Relative Amount of Symbionts in Insect Hosts Changes with Host-Plant Adaptation and Insecticide Resistance

HUI PENG PAN,[1] DONG CHU,[2] BAI MING LIU,[1] WEN XIE,[1] SHAO LI WANG,[1] QING JUN WU,[1] BAO YUN XU,[1] AND YOU JUN ZHANG[1,3]

Environ. Entomol. 42(1): 74–78 (2013); DOI: http://dx.doi.org/10.1603/EN12114

ABSTRACT The impact of symbionts on their hosts depends on their infection density. In the current study, we investigated the effects of host plant and insecticide resistance on the relative amount of symbionts *Portiera*, *Hamiltonella*, *Rickettsia*, and *Cardinium* in the whitefly *Bemisia tabaci* (Gennadius) B biotype. The relative amount of symbionts in three host plant-adapted subpopulations (cucumber, *Cucumis sativus* L.; cabbage, *Brassica oleracea* L.; and cotton, *Gossypium herbaceum* L.) with the same genetic background and insecticide (thiamethoxam)-resistant and -susceptible subpopulations with the same genetic background were measured by quantitative polymerase chain reaction. The results showed that the cucumber population harbored more *Portiera* than the cabbage and cotton populations, the cabbage population harbored more *Hamiltonella* than the cucumber population, *Hamiltonella* amount did not statistically differ between the cotton and cucumber or the cotton and cabbage populations, and the cabbage population harbored more *Rickettsia* and *Cardinium* than the cucumber and cotton populations. In addition, the thiamethoxam-susceptible population harbored more *Portiera* and *Hamiltonella* than the thiamethoxam-resistant population, whereas the thiamethoxam-resistant population harbored more *Rickettsia* than the thiamethoxam-susceptible population. These results indicated that relative amounts of symbionts were affected significantly by host plant-adaption and insecticide resistance, and the response to host plant and insecticide differed among the symbionts.

KEY WORDS *Bemisia tabaci*, symbiont, host plant, insecticide

The sweetpotato whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is a destructive pest of many field crops and protected crops worldwide. It causes serious damage by directly feeding on plants and by vectoring >200 plant viruses (Hogenhout et al. 2008, Nawaz-ul-Rehman and Fauquet 2009, Pan et al. 2012a). *Bemisia tabaci* is recognized as a species complex that comprises a large number of genetically distinct sibling species, biotypes, or both (Brown et al. 1995, Dinsdale et al. 2010, De Barro et al. 2011). Researchers have thus far described >30 whitefly biotypes, of which the B biotype (herein referred to as B) and Q biotype (herein referred to as Q) are the most invasive (De Barro et al. 2011).

The *B. tabaci* associated with the first whitefly outbreak in China, which occurred in the mid-1990s, was the exotic B (Luo et al. 2002). Since then, B rapidly invaded the entire country, and has led to serious yield losses in many crops (Chu et al. 2006). Q first was found in Yunnan Province in 2003 and was considered a new, invasive whitefly in China (Chu et al. 2006). Since its introduction, Q has spread into many parts of China (Pan et al. 2011).

Like many other insects, *B. tabaci* hosts bacterial endosymbionts (Baumann 2005). The symbionts of insects generally are divided into two groups: primary symbionts (referred to as P-symbionts) and secondary symbionts (referred to as S-symbionts) (Baumann 2005, Feldhaar 2011). To date, one P-symbiont (*Portiera*) and six S-symbionts (*Hamiltonella*, *Arsenophonus*, *Cardinium*, *Wolbachia*, *Rickettsia*, and *Fritschea*) have been reported from the *B. tabaci* (Zchori-Fein and Brown 2002, Weeks et al. 2003, Thao and Baumann 2004a, Baumann 2005, Everett et al. 2005, Gottlieb et al. 2006). The P-symbiont *Portiera* provides nutrients that supplement the insufficient nutrients that *B. tabaci* obtains from its restricted diet of plant phloem (Thao and Baumann 2004b, Baumann 2005), whereas S-symbionts play important roles in *B. tabaci* biology and ecology (Kontsedalov et al. 2008, Mahadav et al. 2008, Ghanim and Kontsedalov 2009, Gottlieb et al. 2010, Brumin et al. 2011, Himler et al. 2011). For example, infection by the S-symbiont *Rickettsia* increases the fitness of *B. tabaci* B: relative to uninfected whiteflies, *Rickettsia*-infected whiteflies experienced a 15–30% increase in survival to adulthood, developed to adulthood 1–2 d faster, produced twice as many offspring, and produced a greater proportion of female offspring (Himler et al. 2011).

[1] Department of Plant Protection, Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing 100081, P. R. China.

[2] College of Agronomy and Plant Protection, Qingdao Agricultural University, Qingdao, 266109, P. R. China.

[3] Corresponding author, e-mail: zhangyj@mail.caas.net.cn.

0046-225X/13/0074–0078$04.00/0

The amount of the symbiont (the number of symbionts per host whitefly) is important because it influences both the efficiency of transmission of the symbiont to the offspring and the virulence of the symbiont (Kondo et al. 2005). For example, high bacterial densities in *B. tabaci* are correlated with the insect's ability to detoxify insecticides and other toxic compounds (Ghanim and Kontsedalov 2009). Latest research from our laboratory recently has shown that biotype, sex, host plant, and geographical location affected the diversity and infection frequency of S-symbionts in *B. tabaci* (Chu et al. 2011; Pan et al. 2012b, c), but, few studies have focused on the infection densities of these symbionts.

In the current study we 1) measured the effect of host-plant adaptation on the relative amount of P- and S-symbionts in *B. tabaci* subpopulations, and 2) determined the relative amount of symbionts in insecticide (thiamethoxam)-resistant and -susceptible subpopulations.

Materials and Methods

Plant Cultures. Three crop species from three families, which have been widely cultivated in China, were used in the experiments: cabbage (*Brassica oleracea* L., 'Jingfeng 1'), cucumber (*Cucumis sativus* L., 'Zhongnong 12') and cotton (*Gossypium herbaceum* L., 'DP99B'). The cabbage and cucumber seeds were purchased from the Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing, China, and the cotton seed was purchased from the Monsanto Company, Creve Coeur, MO, United States. All the three field crops were grown in a potting mix (a mixture of peat moss, vermiculite, organic fertilizer, perlite in a 10:10:10:1 ratio by volume) in 1.5-liter pots (one plant per pot) and enclosed in whitefly-proof screen cages under natural light and controlled temperature (26 ± 2°C) in a glasshouse. When these plants grew to the 5–7 true leaf stage, they were used to maintain the corresponding *B. tabaci* laboratory populations (see next section).

***B. tabaci* Host Plant-Adapted Subpopulations.** The three host plant-adapted *B. tabaci* B subpopulations (cotton, cucumber, and cabbage) used in this study were derived from the same parental population. The parental population was established in 2004 by releasing 10 pairs of *B. tabaci* adults, which had been collected from cabbage in Haidian District of Beijing, into one screen cage containing one cabbage plant in a glasshouse (Xie et al. 2011, Pan et al. 2012c). The parental population was identified as a pure B population based on the sequencing of the mitochondria cytochrome oxidase one (*mtCO1*) gene marker (Zhang et al. 2005). After two generations, two more cabbage plants (i.e., three plants in total) were placed in the parental population cage. After one additional generation, two additional host-adapted subpopulations (cucumber and cotton) were established by randomly removing two of the three cabbage plants from the parental population cage (i.e., from the cage with the cabbage population) and transferring one to a cage with one cucumber plant and the other to a cage with one cotton plant. These three populations, which have the same genetic background, have been maintained in the same glasshouse since then. At the time of the current study, the three genetically similar but host-adapted populations had been cultured for over 6 yr (≈105 generations) under the same conditions and without exposure to insecticide.

Table 1. Oligonucleotide primers used in quantitative PCR

Gene	Primer set	Amplicon size (bp)	Primer sequence (5′ to 3′)
Portiera 16S rDNA	Port-F	229	TAGTCCACGCTGTAAACG
	Port-R		AGGCACCCTTCCATCT
Rickettsia gltA	Glta-F	154	CGGATTGCTTTACTTAC
	Glta-R		AAATACGCCACCTCTA
Cardinium 16S rDNA[a]	Card-F	124	ACGGGAGGCAGCAGTA
	Card-R		CCGCAGGGATTGTTTT
Hamiltonella 16S rDNA[b]		243	GCATCGAGTGAGCACAGTTT
			TATCCTCTCAGACCCGCTAGA
Actin[c]		130	TCTTCCAGCCATCCTTCTTG
			CGGTGATTTCCTTCTGCATT

[a] Sequences designed in this study.
[b] Sequences obtained from Brumin et al. (2011).
[c] Sequences obtained from Ghanim and Kontsedalov (2009).

Thiamethoxam-Resistant and -Susceptible Subpopulations of *B. tabaci*. The *B. tabaci* B thiamethoxam-resistant (TH-R) and -susceptible (TH-S) subpopulations were the same populations as described previously (Feng et al. 2009, 2010). A recent comparison of the two subpopulations indicated that the mRNA expression levels of three P450 genes (*CYP6a8*, *CYP4v2*, and *CYP6v5*) were >10-fold higher in the TH-R than in the TH-S subpopulation (Xie et al. 2012). At the time of this study, the resistance ratio was at least 70 and seemed stable (Xie et al. 2012).

Quantitative Real-Time Polymerase Chain Reaction. Females from each of the five populations (cabbage, cucumber, cotton, TH-S, and TH-R) were collected (giving three samples for each population with 20 females per sample), stored at −80°C, and then subjected to DNA extraction with a TIANamp Genomic DNA Kit (Tiangen Biotech Co., Ltd, Beijing, China). The purified DNA from each sample was eluted with 200 μl of AE buffer supplied in the kit.

The symbionts in the samples were quantified by Quantitative Real-Time Polymerase Chain Reaction (qPCR). The gene names, amplicon sizes, and primers are listed in Table 1. qPCR was performed with the Applied Biosystems 7900 real-time PCR instrument with 2×SYBR Green PCR mix (Tiangen, Beijing, China). Amplifications were performed in 10-μl reactions containing 5 μl of 2×SYBR Green PCR mix, 0.5 μl of each primer (10 μM each), 2 μl of template DNA, and 2 μl of ddH_2O. To ensure the validity of the data, each of the genes was quantified in quadruplicate for each sample. The cycling conditions were as follows: 5 min activation at 95°C followed by 40 cycles of 30 s at 95°C, 30 s at 55°C, and 20 s at 72°C. As an endogenous control, the expression of β-actin gene was measured in parallel (Table 1). The mean normalized expression value of each symbiont was calculated by comparing

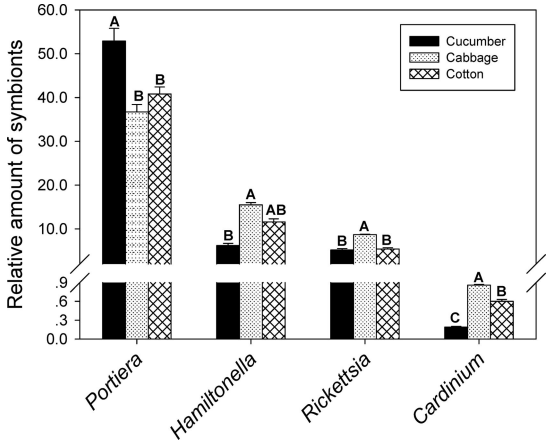

Fig. 1. Relative amount of *Portiera*, *Hamiltonella*, *Rickettsia*, and *Cardinium* in three *B. tabaci* populations (cucumber, cabbage, and cotton) as determined by quantitative PCR (normalized according to the amount of actin gene). Values for relative amount of symbionts are means ± SEM of three replicates for each kind of plant. The data were analyzed with one-way ANOVA. For each kind of symbiont, different letters above the bars indicate significant differences among the three populations (Tukey test, $P < 0.05$).

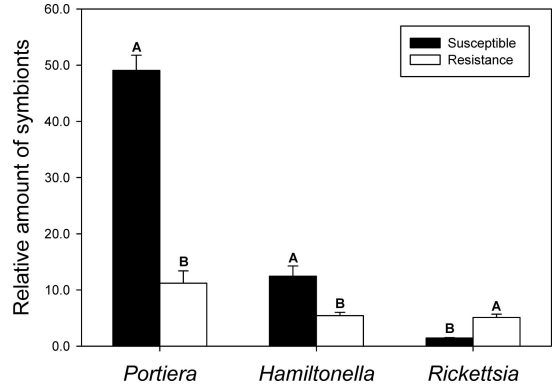

Fig. 2. Relative amount of *Portiera*, *Hamiltonella*, and *Rickettsia* in the thiamethoxam-resistant population and thiamethoxam-susceptible population as determined by quantitative PCR (normalized according to the amount of actin gene). Values for relative amount of symbionts are means ± SEMs of three replicates for each kind of population. The data were analyzed with one-way ANOVA. For each symbiont, different letters above the bars indicates that the amount of the symbiont differed between the resistant and susceptible populations (Tukey test, $P < 0.05$).

the threshold cycle (C_t) of each target gene to that of the whitefly β-actin gene according to the $2^{-\Delta Ct}$ method (Livak and Schmittgen 2001).

Data Analysis. The differences in relative amount of symbionts in *B. tabaci* reared on different host plants or between the TH-S and TH-R populations were analyzed using one-way analysis of variance (ANOVA), and the means were compared by the Tukey test at $P < 0.05$. All statistical analyses were performed with SPSS version 17.0 (SPSS Inc., Chicago, IL).

Results

Effects of Host-Plant Adaptation. The cucumber population harbored more *Portiera* than the cabbage and cotton populations ($F_{2, 6} = 15.771, P = 0.004$) (Fig. 1), and the cabbage population harbored more *Hamiltonella* than the cucumber population ($F_{2, 6} = 9.077$, $P = 0.015$); *Hamiltonella* amount did not statistically differ between the cotton and cucumber or the cotton and cabbage populations (Fig. 1). The cabbage population harbored more *Rickettsia* and *Cardinium* than the cucumber and cotton populations ($P < 0.0001$, respectively) (Fig. 1).

Effects of Insecticide. The TH-S population harbored more *Portiera* and *Hamiltonella* than the TH-R population ($F_{1, 4} = 121.814$, $P < 0.0001$ and $F_{1, 4} = 13.701$, $P = 0.021$, respectively), whereas the TH-R population harbored more *Rickettsia* than the TH-S population ($F_{1, 4} = 31.717$, $P = 0.005$) (Fig. 2).

Discussion

The principal topic addressed in this research was the effect of plant factors on the relative amount of bacterial symbionts in *B. tabaci*, an effect that has been described previously in aphids (Wilkinson et al. 2001; Tsuchida et al. 2002, 2004; Leonardo and Muiru 2003; Chandler et al. 2008). Our recent survey of 61 field *B. tabaci* populations collected from different plant species and locations in China demonstrated that at least three factors, including biotype or genetic group, host plant, and geographical location, affect the infection frequencies of the S-symbionts in *B. tabaci* (Pan et al. 2012b).

In the current study, the three plant-adapted subpopulations derived from the same parental B biotype laboratory population indicate that host plant affects the amount of the S-symbionts in *B. tabaci.* This is because the amounts of P- and S-symbionts were all significantly different among the three plant-adapted subpopulations. This result agrees with that of Pan et al. (2012c), who report that significant variations were exhibited in the infection frequencies of S-symbionts among different host plant-adapted laboratory subpopulations with the same genetic background as the same populations used in this study (Pan et al. 2012c).

Host plants exhibited significant impacts on the amount of the B whiteflies (Fig. 1). However, founder effect, or genetic drift over a period of 6 yr might result in the S-symbiont-host plant association pattern from this experiment. In addition, other factors such as the genetic background, population inertia, and stochasticity might confound the impacts of host plants on the amount of S-symbionts and cause the discrepancies.

Previous studies showed that multiple infections, i.e., infections by different symbionts within the same individuals, are common among diverse insect taxa (Tsuchida et al. 2002, 2004; Simon et al. 2003; Gottlieb et al. 2008; Gueguen et al. 2010; Toju and Fukatsu 2011; Pan et al. 2012b). Although all three host-plant populations contained higher densities of P-symbiont

Portiera than of S-symbionts *Hamiltonella*, *Rickettsia*, or *Cardinium*, the amount of each symbiont often differed among the three populations. In particular, the amount of the *Portiera* was significantly affected by co-infection with the *Hamiltonella* and *Rickettsia* in the cucumber and cabbage populations of *B. tabaci*. This result is consistent with that of Sakurai et al. (2005), who report the P-symbiont *Buchnera* population was significantly suppressed in the presence of *Rickettsia* in aphids (Sakurai et al. 2005). However, the amount of the P-symbiont *Portiera* was not influenced by co-infection with S-symbionts in the cotton population (Fig. 1). This disparity may be because of the fact that different plant species harbor different phytotoxin or secondary metabolites that have antibiotic effects on herbivores.

Our results indicate that selection for resistance to the insecticide thiamethoxam affects the amount of symbionts in *B. tabaci*. The TH-S strain harbored more *Portiera* and *Hamiltonella* than the TH-R strain, whereas the TH-R strain harbored more *Rickettsia* than the TH-S strain. A previous study showed that *Wolbachia* amount were greater in a pesticide-resistant mosquito population than in a susceptible population with the same background; the researchers suspected that mosquitoes may control *Wolbachia* amount less efficiently when they carry resistance genes (Berticat et al. 2002). Like *Wolbachia*, *Rickettsia* also belongs to the Alphaproteobacteria, and it appears that control of *Rickettsia* may be less efficient in thiamethoxam-resistant than in thiamethoxam-susceptible *B. tabaci* populations. This result contrasts with that of Kontsedalov et al. (2008) and Ghanim et al. (2009), who report that the presence and higher amount of *Rickettsia* in *B. tabaci* populations is instrumental in making the whitefly more susceptible to chemical insecticides, whereas absence and lower amount of *Rickettsia* in *B. tabaci* could result in resistance outbreaks. This disparity between our study and Kontsedalov et al. (2008) and Ghanim et al. (2009) may be because of the fact that different populations were screened or different localization patterns of *Rickettsia* in *B. tabaci* in the two studies (Caspi-Fluger et al. 2011).

In this study, the TH-S strain harbored more *Portiera* and *Hamiltonella* (they both belong to the Gammaproteobacteria) than the TH-R strain, which may have resulted from the fitness costs of insecticide-resistance genes (Feng et al. 2009). Further research is needed to determine why different symbionts respond differently to insecticide application or insecticide resistance genes.

Acknowledgements

This research was supported by the National Science Fund for Distinguished Young Scholars (31025020), the 973 Program (2013CB127602), and the Beijing Key Laboratory for Pest Control and Sustainable Cultivation of Vegetables. The granting agencies have no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References Cited

Baumann, P. 2005. Biology bacteriocyte-associated endosymbionts of plant sap-sucking insects. Annu. Rev. Entomol. 59: 155–189.

Berticat, C., F. Rousset, M. Raymond, A. Berthomieu, and M. Weill. 2002. High *Wolbachia* density in insecticide-resistant mosquitoes. Proc. R. Soc. Lond. B Biol Sci. 269: 1413–1416.

Brown, J. K., D. R. Frohlich, and R. C. Rosell. 1995. The sweetpotato or silverleaf whiteflies: biotypes of *Bemisia tabaci* or a species complex? Annu. Rev. Entomol. 40: 511–534.

Brumin, M., S. Kontsedalov, and M. Ghanim. 2011. *Rickettsia* influences thermotolerance in the whitefly *Bemisia tabaci* B biotype. Insect Sci. 18: 57–66.

Caspi-Fluger, A., M. Inbar, N. Mozes-Daube, M. Laurence, M. S. Hunter, and E. Zchori-Fein. 2011. *Rickettsia* 'in' and 'out': two different localization patterns of a bacterial symbiont in the same insect species. PLoS ONE 6: e21096.

Chandler, S. M., T. L. Wilkinson, and A. E. Douglas. 2008. Impact of plant nutrients on the relationship between a herbivorous insect and its symbiotic bacteria. Proc. R. Soc. B Biol. Sci. 275: 565–570.

Chu, D., Y. J. Zhang, J. K. Brown, B. Cong, B. Y. Xu, Q. J. Wu, and G. R. Zhu. 2006. The introduction of the exotic Q biotype of *Bemisia tabaci* (Gennadius) from the Mediterranean region into China on ornamental crops. Fla. Entomol. 89: 168–174.

Chu, D., C. S. Gao, P. J. De Barro, Y. J. Zhang, F. H. Wan, and I. A. Khan. 2011. Further insights into the strange role of bacterial endosymbionts in whitefly, *Bemisia tabaci:* comparison of secondary symbionts from biotypes B and Q in China. Bull. Entomol. Res. 101: 477–486.

De Barro, P. J., S. S. Liu, L. M. Boykin, and A. Dinsdale. 2011. *Bemisia tabaci:* a statement of species status. Annu. Rev. Entomol. 56: 1–19.

Dinsdale, A., L. Cook, C. Riginos, Y. Buckley, and P. J. De Barro. 2010. Refined global analysis of *Bemisia tabaci* (Hemiptera: Sternorrhyncha: Aleyrodoidea: Aleyrodidae) mitochondrial cytochrome oxidase 1 to identify species level genetic boundaries. Ann. Entomol. Soc. Am. 103: 196–208.

Everett, K. D., M. Thao, M. Horn, G. E. Dyszynski, and P. Baumann. 2005. Novel chlamydiae in whiteflies and scale insects: endosymbionts *'Candidatus* Fritschea bemisiae' strain Falk and *'Candidatus* Fritschea eriococci' strain Elm. Int. J. Syst. Evol. Microbiol. 55: 1581–1587.

Feldhaar, H. 2011. Bacterial symbionts as mediators of ecologically important traits of insect hosts. Ecol. Entomol. 36: 533–543.

Feng, Y. T., Q. J. Wu, B. Y. Xu, S. L. Wang, X. L. Chang, W. Xie, and Y. J. Zhang. 2009. Fitness costs and morphological change of laboratory-selected thiamethoxam resistance in the B-type *Bemisia tabaci* (Hemiptera: Aleyrodidae). J. Appl. Entomol. 133: 466–472.

Feng, Y. T., Q. J. Wu, S. L. Wang, X. L. Chang, W. Xie, B. Y. Xu, and Y. J. Zhang. 2010. Cross-resistance study and biochemical mechanisms of thiamethoxamresistance in B-biotype *Bemisia tabaci* (Hemiptera: Aleyrodidae). Pest Manag. Sci. 66: 313–318.

Ghanim, M., and S. Kontsedalov. 2009. Susceptibility to insecticides in the Q biotype of *Bemisia tabaci* is correlated with bacterial symbiont densities. Pest Manag. Sci. 65: 939–942.

Gottlieb, Y., M. Ghanim, E. Chiel, D. Gerling, V. Portnoy, S. Steinberg, Z. Tzuri, A. R. Horowitz, E. Belausov, N. Mozes-Daube, et al. 2006. Identification and localization of a *Rickettsia* sp. in *Bemisia tabaci* (Homoptera: Aleyrodidae). Appl. Environ. Microbiol. 72: 3646–3652.

Gottlieb, Y., M. Ghanim, G. Gueguen, S. Kontsedalov, F. Vavre, F. Fleury, and E. Zchori-Fein. 2008. Inherited intracellular ecosystem: symbiotic bacteria share bacteriocytes in whiteflies. FASEB J. 22: 2591–2599.

Gottlieb, Y., E. Zchori-Fein, N. Mozes-Daubem, S. Kontsedalov, M. Skaljac, M. Brumin, I. Sobol, H. Czosnek, F. Vavre, F. Fleury, et al. 2010. The transmission efficiency of *tomato yellow leaf curl virus* by the whitefly *Bemisia tabaci* is correlated with the presence of a specific symbiotic bacterium species. J. Virol. 84: 9310–9317.

Gueguen, G., F. Vavre, O. Gnankine, M. Peterschmit, D. Charif, E. Chiel, Y. Gottlieb, M. Ghanim, E. Zchori-Fein, and F. Fleury. 2010. Endosymbiont metacommunities, mtDNA diversity and the evolution of the *Bemisia tabaci* (Hemiptera: Aleyrodidae) species complex. Mol. Ecol. 19: 4365–4376.

Himler, A. G., T. Adachi-Hagimori, J. E. Bergen, A. Kozuch, S. E. Kelly, B. E. Tabashnik, E. Chiel, V. E. Duckworth, T. J. Dennehy, E. Zchori-Fein, et al. 2011. Rapid spread of a bacterial symbiont in an invasive whitefly is driven by fitness benefits and female bias. Science 332: 254.

Hogenhout, A., E. D. Ammar, A. E. Whitfield, and M. G. Redinbaugh. 2008. Insect vector interactions with persistently transmitted viruses. Annu. Rev. Entomol. 46: 327–359.

Kondo, N., M. Shimada, and T. Fukatsu. 2005. Infection density of *Wolbachia* endosymbiont affected by co-infection and host genotype. Biol. Lett. 1: 488–491.

Kontsedalov, S., E. Zchori-Fein, E. Chiel, Y. Gottlieb, M. Inbar, and M. Ghanim. 2008. The presence of *Rickettsia* is associated with increased susceptibility of *Bemisia tabaci* (Homoptera: Aleyrodidae) to insecticides. Pest Manag. Sci. 64: 789–792.

Leonardo, T. E., and G. T. Muiru. 2003. Facultative symbionts are associated with host plant specialization in pea aphid populations. Proc. R. Soc. B Biol. Sci. 270: S209–S212.

Livak, K. J., and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25: 402–408.

Luo, C., Y. Yao, R. J. Wang, F. M. Yan, D. X. Hu, and Z. L. Zhang. 2002. The use of mitochondrial cytochrome oxidase mtCOI gene sequences for the identification of biotypes of *Bemisia tabaci* (Gennadius) in China. Acta Entomol. Sin. 45: 759–763.

Mahadav, A., D. Gerling, Y. Gottlieb, H. Czosnek, and M. Ghanim. 2008. Parasitization by the wasp *Eretmocerus mundus* induces transcription of genes related to immune response and symbiotic bacteria proliferation in the whitefly *Bemisia tabaci.* BMC Genomics 9: 342.

Nawaz-ul-Rehman, M. S., and C. M. Fauquet. 2009. Evolution of geminiviruses and their satellites. FEBS Lett. 583: 1825–1832.

Pan, H. P., D. Chu, D. Q. Ge, S. L. Wang, Q. J. Wu, W. Xie, X. G. Jiao, B. M. Liu, X. Yang, N. N. Yang, et al. 2011. Further spread of and domination by *Bemisia tabaci* biotype Q on field crops in China. J. Econ. Entomol. 104: 978–985.

Pan, H. P., D. Chu, W. Q. Yan, Q. Su, B. M. Liu, W. Xie, X. G. Jiao, R. M. Li, N. N. Yang, X. Yang, et al. 2012a. Rapid spread of *Tomato yellow leaf curl virus* in China is aided differentially by two invasive whiteflies. PLoS ONE 7: e34817.

Pan, H. P., X. C. Li, D. Q. Ge, S. L. Wang, Q. J. Wu, W. Xie, X. G. Jiao, D. Chu, B. M. Liu, B. Y. Xu, et al. 2012b. Factors affecting population dynamics of maternally transmitted endosymbionts in *Bemisia tabaci.* PLoS ONE 7: e30760.

Pan, H. P., X. C. Li, and Y. J. Zhang. 2012c. Sex affects the infection frequencies of symbionts in *Bemisia tabaci.* Commun. Integr. Biol. 5: 1–3.

Sakurai, M., R. Koga, T. Tsuchida, X. Y. Meng, and T. Fukatsu. 2005. *Rickettsia* symbiont in the pea aphid *Acyrthosiphon pisum*: novel cellular tropism, effect on host fitness, and interaction with the essential symbiont *Buchnera.* Appl. Environ. Microbiol. 71: 4069–4075.

Simon, J. C., S. Carré, M. Boutin, N. Prunier-Leterme, B. Sabater-Muñoz, A. Latorre, and R. Bournoville. 2003. Host-based divergence in populations of the pea aphid: insights from nuclear markers and the prevalence of facultative symbionts. Proc. R. Soc. B Biol. Sci. 270: 1703–1712.

Thao, M. L., and P. Baumann. 2004a. Evidence for multiple acquisition of *Arsenophonus* by whitefly species (Sternorrhyncha: Aleyrodidae). Curr. Microbiol. 48: 140–144.

Thao, M. L., and P. Baumann. 2004b. Evolutionary relationships of primary prokaryotic endosymbionts of whiteflies and their hosts. Appl. Environ. Microbiol. 70: 3401–3406.

Toju, H., and T. Fukatsu. 2011. Diversity and infection prevalence of endosymbionts in natural populations of the chestnut weevil: relevance of local climate and host plants. Mol. Ecol. 20: 853–868.

Tsuchida, T., R. Koga, H. Shibao, T. Matsumoto, and T. Fukatsu. 2002. Diversity and geographic distribution of secondary endosymbiotic bacteria in natural populations of the pea aphid, *Acyrthosiphon pisum.* Mol. Ecol. 11: 2123–2135.

Tsuchida, T., R. Koga, and T. Fukatsu. 2004. Host plant specialization governed by facultative symbiont. Science 303: 1989.

Weeks, A. R., R. Velten, and R. Stouthamer. 2003. Incidence of a new sex-ratio-distorting endosymbiotic bacterium among arthropods. Proc. R. Soc. B Biol. Sci. 270: 1857–1865.

Wilkinson, T. L., D. Adams, L. B. Minto, and A. E. Douglas. 2001. The impact of host plant on the abundance and function of symbiotic bacteria in an aphid. J. Exp. Biol. 204: 3027–3038.

Xie, W., S. L. Wang, Q. J. Wu, Y. T. Feng, H. P. Pan, X. G. Jiao, L. Zhou, X. Yang, W. Fu, H. Y. Teng, et al. 2011. Induction effects of host plants on insecticide susceptibility and detoxification enzymes of *Bemisia tabaci* (Hemiptera: Aleyrodidae). Pest Manag. Sci. 67: 87–93.

Xie, W., Q. S. Meng, Q. J. Wu, S. L. Wang, X. Yang, N. N. Yang, R. M. Liu, X. G. Jiao, H. P. Pan, B. M. Liu, et al. 2012. Pyrosequencing the *Bemisia tabaci* transcriptome reveals a highly diverse bacterial community and a robust system for insecticide resistance. PLoS ONE 7: e35181.

Zchori-Fein, E., and J. K. Brown. 2002. Diversity of prokaryotes associated with *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae). Ann. Entomol. Soc. Am. 95: 711–718.

Zhang, L. P., Y. J. Zhang, W. J. Zhang, Q. J. Wu, B. Y. Xu, and D. Chu. 2005. Analysis of genetic diversity among different geographical populations and determination of biotypes of *Bemisia tabaci* in China. J. Appl. Entomol. 129: 121–128.

Received 16 April 2012; accepted 22 October 2012.