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## Distribution of *Bemisia tabaci* (Homoptera: Aleyrodidae) biotypes and their associated symbiotic bacteria on host plants in West Africa

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**Abstract.** 1. The whitefly *Bemisia tabaci* is a pest of many agricultural and ornamental crops worldwide and particularly in Africa. *B. tabaci* is a complex of more than 20 biotypes. Effective control of *B. tabaci* calls for a greater knowledge of the local biological diversity in terms of biotypes or putative species. Information is available about biotype distribution in Northern, Eastern, and Southern Africa, but data for Western Africa remain very scarce. At the time of this study, data were available for only three sampling sites in Burkina Faso, where three biotypes have been detected, the native Sub-Saharan Africa non-Silver Leafing (AnSL), the Sub-Saharan Africa Silverleafing (ASL), and the Mediterranean Q biotypes, but no information is available about their respective distributions on host plant species (Gueguen *et al.*, 2010).

2. Our study describes the biotypes and symbiotic bacterial communities of *B. tabaci* sampled in three West African countries, Burkina Faso, Benin, and Togo. A total of 527 individuals were collected from seven cultivated host plants.

3. In the 20 localities studied, we found the same three biotypes AnSL, ASL, and Q previously detected in Burkina Faso. These biotypes display a specific pattern of geographical distribution influenced by the host plant species. In Benin and Togo, the ASL and AnSL biotypes were predominant, while in Burkina Faso, the Q biotype was dominant, with two sub-groups, Q1 and Q3 (recorded to date only in this country), and ASL individuals found in sympatry with Q1 individuals in some localities. As previously reported, each biotype and each genetic group harbours a specific community of symbiotic bacteria.

**Key words.** *Bemisia tabaci*, biotypes, host plant, secondary symbionts, West Africa.

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### Introduction

The whitefly *Bemisia tabaci* (Gennadius) is a significant pest that damages many agricultural crops worldwide. *B. tabaci* is in fact a complex of cryptic species composed of 11 clearly differentiated, high-level groups containing at least 24 distinct

species composed of several low-level groups previously designated as 'biotypes' (De Barro *et al.*, 2010; Dinsdale *et al.*, 2010). Many aspects of this species complex remain unknown, such as the origins of the different species and the process of radiation, the borders between the species and their degree of genetic isolation, the geographical distribution, the biological characteristics and the within-species genetic diversity. *B. tabaci* biotypes can be distinguished using biochemical or molecular markers (allozymes, mitochondrial *Cytochrome Oxidase CO1*, nuclear *Internal Transcribed Spacer ITS*), but their biological significance and taxonomic status are still unclear. These biotypes can differ with regard to the induction of plant silverleafing disorders, host range, fecundity, insecticide resistance, ability to transmit plant viruses (Perring, 2001; De Barro *et al.*, 2010), and in the symbiotic bacterial community they harbour (Gueguen *et al.*, 2010). The situation is in fact even more complex, as significant genetic differentiation is also observed within biotypes. For example, within the Q biotype of the Mediterranean species, the genetic sub-groups designated Q1, Q2, and Q3 have been recognised both by *CO1* sequencing and from the composition of their endosymbiotic bacteria (McKenzie *et al.*, 2009; Gueguen *et al.*, 2010).

In tropical and sub-tropical countries, *B. tabaci* is a pest of primary importance, especially on crops of cassava, cotton, sweet potatoes, tobacco, and tomato. In West Africa, population outbreaks were observed in 1998 in cotton fields in Burkina Faso, Mali, and Ivory Coast inducing severe crop damages (Otoïdobia *et al.*, 2002, 2003). The resulting losses had a severe impact on the economic activity of these countries, as cotton production and trading is one of the main financial resources, especially in Burkina Faso. As a consequence, West African countries make wide use of many pesticides to control *B. tabaci* populations despite their harmful impact on potential natural enemies and on the environment. This practice also leads to high levels of resistance, which have been observed particularly in Burkina Faso (Houndété *et al.*, 2010). As *B. tabaci* biotypes have differing levels of insecticide resistance (Horowitz *et al.*, 2005; Wang *et al.*, 2010) and do not always share the same resistance mutation to a particular chemical class of insecticide (Alon *et al.*, 2006, 2008), pest management programs may inadvertently select for one of these biotypes locally. For instance, the polyphagous B biotype is known to be more susceptible to several chemical compounds than the Q biotype (Horowitz *et al.*, 2005), which explains why the Q biotype sometime displaces the B biotype (Horowitz *et al.*, 2005; Chu *et al.*, 2010; but see Crowder *et al.*, 2010). Another consequence is that the effectiveness of treatments may vary depending on the local composition of biotypes and the chemical classes of insecticides used. Identification of the biotypes present and information about their diversity are thus clearly required for effective control strategies of this pest.

Although major studies on *B. tabaci* have been performed in East Africa, little is known about the distribution and frequency of the various biotypes in West Africa. So far, three biotypes have been reported in this region: the Sub-Saharan African non-Silver Leafing (AnSL) biotype, the Sub-Saharan African

Silverleafing (ASL) biotype, and more recently, the Q biotypes (Berry *et al.*, 2004; Brown & Idris, 2005; Sseruwagi *et al.*, 2005; De la Rua *et al.*, 2006; Gueguen *et al.*, 2010). The AnSL biotype belongs to the Sub-Saharan African clade, and is considered to be the basal group of the species complex, and probably originated in Africa. AnSL has been reported throughout Africa, except in the northern countries near the Mediterranean basin (Berry *et al.*, 2004; Boykin *et al.*, 2007; Gueguen *et al.*, 2010). The closely related ASL and Q biotypes are genetically distant from AnSL. They both belong to the Mediterranean species within the Africa/Middle East/Asia Minor group (Boykin *et al.*, 2007; De Barro *et al.*, 2010). ASL has been reported in Ghana, Ivory Coast, Nigeria, Benin, Togo, and Burkina Faso (Brown & Idris, 2005; De la Rua *et al.*, 2006; Gueguen *et al.*, 2010). Recently, we reported that the Q biotype was present in Burkina Faso, where we detected two (Q1 and Q3) of the three genetic subgroups previously described (Gueguen *et al.*, 2010). However, there is a paucity of data for West Africa, and the precise geographical distribution, the frequency of the biotypes, and their genetic subdivisions, together with their possible association with symbiotic bacteria and host plant species remain largely unknown.

The present study set out to describe the distribution of the *B. tabaci* biotypes across West African countries, and its relationship with the host plant species. A total of 527 individuals collected from 20 localities in three countries (Burkina Faso, Benin and Togo), and from seven host plant species, were sampled during the rainy season in 2007 and 2009. In addition to identifying the biotype and genetic group, we determined the composition of the symbiotic bacteria community they harboured. Indeed, *B. tabaci*, like most phloem-feeding insects, is host to an obligatory primary endosymbiont, the bacterium *Portiera aleyrodidarum*, which is required to provide essential nutrients for its host's survival and development. *B. tabaci* is also infected by several facultative, vertically transmitted, symbiotic bacteria known as secondary endosymbionts (Zchori-Fein & Brown, 2002). Interestingly, each biotype or genetic group is infected by a specific symbiotic community (Chiel *et al.*, 2007; Gueguen *et al.*, 2010). This raises a question about the role of these symbiotic microorganisms in the biology of *B. tabaci*, and in the process of species radiation. Their phenotypic effects remain largely unknown in *B. tabaci*, but in other insect species, bacteria are known to be able to manipulate host reproduction (Stouthamer *et al.*, 1999; Weeks *et al.*, 2003), and to be involved in resistance to natural enemies (Oliver *et al.*, 2003) or host-plant specialisation (Tsuchida *et al.*, 2004). In *B. tabaci*, *Rickettsia* bacteria enhance susceptibility to insecticides (Kontsedalov *et al.*, 2008), but can also enhance whiteflies performance (Himler *et al.*, 2011). Moreover, symbiotic bacteria are thought to play a major role in the virus transmission capacities of the whitefly (Morin *et al.*, 1999; Gottlieb *et al.*, 2010).

The main objective of this study was to provide the first detailed description of the diversity of *B. tabaci* biotypes and their symbiotic bacteria communities in West Africa on several host plant species. This provides a good basis for answering questions about biotype dynamics in relation to insecticide resistance, as well as providing valuable information for developing integrated pest management programmes.

## Materials and methods

### Origin of the whiteflies

Sampling was performed in 2007 and 2009. Live adult *B. tabaci* (males and females) were collected and individually placed in 1.5-ml tubes containing 95% ethanol and kept at  $-20^{\circ}\text{C}$  until they underwent DNA extraction. The samples came from three neighbouring countries in West Africa: Burkina Faso, Benin, and Togo (Fig. 1). A total of 527 individuals were collected at 20 localities and from seven cultivated host plants (cotton, tomato, okra, tobacco, marrow, *Lantana camara*, cassava) (Table 1). At least eight individuals were analysed per locality and per plant. The cotton, tomato, marrow, and okra plantations had all been treated with insecticides (with the exception of the cotton from Kandi Sina in Benin) belonging to several classes: organophosphates, pyrethroids, and neonicotinoids. No treatment had been applied to the cassava and *Lantana camara* (Table 1).

### DNA extraction

Total DNA was extracted from each individual in 26  $\mu\text{l}$  of an extraction buffer containing 50 mM KCl, 10 mM Tris-base pH 8, 0.45% Nonidet P-40, 0.45% Tween 20 and 50  $\text{mg ml}^{-1}$  Proteinase K. Extraction buffer was added to the crude extract, incubated at  $65^{\circ}\text{C}$  for 3 h and then incubated at  $100^{\circ}\text{C}$  for 15 min. A volume of 35  $\mu\text{l}$  of pure water was added to this extract, which was then stored at  $-20^{\circ}\text{C}$  until use.

### Identification of *B. tabaci* biotypes and genetic subgroup

The biotypes were identified on the basis of the mitochondrial marker gene *COI* (*Cytochrome Oxidase I*) after a PCR reaction using the universal *COI* primers C1-J-2195 (5'-TTGATTT TTTGGTCATCCAGAAGT-3') and L2-N-3014 (5'-TCCAA TGCATAATCTGCCATATTA-3') (Khasdan *et al.*, 2005). PCR reactions were performed in 25- $\mu\text{l}$  volumes containing 200  $\mu\text{M}$  dNTP, 200 nM primers, 0.5 IU *Taq* DNA polymerase (Eurobio) and 2  $\mu\text{l}$  of DNA template. Cycling conditions consisted of an initial denaturing step at  $95^{\circ}\text{C}$  for 5 min, followed by 30 cycles of 1 min at  $95^{\circ}\text{C}$  for denaturing, 1 min at  $50^{\circ}\text{C}$  for annealing, 1 min at  $72^{\circ}\text{C}$  for extension, and a final extension of  $72^{\circ}\text{C}$  for 10 min (Tetrad®, Biorad, France). For some individuals (from 2 to 20 individuals per population), the PCR products were then sequenced to determine the biotype. For the others, we used the PCR-RFLP (PCR-Random Fragment Length Polymorphism) test developed and described by H. Henri, G. Terraz, O. Gnankiné, F. Fleury & L. Mouton (unpubl. data). Briefly, digestions of the *mtCOI* amplifications by the *XapI* and *BfmI* restriction enzymes result in different patterns of fragment migration depending on the *B. tabaci* genetic group. Products are visualised in 2% agarose gels containing ethidium bromide ( $0.5 \mu\text{g ml}^{-1}$ ) under UV illumination after a 1-h migration at 100 mV. This PCR-RFLP test can be used to identify all known genetic groups from Africa/Middle East/Asia Minor, and

major clades, including the Q1, Q2, and Q3 groups of the Mediterranean Q biotype.

### Detection and molecular identification of the secondary endosymbiotic bacteria (SS)

Individuals were screened for symbiotic bacterial infection using specific PCR primers that amplify the *16S rRNA* gene for *Hamiltonella*, *Cardinium*, and *Rickettsia*, the *23S rRNA* gene for *Arsenophonus* and *Fritschea* and the *wsp* gene for *Wolbachia* (Table 2). The presence of the obligatory symbiont *Portiera* was also checked to confirm the quality of DNA extraction. DNA was amplified in a final volume of 25  $\mu\text{l}$ , containing 1.5 mM  $\text{MgCl}_2$ , 200  $\mu\text{M}$  dNTPs, 200 nM of each primer, 0.5 IU *Taq* DNA polymerase (Eurobio) and 2  $\mu\text{l}$  of DNA. PCR products were visualised in 1% agarose gels stained with ethidium bromide ( $0.5 \mu\text{g ml}^{-1}$ ) under UV illumination to check for the presence/absence of the various bacteria.

### Phylogenetic analyses

Phylogenetic analyses of *B. tabaci* biotypes were performed on mitochondrial *COI* sequences. The accession numbers of the sequences obtained in the present study and used to construct the tree are listed in Table 3. Sequences of *B. tabaci* from Genbank were also included (Fig. 2). Multiple sequence alignment was carried out using MUSCLE software (Edgar, 2004). The appropriate model of evolution was estimated with jModeltest v 0.1.1 (Posada, 2008). The best likelihood score was evaluated with a corrected version of the Akaike information criterion for small samples (AICc). The model selected was HKY+G. Using this model, the phylogenetic tree was constructed by the maximum likelihood method with PhyML (version 3.0.1) (Guindon & Gascuel, 2003) and edited using Treedyn (Chevenet *et al.*, 2006). The robustness of the each node was assessed from 100 bootstrap replicates.

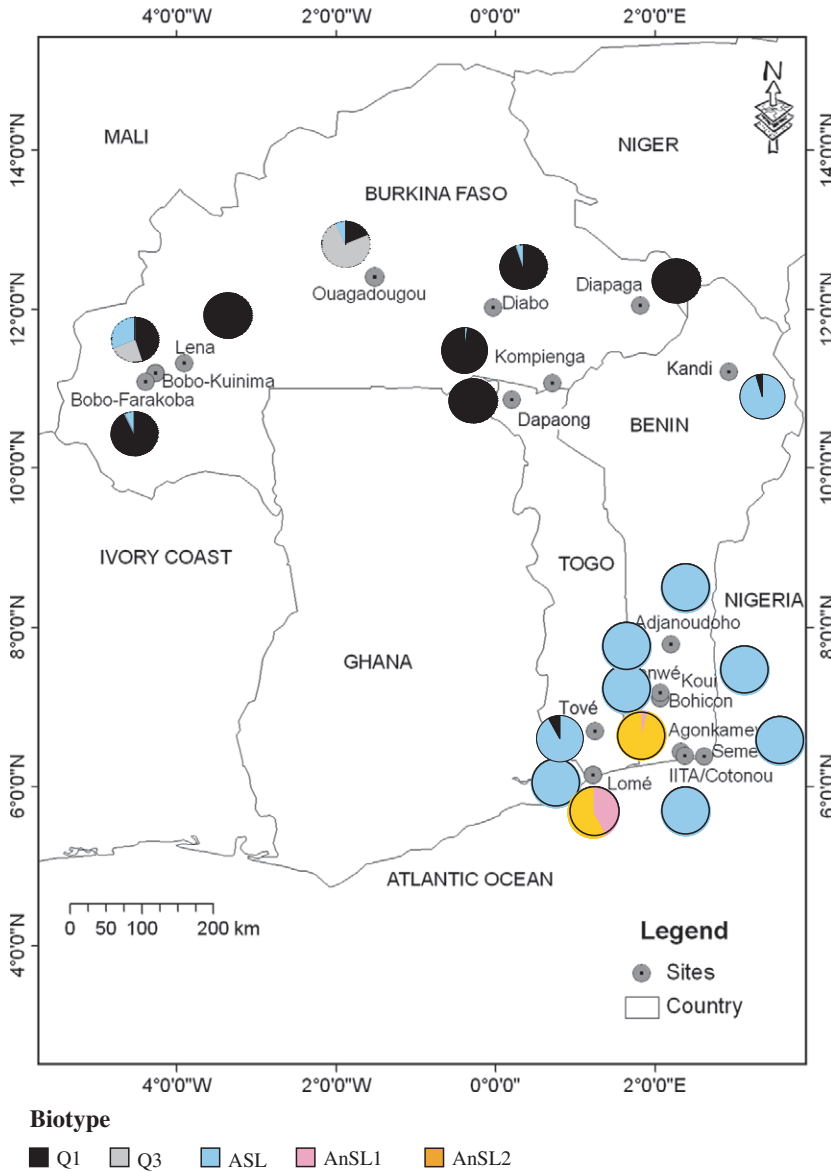
### Statistical analyses

The data collected were subjected to Fisher's exact test with simulated p-values based on 2000 replicates using R statistical software (<http://www.r-project.org/>).

## Results

### Geographical distribution of biotypes

Among the 527 individuals sampled from 20 localities in Burkina Faso, Togo, and Benin, three genetic groups recognised as biotypes were identified (Table 1): Q, ASL and the basal group AnSL. The findings revealed a clear distinction between the different countries (Fig. 1). In Togo and Benin, the African AnSL and the ASL biotypes of *B. tabaci* were predominant with two clearly differentiated genetic groups within the AnSL



**Fig. 1.** Geographical distributions of the samplings sites and of *Bemisia tabaci* biotypes.

biotype, designated AnSL1 and AnSL2 (Fig. 2), which correspond to the Sub Saharan Africa 1 and 2 genetic groups, respectively, described in Dinsdale *et al.* (2010) and Berry *et al.* (2004). These two sub-groups were found on cassava only and occurred together in sympatry (Table 1). However, ASL was the dominant biotype in this area (100% at 8 out of 11 sites). In 2009, the Q biotype was also recorded, but only in the northern parts of these countries. In Tové (Togo) and Kandi (Benin), this biotype was always observed in sympatry with ASL and at a low frequency, whereas at Dapaong (Togo), all individuals belonged to this biotype (Table 1; Fig. 1). The situation was quite different in Burkina Faso, where the Q biotype dominated at most of the sites sampled. The ASL biotype occurred at low frequency and always in sympatry with Q1 (Table 1; Fig. 1), and the AnSL biotype was never detected. However, it should be pointed out that no sample was available from cassava. Sequence analyses revealed the presence of two genetic

sub-groups of the Q biotype: Q1 and the newly described Q3 sub-group recently detected in West Africa (Gueguen *et al.*, 2010). Q1 and Q3 showed different distribution patterns in Burkina Faso in terms both of locality and host plant species. Q3 was only observed in the Western part of the country, and only on *L. camara* and tobacco, whereas Q1 was found at most sampling sites and on most host plants (Fig. 2). Finally, ASL was the only biotype recovered from all three countries, albeit at different frequencies.

*Distribution of biotypes on host plants*

As the samples were not always taken from the same host plants in all three countries, the plants sampled and the locality were not independent. This made it impossible to carry out a global statistical test to find out whether the host plant influences

**Table 1.** Frequencies of *Bemisia tabaci* biotypes related to host plants and localities.

Country	Acronym*	Locality	Host plant	Agricultural practice†	n‡	Year collected	Biotypes present (%§)
Burkina Faso	BF-Cott1a	Kompienga	Cotton	Treated	15	2007	Q1 (100)
	BF-Cott1b		Cotton	Treated	21	2009	Q1 (96), ASL (4)
	BF-Cott2a	Diapaga	Cotton	Treated	16	2007	Q1 (100)
	BF-Cott3a	Lena	Cotton	Treated	18	2007	Q1 (100)
	BF-Cott4a	Diabo	Cotton	Treated	21	2007	Q1 (95), ASL (5)
	BF-Cott4b		Cotton	Treated	20	2009	Q1 (96), ASL (4)
	BF-Tom5a	Bobo/Kuinima	Tomato	Treated	33	2007	Q1 (42), ASL (58)
	BF-Mar5a		Marrow	Treated	31	2007	Q1 (65), ASL (35)
	BF-Okr5a		Okra	Treated	15	2007	Q1 (87), ASL (13)
	BF-Tob5a		Tobacco	Untreated	30	2007	Q3 (86), Q1 (7), ASL (7)
	BF-Cott6a	Bobo/Farako-Bâ	Cotton	Treated	15	2007	Q1 (93), ASL (7)
	BF-Cam7a	Ouagadougou	<i>L.camara</i>	Untreated	44	2007	Q3 (100)
	BF-Cam7b		<i>L.camara</i>	Untreated	20	2009	Q3 (100)
	BF-Tom7a		Tomato	Treated	15	2007	Q1 (73), ASL (27)
Benin	Be-Cott1a	Tanwé	Cotton	Treated	10	2007	ASL (100)
	Be-Cott2a	Koui	Cotton	Treated	18	2007	ASL (100)
	Be-Cott3a	Adjanoudoho	Cotton	Treated	9	2007	ASL (100)
	Be-Tom4a	IITA	Tomato	Treated	10	2007	ASL (100)
	Be-Cott5a	CRA-CF/Bohicon	Cotton	Treated	10	2007	ASL (100)
	Be-Cott5b		Cotton	Treated	11	2009	ASL (100)
	Be-Tom6a	Sémé	Tomato	Treated	16	2007	ASL (100)
	Be-Cas7a	Agonkanmey	Cassava	Untreated	20	2007	AnSL1 (4), AnSL2 (96)
	Be-Cott8b	Kandi Sina	Cotton	Untreated	21	2009	Q1 (4.8), ASL (95.2)
Be-Cott9b	Kandi/Gamassosso	Cotton	Treated	18	2009	Q1 (5.5), ASL (94.5)	
Togo	To-Tom1a	Lome Ferme/UL	Tomato	Treated	13	2007	ASL (100)
	To-Cas2a	Lome Harbour	Cassava	Untreated	20	2007	AnSL1 (42), AnSL2 (58)
	To-Cott3b	Dapaong	Cotton	Treated	29	2009	Q1 (100)
	To-Cott4b	Tové	Cotton	Treated	8	2009	Q1 (8), ASL (92)

\*The first two letters of the acronyms correspond to the country (BF, Burkina Faso; Be, Benin; To, Togo), the second part indicates the host plant and the number refers to a specific locality. The a and b samplings were carried out in 2007 and 2009 respectively.

†This column indicates whether pesticides are used or not.

‡Number of individuals in the sample.

§Percentage of each biotype found at the collecting site.

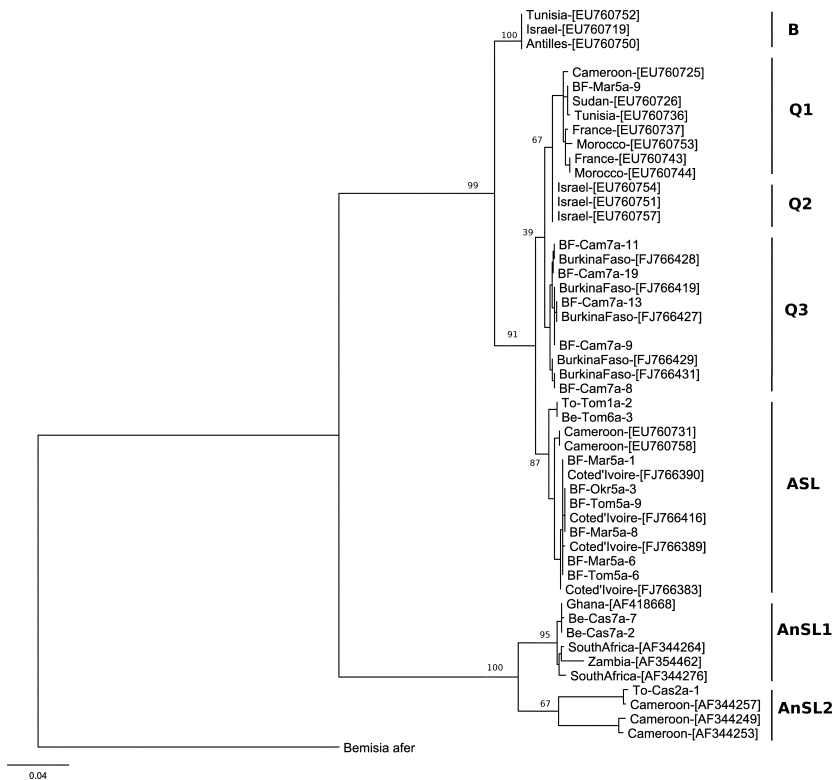
**Table 2.** PCR primers and conditions used in the study.

Symbiont	Targeted gene	Primers	Primer sequences	Annealing temperature (°C)/ product size (bp)	References
<i>Portiera</i>	<i>ADNr16S</i>	28F1098 R	5'-AGAGTTTGATMTGGTCAG-3' 5'-AAAGTTCCCGCCTTATGCGT-3'	58 /~1000	Zchori-Fein and Brown (2002)
<i>Rickettsia</i>	<i>ADNr16S</i>	Rb-FRb-R	5'-GCTCAGAACGAACGCTATC-3' 5'-GAAGGAAAGCATCTCTGC-3'	58 /~900	Gottlieb <i>et al.</i> (2006)
<i>Hamiltonella</i>	<i>ADNr16S</i>	Hb-FHb-R	5'-TGAGTAAAGTCTGGAATCTGG-3' 5'-AGTTCAAGACCGCAACCTC-3'	58 /~700	Zchori-Fein and Brown (2002)
<i>Cardinium</i>	<i>ADNr16S</i>	CFB-FCFB-R	5'-GCGGTGTAATAAGTGAAGAAAC-3' 5'-ACCTMTTCTTAACTCAAGCCT-3'	56 /~400	Weeks <i>et al.</i> (2003)
<i>Wolbachia</i>	<i>wsp</i>	81F415R	5'-TGGTCCAATAAGTGATGAAGAAAC-3' 5'-AAAAATTAACGCTACTCCA-3'	56 /~700	Braig <i>et al.</i> (1998), Vautrin (2008)
<i>Arsenophonus</i>	<i>ADNr23S</i>	Ars-23S-1 Ars-23S-2	5'-CGTTTGATGAATTCATAGTCAAAA-3' 5'-GGTCTCCAGTTAGTGTTACCCAAC-3'	60 /~600	Thao and Baumann (2004)
<i>Fritschea bemisiae</i>	<i>ADNr23S</i>	Frit-FFrit-R	5'-TGGTCCAATAAGTGATGAAGAAAC-3' 5'-GCTCGGTACCCTTTAAATGGCG-3'	60 /~600	Thao <i>et al.</i> (2003)

**Table 3.** Names and accession numbers of samples used for the phylogenetic tree.

Sample	Locality	Country	Host plant	Biotype	Accession number
BF-Tom5a-1	Bobo/Kuinima	Burkina Faso	Tomato	ASL	HQ908636
BF-Tom5a-9	Bobo/Kuinima	Burkina Faso	Tomato	ASL	HQ908637
BF-Mar5a-1	Bobo/Kuinima	Burkina Faso	Marrow	ASL	HQ908638
BF-Mar5a-6	Bobo/Kuinima	Burkina Faso	Marrow	ASL	HQ908639
BF-Mar5a-8	Bobo/Kuinima	Burkina Faso	Marrow	ASL	HQ908640
BF-Okr5a-3	Bobo/Kuinima	Burkina Faso	Okra	ASL	HQ908641
BF-Cam7a-8	Ouagadougou	Burkina Faso	<i>L. camara</i>	Q3	HQ908642
BF-Cam7a-9	Ouagadougou	Burkina Faso	<i>L. camara</i>	Q3	HQ908643
BF-Cam7a-11	Ouagadougou	Burkina Faso	<i>L. camara</i>	Q3	HQ908644
BF-Cam7a-19	Ouagadougou	Burkina Faso	<i>L. camara</i>	Q3	HQ908645
BF-Cam7a-13	Ouagadougou	Burkina Faso	<i>L. camara</i>	Q3	HQ908646
Be-Tom6a-3	Sémé	Benin	Tomato	ASL	HQ908647
Be-Cas7a-2	Agonkanmey	Benin	Cassava	AnSL1	HQ908648
Be-Cas7a-7	Agonkanmey	Benin	Cassava	AnSL1	HQ908649
To-Tom1a-2	Ferme/UL	Togo	Tomato	ASL	HQ908650
To-Cas2a-1	Harbour zone	Togo	Cassava	AnSL2	HQ908651

AnSL, Sub-Saharan Africa non-Silver Leafing; ASL, Sub-Saharan Africa Silverleafing; *Lantana camara*, *L. camara*.

**Fig. 2.** *COI*-gene-based phylogeny of *Bemisia tabaci*.

the distribution of the biotypes. However, it did look as though there was a strict association between the AnSL biotype and cassava, no other biotype having ever been found on this plant, even when ASL was present in the same locality (Table 1). AnSL was never found on cotton crops, regardless of whether they had undergone insecticide treatment or not. Similarly, only the Q3 group was found on *L. camara*. Although Q3 individuals were also observed on tobacco, it was only on *L. camara* that

this biotype was not found in sympatry with other biotypes. Importantly, these two plants were the only ones that had not been treated with insecticides in our Burkina Faso samples, raising the possibility that the distribution of Q3 is mainly determined by insecticide use. In Bobo/Kuinima (Burkina Faso), where four plant species had been sampled in 2007, a significant relationship was found between the plant and the biotype distribution (Fisher's Exact Test,  $P = 0.0005$ ). In particular, Q3 was

only found on tobacco, and was strongly predominant on this plant. Observation of biotype distribution among the host plants also suggests an association between the Q1 biotype and cotton, since when it is present Q1 reaches very high frequencies of up to 100% on this plant. Once again, it is possible that this pattern was mediated by insecticide use.

#### *Prevalence, diversity, and distribution of endosymbiotic bacteria*

The presence of the seven known symbiotic bacteria infecting *B. tabaci* (the primary symbiont *Portiera* and the six secondary symbionts: *Hamiltonella*, *Arsenophonus*, *Rickettsia*, *Cardinium*, *Wolbachia*, *Fritschea*) was checked by specific diagnostic PCR. As expected, all individuals were found to be infected with *Portiera*, which demonstrated the high quality of the DNA extractions. Figure 3 shows the infection status of individuals from all 20 localities and from different host plants with regard to all the secondary endosymbionts except *Fritschea*, which was never detected. As previously reported (Chiel *et al.*, 2007; Gueguen *et al.*, 2010), all genetic groups (AnSL1, AnSL2, ASL, Q1, Q3) were found to host a specific bacterial assemblage (Fisher's Exact Test,  $P = 0.0005$ ): the most common bacteria being *Hamiltonella* for the Q1 biotype, *Arsenophonus* for ASL and AnSL biotypes, and the association *Arsenophonus/Rickettsia* for the Q3 group. However, the infection status of individuals belonging to the ASL biotype is variable, with a strong contrast being observed between Burkina Faso and Togo/Benin (Fisher's Exact Test,  $P = 5 \times 10^{-15}$ ). Indeed, while in Burkina Faso most of the individuals are infected by *Arsenophonus* (80% on average), the level of infection with this secondary symbiont was low in Togo and Benin (less than 12%), and it was not detected at all on some populations in Benin (Fig. 3). Here, we report for the first time the infection status of the basal clade AnSL, which appears to be very similar to that of the ASL biotype, having *Arsenophonus* as the main symbiotic bacterium and a high frequency of uninfected individuals.

In contrast to the findings of a previous study (Gueguen *et al.*, 2010), multiple infections with several secondary symbionts were uncommon in the areas we sampled (13.7%; Fig. 3). In most cases, they involved infection with two species (95.8%) and were mainly observed within the Q3 biotype co-infected by *Rickettsia* and *Arsenophonus*.

#### Discussion

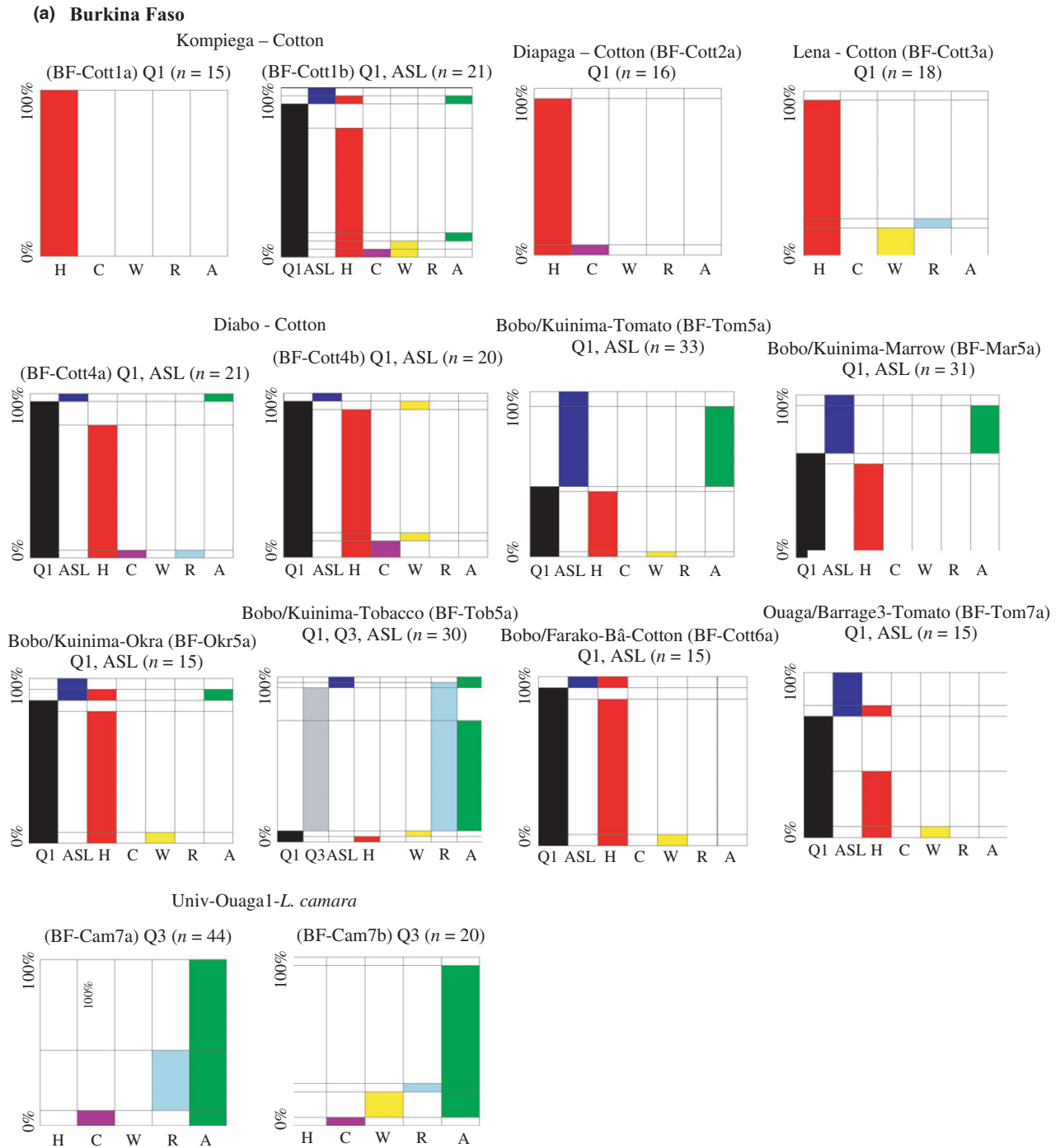
This first detailed analysis of *B. tabaci* biotypes in West African countries provides valuable information about the level of regional biodiversity, including cytoplasmic biodiversity due to infection by symbiotic bacteria related to different host plant species and insecticide treatments. It also extends our knowledge about the geographical range of major biotypes that have spread throughout the world, such as the Q biotype. We identified the same biotypes as had previously been reported in Burkina Faso by Gueguen *et al.* (2010), i.e. the ancestral AnSL (Sub-Saharan Africa non-Silver Leafing), the ASL (SubSaha-

ran Africa Silver Leafing) and the Q biotypes. The frequencies of these biotypes showed marked differences depending on the site sampled. One central finding is that the Q biotype was present at a high frequency in Burkina Faso in 2007 and 2009, whereas it was only detected in the northern parts of Togo and Benin in 2009, which could be explained by a recent introduction into these latter countries of agricultural practices that facilitate its ability to spread. In addition to biotype diversity, the *COI* mitochondrial marker revealed the existence of sub-groups within the AnSL and Q biotypes (designated AnSL1, AnSL2 and Q1, Q3 respectively). The detection of diversity at such a small geographical scale raises numerous questions about the evolution of biotypes, the coexistence and distribution of sub-groups, and the consequences of this diversity for *B. tabaci* management.

These three biotypes (ASL, AnSL, and Q) have also been detected in East Africa, including five sub-groups of the AnSL biotype, but only one, the Q1 sub-group, of biotype Q (Berry *et al.*, 2004; Brown & Idris, 2005; Sseruwagi *et al.*, 2005; De la Rua *et al.*, 2006; Gueguen *et al.*, 2010). Sub-group Q3 had only been detected in Burkina Faso, but this could have been due to a sampling bias, as Q3 appears to be restricted to certain plants (tobacco and *L. camara*). In both East and West Africa, the Q biotype has been found at higher latitudes than the ASL and AnSL biotypes. In northern African countries, such as Morocco and Tunisia, the Q1 biotype is predominant (Gueguen *et al.*, 2010), and a similar trend can also be seen in the present study (Fig. 1). In East Africa, the Q biotype has only been detected in Sudan, which is in the North/Centre region of Africa, and in the Uganda, which borders it to the south. In countries further south (Tanzania, South Africa, Mozambique), AnSL is widespread. However, Cameroon, Benin, and Togo, where we almost exclusively find the AnSL biotype, are at a higher latitude than Uganda, so latitude alone cannot entirely explain the pattern of distribution, and longitude must also be taken into account.

Interestingly, in Burkina Faso, ASL was always found in sympatry with the Q biotype and on the same host plant, particularly the Q1 sub-group, suggesting the possible coexistence of the ASL and Q biotypes at least on a short time scale. However, on all these plants, Q1 was predominant. On cotton, ASL was rarely observed and when present, it never reached frequencies of more than 7%. This situation could indicate competitive interactions between ASL and Q in Burkina Faso, with the outcome of competition depending on environmental conditions, such as host plant species or agricultural practice. Indeed, resistance to neonicotinoids has been shown in this country to be correlated with the presence of the Q1 biotype, with ASL individuals being most susceptible (O. Gnankiné, L. Mouton, A. Savadogo, T. Martin, A. Sanon, F. Vavre, R. Dabire & F. Fleury, unpubl. data). More generally, the Q biotype is known to be highly resistant to several insecticides (Horowitz *et al.*, 2005), which could give it a competitive advantage when insecticides are used. This situation had already been observed among the B and Q biotypes, the latter excluding the former in the context of insecticide treatment (Chu *et al.*, 2010; Crowder *et al.*, 2010). The same phenomenon may occur in Western Africa among resistant Q and susceptible ASL biotypes, explaining





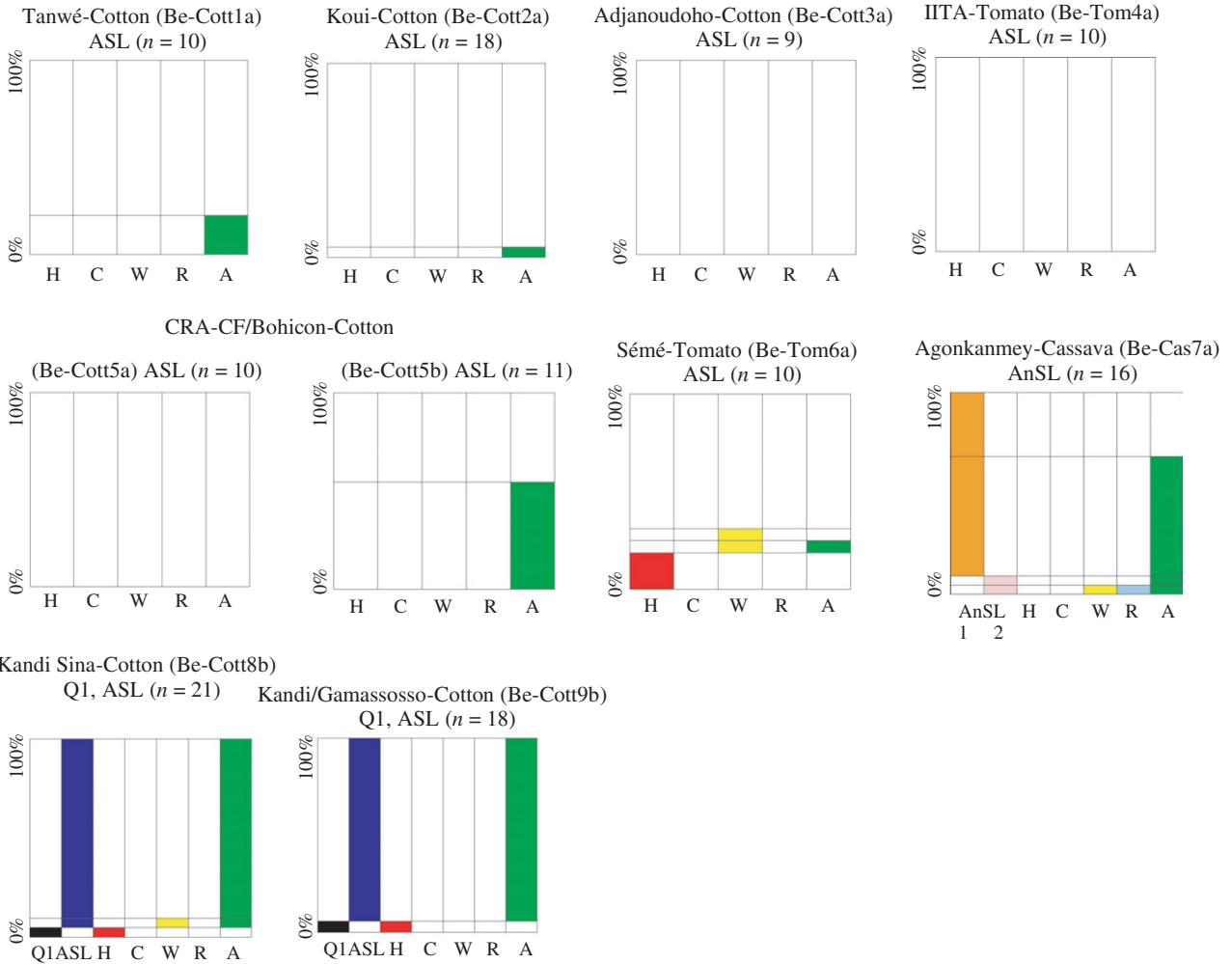
**Fig. 3.** Infection status of *Bemisia tabaci* individuals collected in 16 localities of three Western Africa countries.

why the sensitivity of adult populations of *B. tabaci* can vary greatly from one place and 1 year to another in Burkina Faso (Gnankiné *et al.*, 2007). However, in the localities where we sampled twice, in 2007 and in 2009, the prevalence of these biotypes was similar. This time lapse may have been too short, and other records on the spatial distribution of biotypes in Burkina Faso and in bordering countries spanning several years are

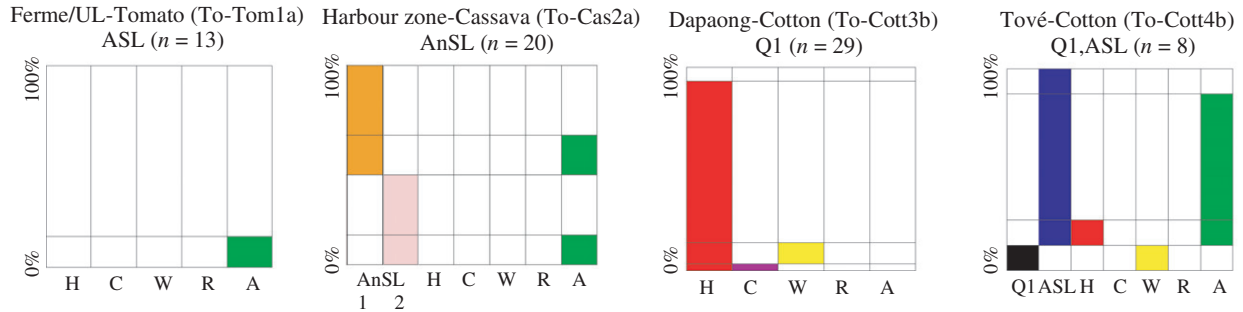
required before we can determine whether the Q biotype is actually spreading, and whether the sympatric situation of ASL and Q is stable or whether one biotype will eventually exclude the other, which could have major repercussions for pest management and agricultural production in this region.

The stable co-existence of biotypes could involve host-plant specialisation; for example, AnSL was the only biotype found

**(b) Benin**



**(c) Togo**



**Fig. 3.** (Continued).

on cassava. An earlier survey in sub-Saharan Africa (Abdullahi *et al.*, 2003) had already highlighted the fact that the populations that develop on cassava seem to be restricted to cassava only, whereas populations from other plants are polyphagous, but do not colonise cassava. Such niche specialisation of the AnSL, with cassava exercising a repulsive action on other biotypes,

could allow it to persist even if other biotypes are present locally. Unfortunately, all our samples collected on cassava originated from Togo and Benin, where ASL was the predominant biotype. Sampling on cassava in Burkina Faso could reveal whether AnSL is also found in this country despite the presence of Q1 and Q3. Q3 also seems to develop specifically in one host plant,

*L. camara*, where no other biotype has been detected, which suggests that even within the Q biotypes, there can be significant ecological and biological differences between sub-groups. However, this effect could also be due to the fact that this plant did not undergo insecticide treatment, something also suggested by the presence of Q3 on untreated tobacco. Further investigations of the ability of Q1, ASL and Q3 to develop on these different plants and on their resistance to insecticides are required to clarify and allow us to predict the dynamics of these biotypes and sub-groups.

Our study also confirms the initial observations that there is a link between symbiotic bacterial communities and the biotypes or genetic groups of *B. tabaci* (Chiel *et al.*, 2007; Gueguen *et al.*, 2010). As previously observed, within the Q biotype, most Q1 individuals harbour *Hamiltonella* and sometimes low frequencies of *Wolbachia* or/and *Cardinium*, whereas Q3 individuals were mostly found to harbour *Arsenophonus* with a high level of co-infection with *Rickettsia*. In the ASL and AnSL biotypes, *Arsenophonus* is the most common bacterium with higher frequency in the AnSL than the ASL biotype. In very few cases, other bacteria were reported such as *Wolbachia*, *Rickettsia*, or *Hamiltonella*. The reasons for these biotype-symbiont specific associations remain unknown, and the first step towards understanding the mechanisms involved will be to determine the phenotypic effects of these bacteria in *B. tabaci*. In aphids, some secondary symbionts are known to be involved in ecological specialisation, such as host plant utilisation (Tsuchida *et al.*, 2004), and so the symbiotic community could play a major role in biotype adaptation and competition.

One noteworthy finding is the fact that the prevalence of secondary symbionts varied considerably between the different localities and genetic groups. Most individuals from Burkina Faso belonged to the ASL and Q biotypes, and displayed high levels of infection, while in Benin and Togo, where the Q biotype is rare, the prevalence of secondary symbionts in ASL, consisting mainly *Arsenophonus*, was very low. Moreover, in Benin, some populations did not harbour any of the 6 secondary symbionts we checked. This is the first description of *B. tabaci* populations in which no secondary symbionts have been recorded. In addition, only a small number of individuals were infected by more than one secondary symbiont, with the exception of individuals belonging to the Q3 biotype, which contrasts with a previous study in which a high level of multiple infections was found (Gueguen *et al.*, 2010). However, it is impossible to rule out the possibility that other bacterial symbionts were in fact present in some *B. tabaci* individuals, and further studies involving more detailed investigations of the symbiotic content are called for. However, despite these limitations, the data reported raise several questions about the dynamics of infection by secondary symbionts and the influence of the environment on symbiotic composition.

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