *Fritschea*

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Introduction

Many insects are infected with maternally inherited intracellular bacteria. These are divided into the primary symbionts present in all individuals and the secondary symbionts (SS) facultatively associated with their insect hosts (Chiel *et al.*, 2007). Many of the different SS organisms have been shown to influence the biology, ecology and evolution of their hosts. One of the most widely studied SS is *Wolbachia*, which is known to occur in many arthropod species. This endosymbiont can alter the reproduction of its hosts principally through parthenogenesis induction and cytoplasmic incompatibility (Werren, 1997). In addition, *Wolbachia* can have both positive and negative influences on host fitness. Some *Wolbachia* improve the reproduction, hence fitness, of their hosts (e.g. Hariri *et al.*, 1998; Vavre *et al.*, 1999), while others reduce it by reducing fecundity (e.g. Min & Benzer, 1997; Wenseleers *et al.*, 2002), life span (McMeniman *et al.*, 2009), ability to disperse (Silva *et al.*, 2000), adult survival and locomotor performance (Fleury *et al.*, 2000). *Wolbachia* has also been implicated in affecting competitive ability and host evolution (Heddi *et al.*, 1999; Zchori-Fein & Brown, 2002) and is associated with host invasiveness (Shoemaker *et al.*, 2000; Tsutsui *et al.*, 2003; Chu *et al.*, 2005). Several other SS organisms have also been shown to play an important role in host fitness (Koga *et al.*, 2003; Zabalou *et al.*, 2004), host nutrition, adaptation to plant hosts (e.g. Douglas, 1998; Leonardo & Muir, 2003; Tsuchida *et al.*, 2004), resistance to parasitoids (Oliver *et al.*, 2005) and to heat stress (Montllor *et al.*, 2002).

The sweet potato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), is an important agriculture pest worldwide (Oliveira *et al.*, 2001). It is considered to be a species complex composed of at least 24 recognizable 'species' based on mitochondrial cytochrome oxidase I (*mtCOI*) maker, which (where data exist) have been shown to be either reproductively isolated or produce hybrid progeny that are considerably less fit than their parents (Dinsdale *et al.*, 2010; Xu *et al.*, 2010). Among them, two *B. tabaci* species, referred as Mediterranean (commonly known as biotype Q) and Middle East Asia Minor 1 (biotype B) (Dinsdale *et al.*, 2010), have become globally invasive.

Over the past decade or so, six SS, viz. *Wolbachia*, *Arsenophonus*, *Rickettsia*, *Hamiltonella*, *Fritschea* and *Cardinium*, have been identified from various members of the *B. tabaci* species complex (e.g. Zchori-Fein & Brown, 2002; Nirgianaki *et al.*, 2003; Thao *et al.*, 2003; Weeks *et al.*, 2003; Chu *et al.*, 2005; Chiel *et al.*, 2007). Among these SS, the distribution of *Wolbachia* and its role in the *B. tabaci* has been the most studied. The term supergroup of *Wolbachia* has been employed to designate the closely related groups based on gene sequences (Lo *et al.*, 2002). Previous studies have shown that *B. tabaci* were infected by *Wolbachia* belonging to supergroup B, but that infections were not found in all members of the species complex (Zchori-Fein & Brown, 2002; Nirgianaki *et al.*, 2003; Chu *et al.*, 2005; Ruan & Liu, 2005). In the case of biotype B, several studies (Zchori-Fein & Brown, 2002; Chu *et al.*, 2005; Ruan & Liu, 2005) failed to detect the presence of *Wolbachia* using both the *wsp* or 16s rDNA genes, whereas another molecular study (Nirgianaki *et al.*, 2003) detected infections in one out of 12 collections using the *wsp* gene. Similarly for biotype Q, no infections were found using the *wsp* gene in four collections from China, Israel and Spain (Chu *et al.*, 2005), whereas a more recent study using the 16s rDNA gene revealed five out of ten populations in Israel to be infected

(Chiel *et al.*, 2007). In contrast, collections belonging to species other than those containing biotypes B and Q appear more likely to be infected with *Wolbachia* (Li *et al.*, 2007).

The removal of some SS including *Wolbachia* and/or reduction of the primary endosymbiont using antibiotics may produce both favourable and unfavourable effects on the fitness of *B. tabaci* (Ruan *et al.*, 2006). In individuals belonging to the species associated with Asia that were infected with *Wolbachia* and *Arsenophonus*, treatment of adults with the antibiotics tetracycline or ampicillin trihydrate lead to an increase in the rate of development of their offspring, but did not significantly affect their survival, whereas treatments with another antibiotic, rifampicin, significantly retarded development and reduced progeny survival. The lack of detection of *Wolbachia* in some species belonging to the *B. tabaci* species complex suggests that it may not play a role; however, advances in DNA technology have demonstrated considerable increases in the level of sensitivity, suggesting that previous negative findings may have been false (Li *et al.*, 2007; Qu *et al.*, 2009).

Recent studies have shown that SS may play a major role in the biology of *B. tabaci*. A recent study of the distribution of SS in Israeli populations of *B. tabaci* biotypes B and Q found both *Hamiltonella* and *Rickettsia* in biotype B and *Arsenophonus*, *Wolbachia* and *Rickettsia* in biotype Q (Chiel *et al.*, 2007). *Fritschea* and *Cardinium* were not found in either biotype (Chiel *et al.*, 2007). Quantitative real-time PCR and FISH analyses showed that proliferation of *Rickettsia* is strongly induced when the *B. tabaci* was parasitized by the parasitic wasp *Eretmocerus mundus* (Mercet) (Hymenoptera: Aphelinidae), which suggests that it may be involved in the insect's resistance to various environmental stresses (Mahadav *et al.*, 2008). For example, *Rickettsia* in biotype B increased its susceptibility to several insecticidal compounds (Kontsedalov *et al.*, 2008), while double infections *Rickettsia*-*Arsenophonus* or *Wolbachia*-*Arsenophonus* had a significant influence on the survival of biotype Q exposed to the insecticides thiamethoxam, imidacloprid, pyriproxyfen and spiromesifen (Ghanim & Kontsedalov, 2009).

China has several indigenous members of *B. tabaci* complex whitefly (Qiu *et al.*, 2007), but in recent years both biotypes B and Q have successfully invaded and spread across large parts of the country (Luo *et al.*, 2002; Chu *et al.*, 2006). It appears that while biotype B invaded China in the mid-1990s (Luo *et al.*, 2002), biotype Q was a more recent arrival being first detected in Kunming, Yunnan Province, in 2003 and subsequently in the Beijing, Henan and Shandong Provinces (Chu *et al.*, 2006, 2007). Biotype B showed higher fecundity (Muniz & Nombela, 2001; Nombela *et al.*, 2001) and competitive ability than biotype Q under laboratory conditions (Pascual & Callejas, 2004), while biotype Q was found highly resistant to many insecticides, such as the neonicotinoid group of pesticides (Nauen *et al.*, 2002; Rauch & Nauen, 2003; Horowitz *et al.*, 2005). Recent studies have suggested that biotype Q has begun to displace biotype B (Chu *et al.*, 2007, 2010). A possible contributor to this successful displacement could be the presence of SS; and, thus, the determination of distribution of SS within biotypes B and Q should provide useful baseline information with which to examine the causes of this displacement.

The infection status of *B. tabaci* in China, with respect to SS, is poorly known, with studies focusing only on *Wolbachia* (Chu *et al.*, 2005; Ruan & Lui, 2005; Lin & Li, 2008). It would be useful to know the extent of infections and whether infections may have been derived locally or were present in the invaders

before they established in China (Chu *et al.*, 2007, 2010). The aim of the present study is to clarify the infection status of *Wolbachia*, *Arsenophonus*, *Rickettsia*, *Hamiltonella*, *Fritschea* and *Cardinium* in biotypes B and Q in China over the period 2005–2009. We then focus more closely on those SS that have been associated with insecticide resistance, *Wolbachia*, *Rickettsia* and *Arsenophonus* (Kontsedalov *et al.*, 2008; Ghanim & Kontsedalov, 2009) and compare their infection levels in biotype Q from China and its home range (Mediterranean countries).

Materials and methods

Whitefly samples and determination of *B. tabaci* biotypes

A total of 93 Chinese collections of *B. tabaci* on different hosts were made across seven sites (Liaocheng, Dezhou, Jinan, Zibo, Shouguang, Zaozhuang and Linyi) in Shandong Province of China between 2005 and 2009. At each location in each year, the collections were made from a range of host plants. For host plants, a collection involved sampling whitefly from every second available plant until at least 100 adults had been collected. A further 14 collections were provided by researchers from different Mediterranean countries (Bosnia-Herzegovina (one collection), Greece (two collections), Croatia (three collections), Cyprus (one collection), Egypt (one collection), Italy (one collection), Morocco (two collections), Spain (two collections) and USA (one collection). The adults were collected live and placed into tubes containing 95% ethanol and then stored at -20°C . Individual adults were subjected to lysis and DNA extraction following the procedure of Frohlich *et al.* (1999). The identity of *B. tabaci* from China determined, based on the CAPS (cleavage amplified polymorphic sequence) of *mtCOI* (mitochondrial cytochrome oxidase I) with the restriction endonucleases *VspI* and *StuI*. The *mtCOI* fragment (~620 bp) amplified by a set of PCR primers C1-J-2195 (5'-TTGATTTTGGTCATCCAGAAAGT-3') and R-BQ-2819 (5'-CTGAATATCGRCGAGGCATTCC-3') was first cleaved by *VspI*; uncut products were then cleaved by *StuI*. In the regions of China, all of the *mtCOI* cut by *VspI* have previously been shown to belong to biotype Q, whereas those cut by *StuI* were biotype B (Chu *et al.*, 2010). The identity of *B. tabaci* from Mediterranean countries was determined through sequencing of the COI (Chu *et al.*, 2006). Identity of the sequences was determined by comparing these unknown sequences with the 24 recently published consensus sequences (Dinsdale *et al.*, 2010).

Determination of secondary symbionts

Wolbachia 16S rDNA was amplified using the primers 16s315f (5'-GCATGAGTGAAGAAGGCC-3') and 16s628r (5'-AGATAGACGCCT TCGCCA-3') in Li *et al.* (2007). Five μl of PCR product was electrophoresed on 2% agarose gels. To confirm the presence of *Wolbachia*, the 314 bp product was digested with *NheI* following the methodology of Li *et al.* (2007). Only *Wolbachia* 16S rDNA product is cut by this enzyme and produces two bands (118, 196 bp). Digestion with *VspI* was done using 5 μl of PCR product in 10 μl reaction according to the manufacturer's instructions (Qu *et al.*, 2009). Sequencing of this fragment (Qu *et al.*, 2009) has confirmed it as being *Wolbachia* 16S rDNA. Digestion with *VspI* followed product separation using agarose gels revealed three banding patterns: (i) one band (314 bp) is expected for *Wolbachia*

belonging to supergroup A; (ii) three bands (164, 94, 56 bp) for supergroup B *Wolbachia*; and (iii) four bands (314, 164, 94, 56 bp) created by double infection. The presence of *Hamiltonella*, *Rickettsia*, *Arsenophonus*, *Fritschea* and *Cardinium* was determined using primers from Chiel *et al.* (2007). At least ten adults were taken from each collection, DNA extracted (Frohlich *et al.*, 1999) and the product then amplified using the methodology described in Chiel *et al.* (2007). The amplified products were then sequenced and their identity confirmed by comparing the sequences against those in GenBank using a standard BLAST comparison.

Statistical analysis

To determine the difference in the frequency of each SS in biotypes B and Q, all data for biotype B from China were pooled, respectively, and then independent sample *t*-tested to test the difference with those from the collections made from the Mediterranean region. The percentage of SS in each collection was arcsine transformed prior to analysis. We repeated the analysis for the comparison of the percentage infection of each SS species in biotype Q from China and those from the Mediterranean region.

The difference in the frequency of infection for each SS in biotype B across different host plants was analysed using one-way analysis of variance (ANOVA). Percentage data were arcsine transformed, host plants was regarded as factor and the percentage of each SS in each collection was the dependant variable. Biotype Q was analysed in the same way.

Results

The presence of different SS in biotypes B and Q from China and their changes in percentage infection during 2005–2009

In total, 373 individuals belonging to biotype B and 1830 to biotype Q were used to screen for the presence of SS. Biotype B was found to be the most common and widely distributed of the two biotypes found in China from 2005 to 2006 and biotype Q from 2007 to 2009. All SS species except *Fritschea* were detected in both biotypes B and Q. For biotype B, the percentage infection of *Hamiltonella* was highest (92.0%) followed by *Rickettsia* (70.2%), with *Arsenophonus* the lowest (1.1%). For biotype Q, the percentage infection of *Hamiltonella* was again highest (73.3%), with *Arsenophonus* again the lowest (0.7%). The percentage infections by *Wolbachia*, *Rickettsia* and *Hamiltonella* in biotype B were each significantly higher than those in biotype Q ($P < 0.05$). However, the percentage infection with *Cardinium* in biotype B was significantly lower than that in biotype Q ($P < 0.05$) (table 1).

Among the five SS, *Wolbachia* in the infected individuals of both biotypes B and Q belonged to supergroup A. Of the collections examined, 33.3% of collections containing biotype B and 12.5% of those with biotype Q were infected with *Wolbachia*. In terms of individual frequencies, 12.6% of biotype B and 2.1% of biotype Q individuals were found to be infected (table 1).

The percentage infections in biotypes B and Q varied over the period 2005–2009 (fig. 1). The percentage of biotype B infected with *Wolbachia* ranged from 5.9% to 36.4% during 2005–2008 and declined to zero in 2009. The percentage of biotype Q infected with *Wolbachia* ranged from 1.0% to 8.4% during 2005–2008 and also declined to zero in 2009. The percentage of biotype B infected with *Rickettsia* ranged from

Table 1. Percentage infections for the different SS in *Bemisia tabaci* biotypes B and Q.

Biotype	Population	N ^a	W ^b	R	A	H	C	None	Reference
B	China (Shandong)	373	12.6	70.2	1.1	92.0	4.8	5.1	Present study
B	Israel	205	0.0	64.0	0.0	100.0	0.0	0.0	Chiel <i>et al.</i> (2007)
Q	China (Shandong)	1830	2.1	4.1	0.7	73.3	12.2	21.6	Present study
Q	Mediterranean	130	0.0	0.8	2.3	–	–	–	Present study
Q	Israel	150	33.0	74.0	87.0	0.0	0.0	3.3	Chiel <i>et al.</i> (2007)
Q	Cyprus, Croatia, USA	47	36.2	90.4	12.8	–	–	–	Present study

N^a, number of individuals tested from biotypes Q and B populations.

^b, based on the method in Li *et al.* (2007).

W, *Wolbachia*; R, *Rickettsia*; A, *Arsenophonus*; H, *Hamiltonella*; C, *Cardinium*; –, undetected.

66.3% to 83.3% during 2005–2009, whereas for biotype Q it ranged from 14.3% to 16.2% in 2006 and 2007 before declining to almost zero in 2008 and 2009. *Arsenophonus* was nearly always <5.0% in both biotypes B and Q. *Hamiltonella* was the most frequently encountered infection maintaining levels above 68.3% in both biotypes B and Q over the period. *Cardinium* maintained a low presence of <6.5% in biotype B, while in biotype Q ranged between 7.6% and 17.3% (fig. 1).

The difference of percentage infection of SS in biotypes B or Q from different locations and host plants in China

The percentage infection with *Wolbachia* in biotype B from DeZhou (68.2%) was the highest, while in all other locations was <13.1%. The percentage infection of *Arsenophonus* in biotype B from most locations was higher than 53.3% except at ShouGuang where only 9.4% of individuals were infected. The percentage infection of *Rickettsia* in biotype B from all locations was lower than 4.6%, while those for *Hamiltonella* exceeded 68.8%. The percentage infection with *Cardinium* in biotype B from LiaoCheng (18.8%) was the highest, while at other locations was lower than 4.6%.

The percentage infection with *Wolbachia*, *Arsenophonus*, *Rickettsia* and *Cardinium* in biotype Q from all locations was <16.9%. The percentage infection with *Hamiltonella* in biotype Q from all locations exceeded >29.3% and was usually >83.4%. The difference of the percentage infection for each SS in biotypes B and Q did not differ significantly across the different host plant species ($P > 0.05$).

Percentage infections of Wolbachia, Rickettsia and Arsenophonus in biotype Q from Mediterranean regions and China

A total of 177 individuals from Bosnia-Herzegovina, Crete, Croatia, Cyprus, Egypt, Italy, Morocco, Spain and a non-Mediterranean population from the USA were examined for the presence of *Wolbachia*, *Rickettsia* and *Arsenophonus*. Only biotype Q from Cyprus was found to be infected by all three symbionts, viz., *Wolbachia* (85.0%), *Rickettsia* (100.0%) and *Arsenophonus* (30.0%). *Wolbachia* in the infected individuals from Cyprus belonged to supergroup A. Biotype Q from Italy was infected by *Rickettsia* (9.1%) and *Arsenophonus* (9.1%). *Rickettsia* was also found in biotype Q from Croatia and the USA with percentage infections of 70.0% and 100.0%, respectively. *Arsenophonus* was also found in biotype Q from Morocco and Spain.

Based on the analysis of *mtCOI* obtained from biotype Q individuals in each of the collections used in this study (not

shown), two distinct subgroups within biotype Q were identified and found to be consistent with previously published research (Chu *et al.*, 2008). Biotype Q from Bosnia-Herzegovina, Crete, Croatia (Croatia (1) and Croatia (2)), Egypt, Italy, Morocco, Spain and China belonged to one subgroup (I) while those from Cyprus, Croatia (Croatia (3)) and USA belonged to the second subgroup (II). The percentage infections of *Wolbachia*, *Rickettsia* and *Arsenophonus* in biotype Q (subgroup I) from China and from the Mediterranean region was <4.1%. While percentage infections with *Wolbachia*, *Rickettsia* and *Arsenophonus* in biotype Q (subgroup II) from Cyprus, Croatia (Croatia (3)) and USA was 36.2%, 90.4% and 12.8%, respectively. The percentage infection of *Rickettsia* in biotype Q from Cyprus, Croatia (Croatia (3)) and USA was always >70.0%.

Discussion

The distribution of the SS in the biotypes B and Q

Our study has demonstrated the presence of the endosymbionts *Wolbachia*, *Arsenophonus*, *Rickettsia*, *Hamiltonella* and *Cardinium* in both *B. tabaci* biotypes B and Q from China. This is a considerable departure from previously published data (Chiel *et al.*, 2007; Ahmed *et al.*, 2009). In the earlier study, only *Rickettsia* (64.0%) and *Hamiltonella* (100.0%) were detected in biotype B from Israel and only *Wolbachia* (33.0%), *Rickettsia* (74.0%) and *Arsenophonus* (87.0%) were detected in biotype Q from Israel (Chiel *et al.*, 2007). Based on these findings, the authors speculated on the possible roles of SS. However, our study showed that percentages of infection in biotype B were similarly high for *Rickettsia* and *Hamiltonella*, whereas percentages of infection by the remaining four SS were always below 25.7% with *Arsenophonus* and *Cardinium* being particularly low.

We also found *Wolbachia*, *Rickettsia* and *Arsenophonus* in biotype Q from China. However, the percentage of individuals in China infected with *Wolbachia*, *Rickettsia* and *Arsenophonus* were all very much lower than the percentages reported for Israel (Chiel *et al.*, 2007), and the percentage of *Wolbachia* was much lower than that for China (42.5%) (Ahmed *et al.*, 2009). Furthermore, in China infections with *Hamiltonella* and *Cardinium* were detected, whereas in Israel they were absent. In addition, our study showed that percentage infections with *Wolbachia*, *Rickettsia*, *Hamiltonella* and *Cardinium* differed significantly for both biotypes B and Q from China, whereas those for *Arsenophonus* did not. Our findings, thus, present quite a different picture to that provided by previous studies (Chiel *et al.*, 2007) and suggests that much more work is needed to analyse the roles played by SS. Why diversity of SS

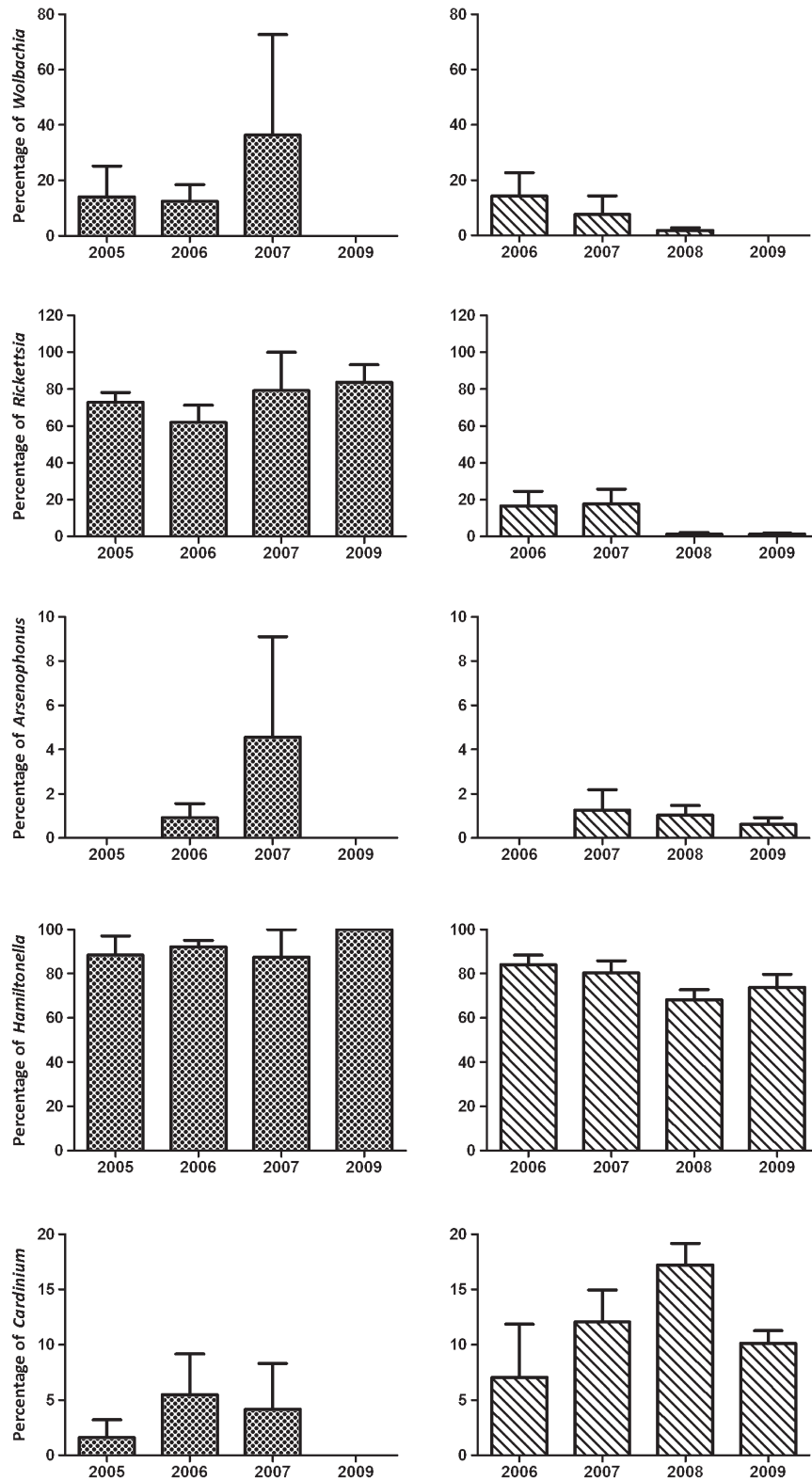


Fig. 1. The distribution of the different symbionts within *B. tabaci* biotypes B and Q from China during 2005 to 2009 (▨, biotype B; ▨, biotype Q).

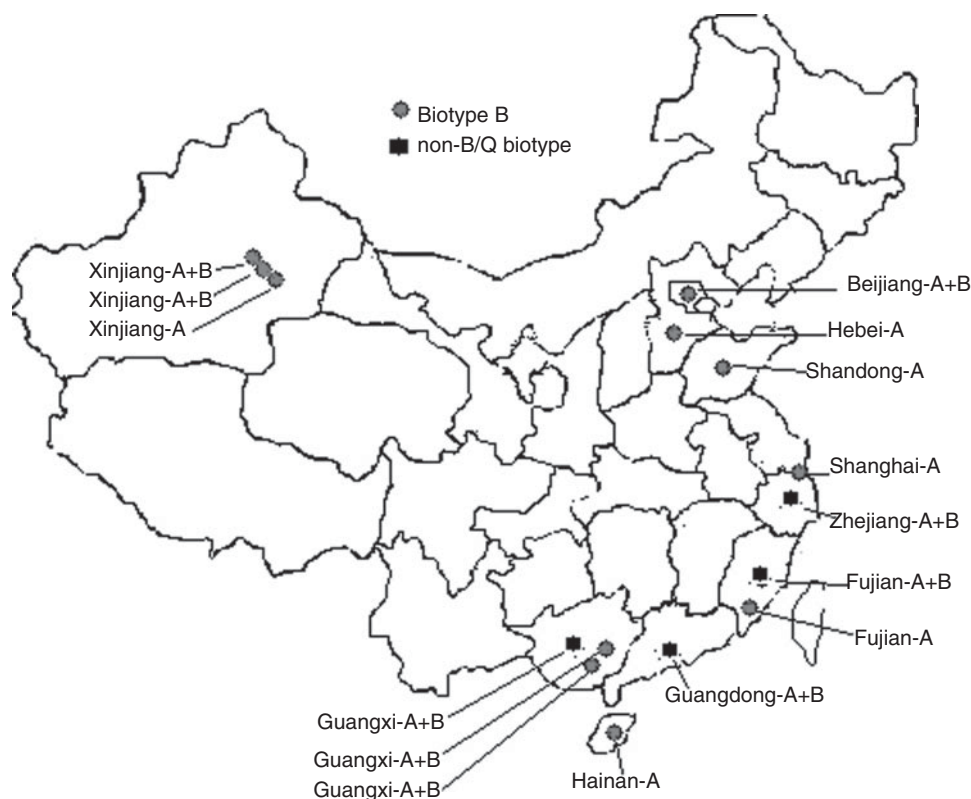


Fig. 2. *Wolbachia* infection status of B and non-B/Q biotype *B. tabaci* using PCR-RFLP analysis of 16s rDNA in China based on references (Li *et al.*, 2007; Guo & Li, 2008; Lin & Li, 2008). Note: A, B, A+B indicates *Wolbachia* supergroup A, B, both A and B, respectively.

infections in China should differ so markedly with those from Israel is unclear; there may be underlying biological explanations, but, equally, the patterns observed may reflect different levels of sampling effort.

The change in percentage infection of SS in biotypes B and Q from China during 2005–2009 and their influential factors

A key finding from this study is that the relative abundance of several of the SSs remain extremely fluid over time, suggesting that existence of complex ecological/genetic/physiological relationships that have yet to be understood. For both biotypes B and Q, the percentage infection varied across the period 2005–2009, but there was no obvious association with different host plants. This is a departure from earlier work (Chiel *et al.*, 2007); however, we are reluctant to draw too many conclusions from this as sampling effort was quite different between the two studies. In our present study, the percentage infections of *Hamiltonella* and *Arsenophonus* were consistent in Q over the period 2005–2009. *Hamiltonella* was always the most commonly detected SS, while infections with *Arsenophonus* remained at <5% over this period. Interestingly, the other two SSs, *Wolbachia* and *Rickettsia*, decreased during 2008–2009, whereas infections with *Cardinium* decreased in 2009. *Wolbachia* become undetectable in 2009 and *Rickettsia* declined to almost zero. The process(es) driving these widespread changes in infection frequencies is/are unknown, but the findings reveal that infection levels show considerable instability over time rather than being

fixed. The data also suggest that considerable changes in infection profiles over quite short time periods may be expected. This adds a further complication to comparisons across different studies.

For biotype B, the percentage infections of all SSs varied over the period 2005–2009. *Hamiltonella* and *Rickettsia* were the most frequently encountered SS. In contrast, infections with *Wolbachia*, *Arsenophonus* and *Cardinium* were low during 2005–2007 and each was absent in 2009.

In a recent study of insecticide resistance in field populations of biotypes B and Q from Shandong Province, we found that the biotype Q had higher levels of resistance to many insecticides compared to those in biotype B (Chu *et al.*, 2010). These observations reflect those of earlier work by Kontsedalov *et al.* (2008), who showed an association to exist between the presence of *Rickettsia* with increased susceptibility to acetamiprid, thiamethoxam, spiromesifen and especially pyriproxyfen insecticides. Similarly, another study (Ghanim & Kontsedalov, 2009) showed that the insecticides thiamethoxam, imidacloprid, pyriproxyfen and spiromesifen were all more effective against biotype Q with *Rickettsia*-*Arsenophonus* and *Wolbachia*-*Arsenophonus* infections than those with only *Arsenophonus* infections. Our study similarly suggests that infections with *Wolbachia* and *Rickettsia* may increase susceptible to insecticides and that the loss of these particular SS infections leads to increased resistance (Ghanim & Kontsedalov, 2009). Hence, our data is consistent with the hypothesis of Ghanim & Kontsedalov (2009), although more work is required to confirm this apparent relationship.

Table 2. Percentage of infection with *Wolbachia* within collections of *Bemisia tabaci* biotypes B and Q.

Reference	Biotypes	Collection sites*	Total number tested*	Infected (%)			Total frequency (%)
				A	B	A + B	
Nirgianaki <i>et al.</i> , 2003	B	USA, 2(0); Antigua, 1(0); Australia, 1(0); Brazil, 1(0); Cyprus, 1(0); Egypt, 1(0); Israel, 1(0); Japan, 1(0); New Zealand, 1(0); Taiwan, 1(0); Yemen, 1(1)	12(1)		8.3		8.3
	Non-B	Australia, 1(1); Benin, 1(1); Colombia, 1(1); Greece, 1(1); Hainan, 1(1); India, 1(0); Kenya, 5(2); New Zealand, 1(0); Nigeria, 2(0); Pakistan, 1(1); Portugal, 1(0); Singapore, 1(0); Spain, 6(1); Sri Lanka, 1(0); Sudan, 1(0); Turkey, 1(1)	26(10)		38.5		38.5
Zchori-Fein & Brown, 2002	B	Israel, 1(0); USA, 1(0)	2(0)				0
	Non-B	Australia, 1(0); USA, 3(3); Mexico, 3(1); Brazil, 1(0); Cameroon, 1(0); India, 1(0); Kenya, 1(0); Tanzania, 3(0); Uganda, 4(3)	18(7)				38.3
Hong <i>et al.</i> , 2005	B	Taiwan, 129(25)	129(25)				19.4
	Non-B	Taiwan, 73(52)	73(52)				71.2
Chu <i>et al.</i> , 2005	B	Israel, 1(0); Spain, 1(0); USA, 3(0); Australia, 1(0); China, 10(0)	16(0)				0
	Q	Israel, 1(0); Spain, 1(0); China, 2(0)	4(0)				0
	Non-B/Q	China, 2(1); Kenya, 1(1); Pakistan, 1(0)	4(2)		50		50
Chiel <i>et al.</i> , 2007	B	Israel, 18(0)	18(0)				0
	Q	Israel, 10(5)	10(5)				50
Li <i>et al.</i> , 2007	B	China, 10(10); Israel, 3(3)	13(13)	23		77	100
	Q	Israel, 1(1)	1(1)	100			100
	Non-B/Q	China, 4(4)	4(4)		25	75	100
Lin & Li, 2008	B	Fujian, China, 11(11)	11(11)	100			100
	Non-B/Q	Fujian, China, 6(6)	6(6)			100	100
Guo & Li, 2008	B	China, 9(9)	9(9)	100			100
	Non-B/Q	China, 4(4)	4(4)			100	100
Present study	B	Shandong, China, 21(7)	21(7)	33.3			33.3
	Q		83(9)	10.8			10.8
	Q(China)	Shandong, China, 72(9)	72(9)	12.5			12.5
	Q(Mediterranean)	Crete, 2(0); Egypt, 1(0); Italy, 1(0); Morocco, 2(0); Spain, 2(0); Croatia, 2(0); Bosnia and Herzegovina, 1(0)	11(0)				0

* The number of infected populations is indicated in parentheses.

The potential role of *Wolbachia* in biotypes B and Q

Wolbachia is known to affect a range of biological processes in arthropods (Ros *et al.*, 2009), but their role in *B. tabaci* is unknown (Chiel *et al.*, 2007). Phylogenetic reconstructions using the *ftsZ* gene have been used to identify eight supergroups (A to H) within *Wolbachia* (Lo *et al.*, 2002; Ros *et al.*, 2009). Of these, all but C and D have been detected in arthropods. A and B are the most widely distributed across arthropods.

In China, *Wolbachia* infections in both invader and indigenous populations belonged mostly to supergroup A as a single infection, whereas the indigenous populations tended to carry *Wolbachia* from supergroup B as a co-infection with no single infections with supergroup B being observed (Li *et al.*, 2007; Guo & Li, 2008; Lin & Li, 2008) (fig. 2). Their data showed that the *Wolbachia* infections were closely related to the genetic group structure of *B. tabaci*, and that infection percentages were much higher than those reported previously (Li *et al.*, 2007; Guo & Li, 2008; Lin & Li, 2008). *Bemisia tabaci* populations *sensu lato* are known to have a complex genetic structure (De Barro *et al.*, 2000; Boykin *et al.*, 2007), and it has recently been shown that at 3.5% *mtCOI* divergence there is a

distinct break in the frequency distribution (Dinsdale *et al.*, 2010). If the observed genetic variation is due to differentiation among populations of the one species, it would be expected that there would be no distinct gaps and that variation, although clumped, would be relatively continuous. This was not the case. Further, Xu *et al.* (2010) has shown that, while the data is patchy, sampled populations show marked levels of reproductive isolation between the groups identified at this level of sequence divergence with either no female progeny produced, or progeny with reduced fitness relative to their parents. One possible driver behind this structure is between genetic group cytoplasmic incompatibility caused by *Wolbachia*.

Several early studies (Zchori-Fein & Brown, 2002; Nirgianaki *et al.*, 2003; Chu *et al.*, 2005; Hong *et al.*, 2005) showed that *B. tabaci* infected with *Wolbachia* belonged primarily to genetic groups other than those to which biotypes B and Q belonged (tables 2 and 3). The data, however, are quite variable.

More recent studies (Chiel *et al.*, 2007) found no biotype B individuals to be infected but that a third of biotype Q individuals in half the collections were (tables 2 and 3). In a series of recent studies from China (Li *et al.*, 2007; Guo & Li,

Table 3. Percentage of infection with *Wolbachia* within *Bemisia tabaci* biotypes B and Q individuals.

Reference	Biotypes	Collection sites*	Total number tested*	Infected (%)			Total frequency (%)
				A	B	A + B	
Nirgianaki <i>et al.</i> , 2003	B	USA, 26(0); Antigua, 9(0); Australia, 13(0); Brazil, 13(0); Cyprus, 13(0); Egypt, 12(0); Israel, 13(0); Japan, 9(0); New Zealand, 13(0); Taiwan, 3(0); Yemen, 9(9)	133(9)		6.8		6.8
	Non-B	Australia, 13(13); Benin, 13(13); Colombia, 13(13); Greece, 13(13); Hainan, 13(13); India, 9(0); Kenya, 49(25); New Zealand, 13(0); Nigeria, 23(0); Pakistan, 13(13); Portugal, 12(0); Singapore, 13(0); Spain, 61(13); Sri Lanka, 13(0); Sudan, 13(0); Turkey, 13(13)	287(129)		44.9		44.9
Hong <i>et al.</i> , 2005	B	Taiwan, 232(41)	232(41)				17.7
	Non-B	Taiwan, 124(83)	124(83)				66.9
Ruan & Liu, 2005	B	Zhejiang, China, 20(0)	20(0)				0
	Non-B	Zhejiang, China, 20(5)	20(5)				25.0
Chiel <i>et al.</i> , 2007	B	Israel, 205(0)	205(0)				0
	Q	Israel, 150(50)	150(50)				33.3
Li <i>et al.</i> , 2007	B/Q	China, Israel, 260(221)	260(221)	67.0	14.9	5.0	85.9
	Non-B/Q	China, 104(72)	104(72)	19.4	43.8	6.1	69.2
Lin & Li, 2008	B	Fujian, China, 96(95)	96(95)	99.0			99.0
	Non-B/Q	Fujian, China, 57(57)	57(57)	56.1	26.3	17.5	100
Guo & Li, 2008	B	China, 64(63)	64(63)	98.4			98.4
	Non-B/Q	China, 35(19)	35(19)			54.3	54.3
Present study	B	Shandong, China, 373(30)	373(47)	12.6			12.6
	Q		1960(38)	1.9			1.9
	Q(China)	Shandong, China, 1830(40)	1830(38)	2.1			2.1
	Q(Mediterranean)	Crete, 18(0); Egypt, 4(0); Italy, 11(0); Morocco, 23(0); Spain, 31(0); Croatia, 28(0); Bosnia and Herzegovina, 15(0)	130(0)	0			0

* The number of infected individuals is indicated in parentheses.

2008; Lin & Li, 2008), all collections of biotype B contained individuals with infection levels ranging from 98.4–99.0% (tables 2 and 3). Our results are in agreement with these but suggest that levels of infection were much higher than previously recorded. It seems, then, that levels of infection are increasing. Even so, Li *et al.* (2007) showed, using their revised method for detecting *Wolbachia* infections, that increased detection sensitivity and, so, the observed increase is more likely to be due to an improvement in methodology. According to Li *et al.* (2007), co-infection within biotype B individuals was lower than that for non-B/Q biotype individuals (table 2). Furthermore, Guo & Li (2008) and Lin & Li (2008) observed only single supergroup A infection in *B. tabaci* biotype B. Our study reveals similar levels of infection in biotypes B and Q. Yet our percentage of infected biotype B individuals, 12.6%, is far less than the 98.4% observed in Guo & Li (2008) and Lin & Li (2008). Undoubtedly, then, there is considerable variability in infection status of *B. tabaci* across China.

In conclusion, a review of past studies plus the new data generated by this study showed that in China (i) *Wolbachia* infections in biotype Q are less common than infections in biotypes B and non-B/Q *B. tabaci*; (ii) *Wolbachia* infections within biotype Q belong primarily to supergroup A as a single infection; (iii) *Wolbachia* infections in biotype B are more widespread and are primarily single infections belonging to supergroup A; (iv) to date, there are no records of single

infection by supergroup B in either biotype B or Q; and (v) single infection with supergroup B or coinfections with supergroups A and B are mainly confined to the non-B/Q biotype *B. tabaci*. Phylogenetic analysis shows that biotypes B and Q are closely related (Boykin *et al.*, 2007; Dinsdale *et al.*, 2010) but different species (Dinsdale *et al.*, 2010). Together, biotypes B and Q belong to a cluster of African, Mediterranean, Middle East and Asia Minor species which are quite distinct from those occurring in Asia, Australia and the New World (Dinsdale *et al.*, 2010). While the data is not yet unequivocal, supergroup B infections are far more common in these latter groups, which mean that *Wolbachia* may well have played a role in the past in the speciation process of the *B. tabaci* cryptic species complex.

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