

Sex affects the infection frequencies of symbionts in *Bemisia tabaci*

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While biotype, host plant and geographical location are known to affect the infection dynamics of the six secondary symbionts (S-symbionts) including Hamiltonella, Arsenophonus, Cardinium, Wolbachia, Rickettsia and Fritschea in *Bemisia tabaci*, it remains unclear whether sex of *B. tabaci* has an impact on the infection frequencies of the six S-symbionts. To address this issue, gene-specific PCR were conducted to screen for the presence of the six S-symbionts in five host plant-adapted laboratory sub-populations with the same genetic background. Significant variations were exhibited in the infection rates of Rickettsia, Cardinium, Rickettsia + Hamiltonella (RH), Rickettsia + Cardinium (RC), Hamiltonella + Cardinium (HC) and Rickettsia + Hamiltonella + Cardinium (RHC) among the five host plant-adapted sub-populations. Moreover, Rickettsia, Hamiltonella, Cardinium, RH, RC, HC and RHC were present at a significantly higher frequency in the females than in the males of the five host plant-adapted sub-populations. This indicates that sex is another important factor affecting the population dynamics of S-symbionts in *B. tabaci*.

The whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is one of the most polyphagous and destructive pests.¹ It is known to harbor one P-symbiont (Portiera) and six S-symbionts (Hamiltonella, Arsenophonus, Cardinium, Wolbachia, Rickettsia and Fritschea).² Because of different (obligatory vs. facultative) mutualistic relationships they form with their host *B. tabaci*, the P-symbiont Portiera infects every individual of any *B. tabaci* populations, whereas the infection frequencies of the six S-symbionts vary greatly among *B. tabaci* populations.^{3–8} Our recent survey of 61 (17 B biotype and 44 Q biotype) field populations collected from different plant species and locations in China has demonstrated that at least three factors including biotype or genetic group, host plant and geographical location affect the infection dynamics of the S-symbionts in *B. tabaci*.⁹

However, it has not been addressed whether sex also influences the infection frequencies of S-symbionts in any insects including *B. tabaci*. In our recent survey of the 61 field populations, we found that the female adults of two B- and four Q-biotype field populations had significantly higher infection frequencies of Hamiltonella, RHC, RH and HC than did their male adults.⁹ To examine if sex is another key factor governing the infection dynamics of the six S-symbionts in *B. tabaci*, gene-specific PCR described in Pan et al. (2012) were conducted to screen for the presence of the P-symbiont Portiera and the six S-symbionts in five host-adapted but genetically-similar B biotype laboratory sub-populations. The five sub-populations were derived from the same parental population (cabbage population) and had been

maintained on cabbage, poinsettia, cucumber, cotton, or tomato for 5 y (approximately 75 generations) under the same conditions.

The data obtained showed that the five sub-populations were 100.0% infected by the P-symbiont Portiera. But none of them harbored Wolbachia, Fritschea and Arsenophonus (data not shown). Rickettsia, Hamiltonella and Cardinium were present alone or together in the five host plant-adapted sub-populations (Table 1). Hamiltonella was harbored by 79–100% of the females of the five sub-populations, but the males of the cotton, cucumber, cabbage and poinsettia sub-populations did not contain it (Table 1). 8.3% of the females of the cotton sub-population harbored Rickettsia, whereas no males of the cotton sub-population had it. When Rickettsia, Hamiltonella, or Cardinium were found in both females and males of either sub-population, the infection frequency was always higher in females than in males except for Cardinium in the cabbage sub-population. As a result, all the RH, RC, HC and RHC co-infections were detected in a proportion of the females of the cotton, cucumber, tomato and cabbage sub-populations, whereas these co-infections were often not present in the males of the five sub-populations (Table 1).

Pooling the data of the five sub-populations together, the females of the five sub-populations had significantly greater infection frequencies of Rickettsia, Hamiltonella, Cardinium, RH, RC, HC and RHC than did the males of the five sub-populations (Table 2). No significant differences were found between the observed and expected double and triple co-infection frequencies in both male and female whiteflies (Table 2). Pooling the data of the both sexes of each sub-population together, the five

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Table 1. Diversity and infection frequencies of symbionts in the five host plant-adapted but genetically similar sub-populations of *B. tabaci* B biotype

Population	Sex	Infection Frequency (%) By							
		RHC	RH	RC	HC	R	H	C	
Cotton	♀	4.2	8.3	4.2	87.5	8.3	87.5	100.0	
	♂	-	-	-	-	-	-	25.0	
Cucumber	♀	25.0	79.2	25.0	25.0	100.0	79.2	25.0	
	♂	-	-	-	-	16.7	-	25.0	
Cabbage	♀	79.2	79.2	87.5	91.7	87.5	91.7	100.0	
	♂	-	-	66.7	-	66.7	-	100.0	
Tomato	♀	91.7	100.0	91.7	91.7	100.0	100.0	91.7	
	♂	-	8.3	-	-	83.3	25.0	29.2	
Poinsettia	♀	-	-	-	70.8	-	100.0	70.8	
	♂	-	-	-	-	-	-	25.0	

Table 2. Impacts of sex on the diversity and infection frequency of S-symbionts in the five host plant-adapted but genetically similar sub-populations of *B. tabaci* B biotype*

Sex	Infection Frequency (%) By										
	RHC		RH		RC		HC		R	H	C
	exp	obs	exp	obs	exp	obs	exp	obs			
♀	42.1a	40.0Aa	54.3a	53.3Aa	45.9a	41.7Aa	71.1a	73.3Aa	59.2A	91.7A	77.5A
♂	0.7a	0.0Ba	1.7a	1.7Ba	13.6a	13.3Ba	2.0a	0.0Ba	33.3B	5.0B	40.8B

*The frequencies sharing the same upper case letter are not significantly different at $p < 0.05$ (multiple comparisons with Bonferroni corrections). The expected (exp) and observed (obs) co-infection frequencies that share the same lower case letter are not significantly different at $p < 0.05$ (nonparametric tests χ^2).

host plant-adapted sub-populations had significant differences in the infection frequencies of Rickettsia, Cardinium, RH, RC, HC and RHC (Table 3). The infection frequency of Hamiltonella varied among the five sub-populations, but the variations were not significant ($p = 0.206$; Table 3).

Evidently, the results of this study and our recent finding of higher infection frequencies of Hamiltonella, RHC, RH and HC in the female adults of two B- and four Q-biotype field populations indicate that sex of the hosts is another key factor determining the infection dynamics of the six S-symbionts in *B. tabaci*.⁹ To our knowledge, this is the first report of sex acting

as an important factor for the infection dynamics of S-symbionts in *B. tabaci*. The female-biased infection rate of *B. tabaci* by Rickettsia, Hamiltonella and Cardinium suggests that they may confer a strong male-killing effect. Alternatively, they may undergone a male-specific degeneration process and thus disappear in male adults as the β - and gamma-proteobacteria in the mealybugs.¹⁰ More experiments are necessary to resolve these two possibilities.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Table 3. Impacts of host plant on the diversity and infection frequency of S-symbionts in the five host plant-adapted but genetically similar sub-populations of *B. tabaci* B biotype*

Host plant	Infection Frequency (%) By										
	RHC		RH		RC		HC		R	H	C
	exp	obs	exp	obs	exp	obs	exp	obs			
Cotton	1.1a	2.1Ba	1.8a	4.2Ba	2.6a	2.1Ca	27.4a	43.8Aa	4.2C	43.8A	62.5B
Cucumber	5.8a	12.5Ba	23.1a	39.6Aa	14.6a	12.5Ca	9.9a	12.5Ba	58.4B	39.6A	25.0C
Cabbage	35.4a	39.6Aa	35.4a	39.6Aa	77.1a	77.1Aa	45.9a	45.9Aa	77.1AB	45.9A	100.0A
Tomato	34.7a	45.9Aa	57.3a	54.2Aa	55.5a	45.9Ba	37.8a	45.9Aa	91.7A	62.5A	60.5B
Poinsettia	0.0a	0.0Ba	0.0a	0.0Ba	0.0a	0.0Ca	24.0a	35.4ABa	0.0C	50.0A	47.9BC

*The frequencies sharing the same upper case letter are not significantly different at $p < 0.005$ (multiple comparisons with Bonferroni corrections). The expected (exp) and observed (obs) co-infection frequencies that share the same lower case letter are not significantly different at $p < 0.05$ (nonparametric tests χ^2).

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References

1. Inbar M, Gerling D. Plant-mediated interactions between whiteflies, herbivores, and natural enemies. *Annu Rev Entomol* 2008; 53:431-48; PMID:17877454; <http://dx.doi.org/10.1146/annurev.ento.53.032107.122456>
2. Baumann P. Biology bacteriocyte-associated endosymbionts of plant sap-sucking insects. *Annu Rev Microbiol* 2005; 59:155-89; PMID:16153167; <http://dx.doi.org/10.1146/annurev.micro.59.030804.121041>
3. Ahmed MZ, Ren SX, Xue X, Li XX, Jin GH, Qiu BL. Prevalence of endosymbionts in *Bemisia tabaci* populations and their in vivo sensitivity to antibiotics. *Curr Microbiol* 2010; 61:322-8; PMID:20217091; <http://dx.doi.org/10.1007/s00284-010-9614-5>
4. Ahmed MZ, Shatters RG, Ren SX, Jin GH, Mandour NS, Qiu BL. Genetic distinctions among the Mediterranean and Chinese populations of *Bemisia tabaci* Q biotype and their endosymbiont *Wolbachia* populations. *J Appl Entomol* 2009; 133:733-41; <http://dx.doi.org/10.1111/j.1439-0418.2009.01442.x>
5. Chiel E, Gottlieb Y, Zchori-Fein E, Mozes-Daube N, Katzir N, Inbar M, et al. Biotype-dependent secondary symbiont communities in sympatric populations of *Bemisia tabaci*. *Bull Entomol Res* 2007; 97:407-13; PMID:17645822; <http://dx.doi.org/10.1017/S0007485307005159>
6. Chu D, Gao CS, De Barro P, Zhang YJ, Wan FH, Khan IA. 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* 2011; 101:477-86; PMID:21329550; <http://dx.doi.org/10.1017/S0007485311000083>
7. Gueguen G, Vavre F, Gnankine O, Peterschmitt M, Charif D, Chiel E, et al. Endosymbiont metacommunities, mtDNA diversity and the evolution of the *Bemisia tabaci* (Hemiptera: Aleyrodidae) species complex. *Mol Ecol* 2010; 19:4365-76; PMID:20723069; <http://dx.doi.org/10.1111/j.1365-294X.2010.04775.x>
8. Skaljac M, Zanic K, Ban SG, Kotsedalov S, Ghanim M. Co-infection and localization of secondary symbionts in two whitefly species. *BMC Microbiol* 2010; 10:142; PMID:20462452; <http://dx.doi.org/10.1186/1471-2180-10-142>
9. Pan HP, Li XC, Ge DQ, Wang SL, Wu QJ, Xie W, et al. Factors affecting population dynamics of maternally transmitted endosymbionts in *Bemisia tabaci*. *PLoS One* 2012; 7:e30760; PMID:22383972; <http://dx.doi.org/10.1371/journal.pone.0030760>
10. Kono M, Koga R, Shimada M, Fukatsu T. Infection dynamics of coexisting beta- and gammaproteobacteria in the nested endosymbiotic system of mealybugs. *Appl Environ Microbiol* 2008; 74:4175-84; PMID:18469124; <http://dx.doi.org/10.1128/AEM.00250-08>