Detection of genetically isolated entities within the Mediterranean species of Bemisia tabaci: new insights into the systematics of this worldwide pest

Laurence Mouton, Olivier Gnankiné, Hélène Henri, Gabriel Terraz, Guillaume Ketoh, Thibaud Martin, Frédéric Fleury, Fabrice Vavre

Detection of genetically isolated entities within the Mediterranean species of *Bemisia tabaci*: new insights into the systematics of this worldwide pest

Laurence Mouton[*,1], Olivier Gnankiné[*,2], Hélène Henri[1], Gabriel Terraz[1], Guillaume Ketoh[3], Thibaud Martin[4], Frédéric Fleury[1], Fabrice Vavre[1]

[*]Joint first authors

[1]Laboratoire de Biométrie et Biologie Evolutive, UMR 5558, Université de Lyon, CNRS, Villeurbanne, France.

[2]Laboratoire d'Entomologie Appliquée, Université de Ouagadougou, Burkina Faso.

[3]Faculté des Sciences, Université de Lome, B.P. 1515, Lome, Togo.

[4]UR Hortsys, CIRAD, Montpellier, France.

Key words: MED species, *B. tabaci* complex species, microsatellites, insecticide resistance, bacterial symbionts

Corresponding author
L. Mouton
Université de Lyon, Université Lyon1, Laboratoire de Biométrie et Biologie Evolutive, UMR CNRS 5558, 43 Bd du 11 Novembre 1918, 69622 Villeurbanne Cedex, France.
Phone: ++33 (0)4 72 43 29 10
Fax: ++33 (0)4 78 89 27 19
e-mail: laurence.mouton@univ-lyon1.fr

Running title
Within-species genetically isolated entities in *B. tabaci*

ABSTRACT

BACKGROUND: The taxonomy of the species complex *Bemisia tabaci*, a serious agricultural pest worldwide, is not well resolved yet, even though species delimitation is critical for designing effective control strategies. Based on a threshold of 3.5% mitochondrial (*mtCOI*) sequence divergence, recent studies identified 28 putative species. Among them, mitochondrial variability associated with particular symbiotic compositions (=cytotypes) can be observed, like in MED, which raises the question of whether it is a single or a complex of biological species.

RESULTS: Using microsatellites, we investigated the genetic relatedness of Q1 and ASL cytotypes that belong to MED. Samples of the two cytotypes were collected in West Africa where they live in sympatry on the same hosts. Genotyping revealed a high level of differentiation without evidence of gene flow. Moreover, they differed highly in frequencies of resistance alleles to insecticides, which were much higher in Q1 than in ASL.

CONCLUSION: Q1 and ASL are sufficiently reproductively isolated so that the introgression of neutral alleles is prevented, suggesting that they are actually different species. This indicates that nuclear genetic differentiation must be investigated within groups with less than 3.5% *mtCOI* divergence in order to elucidate the taxonomy of *B. tabaci* at a finer level. Overall, these data provide important information for pest management.

1 INTRODUCTION

The whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is one of the most devastating agricultural pests worldwide. Highly polyphagous, it causes damage in various agricultural crops either directly by sucking phloem sap or indirectly by excreting honeydew onto the surface of leaves and fruits.[1] Furthermore, *B. tabaci* is a major vector of several hundred plant viruses (Begomovirus, Crinivirus, Ipomovirus, Torradovirus) that are responsible for severe crop losses.[2]

B. tabaci is a complex of cryptic species whose species delimitation is critical if effective control strategies are to be devised.[3] Recently, important progress has been made at the taxonomic level with the definition of 28 genetic groups based on a threshold of 3.5% mitochondrial Cytochrome Oxidase I (*mtCOI*) sequence divergence that are regarded as cryptic species.[4-7] However, two recent studies from Boykin *et al.*[3] and Lee *et al.*[8] have indicated that this number is probably underestimated, as there is important variability within some species. This is notably the case within the Mediterranean (MED) species, with six mitochondrial haplotypes identified so far, generally associated with particular symbiotic communities in which they are in linkage disequilibrium.[4,9,10] The biological significance of these mitochondria-endosymbiont assemblages (referred to hereafter as cytotypes) is still unclear, but they call into question whether they are also associated with nuclear differentiation, in other words, whether putative species, especially MED, are really a single or a complex of biological species. Clearly, additional data are needed, notably data indicating whether interbreeding occurs in the field between cytotypes, before we can reliably define the taxonomy and systematics of *B. tabaci*.[7] However, field population studies are difficult to perform because many factors, such as host plant specialization and geographical barriers, can limit genetic exchanges. The ideal way to avoid these confounding effects is to study cytotypes that live sympatrically, but this situation is uncommon in *B. tabaci*.[11-13]

In the present study, we took advantage of the situation recently observed in Burkina Faso (West Africa), where two cytotypes belonging to the putative MED species, Q1 and Africa Silver Leafing (ASL), live in sympatry on the same host plants,[14] to investigate their genetic relatedness at the nuclear level. These two cytotypes present less than 3.1% *mtCOI* sequence variation.[15] Despite the occurrence of another cytotype classified within the MED species, the cytotype Q3, in this country, we did not include it since it develops specifically on a restricted number of host plants, especially *Lantana camara*, an ornamental plant where no other cytotype has been detected so far.[14]

For this study, we used seven microsatellite loci already described in the literature[16-19] as neutral markers to explore the genetic relatedness of Q1 and ASL cytotypes. Moreover, as insecticides are intensively used by farmers on cotton and vegetables in West Africa,[20] we also investigated the presence of alleles associated with insecticide resistance. In *B. tabaci*, two mutations in the *para*-type voltage-gated sodium channel gene, L925I and T929V, and one mutation in the acetylcholinesterase enzyme *ace1* (F331W) confer resistance to pyrethroids and organophosphates, respectively.[21-24] Previous studies have shown that the use of these insecticides results in the development of resistant *B. tabaci* populations,[25,26] which is undermining the current management of this pest.[27]

The data obtained clarify the degree of interbreeding between Q1and ASL and thus provide important biological insights for *B. tabaci* systematics. They also provide information about the frequencies and spatial distribution of the resistant mutation alleles in Burkina Faso.

2 EXPERIMENTAL METHODS

2.1 *Bemisia tabaci* samples

Sampling was performed in several locations in Burkina Faso (West Africa; Figure 1). The samples used for microsatellite genotyping were collected in 2007 (Table 1), and those used for investigating the presence of insecticide resistance alleles were collected in 2009 (Table 2), except those from Bobo/Kuinima (2007). Adult whiteflies were collected from cultivated vegetables (tomato, eggplant, marrow, and cucumber) and industrial crops (cotton and tobacco), and stored in ethanol 95% at -20°C until use. The origin of the samples (location and host plant) and the number of individuals are summarized in Tables 1 and 2.

2.2 DNA extraction

Total DNA was extracted from individual insects in 25 µl 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 mg/ml proteinase K. After 3 h at 65°C, samples were incubated at 100°C for 15 min. Pure water (35 µl) was then added to the extract.

2.3 Identification of *B. tabaci* cytotypes

Cytotypes were identified using the Polymerase Chain Reaction-Random Fragment Length Polymorphism (PCR-RFLP) diagnostic assay based on the mitochondrial marker cytochrome oxidase 1 gene sequence (*mtCO1*) described in Henri e*t al.*[15] that can be used to discriminate between all the known cytotypes detected in West Africa.[14]

2.4 Microsatellite genotyping

The genetic structure analysis performed using microsatellite markers was done on 135 individuals collected from five localities in Burkina Faso (Table 1). *B. tabaci* is a haplo-

diploid species, *i.e*., males hatch from unfertilized eggs and thus are haploids, and therefore genotyping was done on females only to estimate observed heterozygosity.

Genotyping was performed on seven microsatellite loci (Table S1). PCR reactions were performed on each primer pair separately in 12.5 µl volumes containing 200 µM dNTPs, 200 nM primers (fluorescently labelled at the 5' end), 0.5 IU *Taq* DNA polymerase (Eurobio, Courtaboeuf, France), and 1µl of DNA template. The cycling conditions were as follows: initial denaturing at 94°C for 2 min, followed by 35 cycles of 30 sec at 94°C for denaturing, 30 sec at 52 or 57°C depending on the primer set (Table S1) for annealing, and 1 min at 72°C for elongation, with a final elongation phase at 72°C for 10 min.

Fluorescent amplicons and a size standard (GS400Rox Size Standard, Applied Biosystems, Warrington, UK) were loaded onto an ABIPrism3100-Avant Genetic Analyser (Applied Biosystems) automated sequencer. Allele sizes were calculated using GeneMapper software (Applied Biosystems). Null alleles were searched for using Micro-Checker (freely available at: http://www.microchecker.hull.ac.uk/[28]).

2.5 Genetic data analysis

2.5.1 Population differentiation tests

We used GENEPOP[29] to assess the linkage disequilibrium between all pairs of loci and to test for Hardy-Weinberg equilibrium within populations and cytotypes. We considered a population to be whiteflies collected in the same locality and on the same host plant. Bonferroni correction was applied for the Chi-squared tests performed to analyze the linkage disequilibrium. The mean number of alleles per locus, observed and expected heterozygosities, and F statistic parameters of Weir and Cockerham[30] were calculated using GENETIX.[31]

2.5.2 Inferences on population structure

Two programs based on different assumptions were used to assess the level of population structure, STRUCTURE[32] and INSTRUCT (http://cbsuapps.tc.cornell.edu/InStruct.aspx). The first assumes Hardy-Weinberg equilibrium for all loci, while the second does not. As data were congruent, we focused mainly on STRUCTURE (see De Barro[33]). This free software uses a Bayesian clustering approach to determine the number of subpopulations (K) present on the basis of the individual genotypes. The admixture model was used, with a burning period of 60,000 iterations followed by 600,000 iterations. Log-likelihood estimates were calculated for K=1 to 10, with 10 replicates of each. The modal value of ΔK, a quantity based on the second order rate of change of the log probability of data with respect to K, was used to determine the number of clusters.[34] We also performed assignment tests with GENECLASS, using the Bayesian method (available at http://www1.montpellier.inra.fr/URLB/geneclass/geneclass.html[35]).

2.6 Identification of susceptible and resistant alleles of the sodium channel and *ace1* genes

Resistant and susceptible alleles in the *para*-type voltage-gated sodium channel and *ace1* genes were detected using the diagnostic assays developed by Tsagkarakou *et al.*[36] Briefly, *ace1*-susceptible (F331) and -resistant (W331) alleles, as well as susceptible (L925) and resistant (I925) *para*-type voltage-gated sodium channel alleles were detected using PCR-RFLP, and the T929V resistant mutation was detected by PCR using primer pairs that amplified only one of the two alleles (V929 or T929). Several PCR products were sequenced for each susceptible and resistant allele and each country.

3 RESULTS

3.1 Microsatellite variability

One hundred and thirty-five females from five sites and four host plants belonging to Q1 and ASL cytotypes were genotyped on the basis of seven microsatellite loci, with 6.3% missing data (Tables 1 and 3). ASL individuals were detected at a single location but from three different host plants; thus we considered three populations for this cytotype. No null alleles were detected using Micro-Checker. The number of alleles ranged from 4 to 31, with an average of 12.1 per locus in the whole sample, and from 1 to 29 per cytotype, with averages of 11 for Q1 and 5 for ASL. One allele was fixed for locus 145 in ASL.

Heterozygote deficiency was detected for all loci and in all populations, leading to high F_{IS} values. This deficit persisted even when samples were grouped by cytotype with a highly significant multilocus F_{IS} (0.188 for Q1 and 0.218 for ASL; $p<0.001$). No linkage disequilibrium was found between the loci, implying that the seven microsatellite loci carried independent genetic information.

3.2 Genotype-based inferences about population structure

The clustering analysis carried out using STRUCTURE and INSTRUCT on all the individuals of *B. tabaci* was performed from K=1 to K=10 subpopulations (Figure 2; Table S2). The highest likelihood value was obtained for K=2, and this was supported by using ΔK as the predictor of the real number of clusters. The two clusters defined strictly corresponded to the cytotypes Q1 and ASL, demonstrating that they correlated with genetic differentiation at the nuclear level (Figure 2). Assignment tests using GENECLASS gave similar results, since all the individuals were correctly assigned to their cytotype (86 Q1 and 49 ASL). The overall differentiation among all the samples in clusters was supported by a high F_{ST} of 0.183 ($p<0.001$).

Analyses were also done on each cytotype separately using STRUCTURE, from K=1 to K=5 (Table S2). The highest likelihood value was obtained for K=1, indicating that there was no differentiation among populations within these cytotypes. Genetic differentiation within cytotypes was also analyzed by computing F_{ST} estimates and the result supported the previous findings since values of F_{ST} were not significant for Q1 or ASL ($p>0.5$), with values of 0.009 and 0.003, respectively.

Our data do not comply with one of the criteria required by STRUCTURE, since there was a deficiency of heterozygotes. However, all the other analyses, which do not require Hardy-Weinberg equilibrium, did support the validity of the results, since they were confirmed using INSTRUCT, the assignment tests of GENECLASS, and F values.

3.3 Detection of sodium channel and *ace1* resistant mutations and allele frequencies

We used either PCR-RFLP or specific PCR, as described in Tsagkarakou *et al.*,[36] to look for resistant mutations in the *ace1* (F331W) and in the *para*-type voltage-gated sodium channel (L925I and T929V) genes in samples collected in Burkina Faso. Our results are shown in Table 2. For both genes, the frequencies of resistant alleles were very variable, depending on the cytotype. Overall, the frequencies of the F331W mutation in the *ace1* gene were 0.98 and 0.59 for Q1 and ASL, respectively. For the *para*-type voltage-gated sodium channel gene, only one of the two resistant mutations described in *B. tabaci* was found, L925I; this corresponds to the allele designated r1 by Alon *et al.*[23] It was almost fixed in the Q1 cytotype, with a frequency of 0.99, whereas it was rarely found in the ASL populations (frequency: 0.02).

Within cytotypes, while frequencies of the L925I mutation were homogeneous in all the populations (Fisher's Exact Test, $p>0.05$ for the two cytotypes), frequencies of the *ace1* resistant allele were significantly different (Fisher's exact test, $p<0.05$), and varied depending

on the host plant species ($p=0.01$ for Q1 and $p<0.001$ for ASL). The genotype frequencies did not differ significantly from those expected under Hardy-Weinberg equilibrium for each gene ($p>0.22$ for both Q1 and for ASL). There was no linkage disequilibrium between the two genes in either of the two cytotypes (Fisher's exact test, $p>0.10$).

4 DISCUSSION

The genetic relatedness between the Q1and ASL cytotypes classified in the MED putative species of *B. tabaci* by Dinsdale *et al.*[4] and De Barro *et al.*[6] have been characterized on the basis of microsatellite markers. Genetic data analysis revealed low F_{ST} values within the Q1 and ASL cytotypes between populations collected from locations 23 to 655 km apart, and from different host plant species (eggplant, tomato, and marrow), indicating low levels of genetic differentiation. This suggests that on this geographic scale, gene flows occur between populations found on different plants, as previously observed by De Barro.[33]

On the other hand, analyses of genetic relationships between cytotypes have revealed that ASL and Q1 individuals are genetically isolated. Indeed, clustering methods assigned the 135 females genotyped into two clusters strictly corresponding to the two cytotypes identified on the basis of the mitochondrial *mtCOI* marker. These data are corroborated by the high F_{ST} values between Q1 and ASL, further suggesting that Q1 and ASL do not interbreed. On the basis of these results, we propose that MED is composed of at least two different species.

This reproductive isolation is not due to physical barriers, since ASL and Q1 live in sympatry on the same host plants. It is also unlikely that there is any cytoplasmic incompatibility (CI) due to bacteria even if Q1 and ASL individuals do not harbor the same symbiotic community.[9,14] Each of these cytotypes harbors a distinct and highly prevalent bacterium (*Hamiltonella* in Q1, *Arsenophonus* in ASL[14]), but neither of them has been shown to induce CI in either of their hosts. Prevalences of the other bacteria they harbor are too low to lead to reproductive isolation due to CI (less than 10% each[14]). It is thus more likely that the lack of gene flow between Q1 and ASL involves either pre-mating behavioral isolation resulting in the absence of mating, or post-mating incompatibility, such as nuclear or nucleo-cytoplasmic incompatibilities, resulting in non-viable hybrids.

Four other cytotypes are classified in the MED putative species, Q2, Q3, J, and L.[6] Recently, a study based on population genetics on field samples and laboratory crossing experiments has shown that Q1 and Q2 interbreed,[37] indicating that they belong to the same species. At the time, there are no data concerning the other cytotypes. Studies considering all cytotypes are required to clarify the systematics of the MED putative species, even though this is not an easy task since except Q1/Q2 and Q1/ASL, they do not live sympatrically. More generally, such studies are required on all the putative species where several cytotypes have been detected, such in the New World.[6] This is all the more important, given that species delimitation is critical for optimizing integrated pest management programs.

More generally, the present study taken together with previous data call into question the use of the *mtCOI* gene with a 3.5% threshold for the *B. tabaci* taxonomy. Indeed, Lee *et al.* [8] highlighted that the variability within *B. tabaci* putative species has been underestimated for at least six of them[8]. Moreover, it has been shown that high *mtCOI* distances (up to 7 %) is not inconsistent with possible hybridization between *B. tabaci* cytotypes[38]. Besides there can be a bias to use mitochondrial markers because mitochondria are subjected to indirect selection caused by linkage disequilibrium with maternally inherited endosymbionts[39]. According to Galtier *et al.*[40] mitochondrial DNA should not be used for population genetic studies and phylogenetic analysis but should be restricted to the description of biodiversity. Alternative approaches based on nuclear markers should thus be used for *B. tabaci* species identification. Among them microsatellites are still the most common markers which could help to distinguish true species but recent methods based on NGS (New Generation Sequencing) tools like RADseq (Restriction-site Associated DNA markers) could also be used. Indeed, RADseq markers give the possibility to obtain genome wide data without previous information and has been proven to be a powerful tool for phylogenetic inferences even for non-model organisms.[41,42]

The mutation that confers resistance to organophosphates was found in both Q1 and ASL but, while this resistant allele was almost fixed in Q1 (mean: 0.98, standard error [s.e.]: 0.02), its frequency was intermediate in ASL (mean: 0.59, s.e.: 0.08). The high selection pressure due to the repetitive organophosphates treatments applied in western Africa, particularly on cotton and vegetables,[20,27] has probably driven the spread of this mutation. On the other hand, while the resistant mutation on the *para*-type voltage-gated sodium channel gene that confers resistance to pyrethroids was also almost fixed in Q1 populations, it was rarely detected in ASL.

ASL and Q1 have always been found in sympatry and thus have been exposed to the same insecticide compounds.[14] This raises the question of how ASL can be maintained. One hypothesis would be that ASL individuals use other mechanisms for insecticide resistance, like metabolic resistance. Indeed, detoxifying enzymes such as esterases, glutathione S-transferases, and cytochrome P450-dependent monooxygenases are involved in resistance to numerous insecticide classes.[23,43,44] An alternative explanation would be that the coexistence of the two cytotypes is recent and that either ASL has just arrived in West Africa and has not yet been counter-selected or that Q1 has just arrived and that ASL has not gone to extinction yet because it also encounters some plant refuges. However, the present study revealed the presence of Q1 and ASL for at least 2007, *i. e.*, 2 years before the survey of resistant alleles was performed. Monitoring the situation across years will help understanding of whether this situation is at equilibrium or not.

5 CONCLUSIONS

While the taxonomy and systematics of *B. tabaci* have been recently clarified, species boundaries remain mainly based on divergence of mitochondrial sequences. This allows a clearer representation of the genetic diversity of this species complex on a large scale, and makes it possible to define major groups and putative species. However, genetic diversity at the mitochondrial level has also been detected on a finer scale, and our results suggest that cytotypes within putative species could also be associated with nuclear variation, making it possible that some genetically isolated entities still exist on a phylogenetic scale finer than 3.5% *mtCO1* sequence divergence. This study demonstrates that reproductive compatibility must be backed up by observations of gene flows to support species delimitation. Moreover, our data indicate the presence of resistant alleles for organophosphates and pyrethroids in Burkina Faso where insecticides are widely used. However, while Q1 and ASL share the same host plants, they differ in the frequencies of resistant mutations. This last point is of particular importance for the choice of the strategy employed to control *B. tabaci*. Overall, these data provide new insights into the biological significance of *B. tabaci* cytotypes and are important for management of this pest.

ACKNOWLEDGMENTS

This work was funded by UNESCO (France) and CNRS (IFR41-UMR5558). These results have been achieved within the framework of the first call on Mediterranean agriculture carried out by ARIMNet, with funding from MOARD (IL), ANR (FR), INIA (ES), NAGREF-DEMETER (GR), and GDAR (TR). The authors are grateful to the INERA Institute in Burkina Faso, University of Lomé, for sample collection and technical assistance. G. Terraz is the recipient of a PhD studentship from the Rhône-Alpes region (“Program Cible” Grant). We thank S. Charlat for useful discussions.

TABLES AND FIGURES

Table 1. Samples of *B. tabaci* from Burkina Faso genotyped for genetic structure analyses and inbreeding coefficient (F_{IS}) of each population

Acronym[a]	Locality	Host plant	Biotype	N_{ind}	F_{IS}
Cot1	Kompienga	Cotton	Q1	15	0.224
Cot2	Diapaga	Cotton	Q1	14	0.223
Cot3	Lena	Cotton	Q1	15	0.190
Cot6	Bobo/Farako-Bâ	Cotton	Q1	14	0.276
Egg5	Bobo/Kuinima	Eggplant	Q1	8	0.119
			ASL	1	
Tom5		Tomato	Q1	9	0.041
			ASL	34	0.039
Mar5		Marrow	Q1	11	0.068
			ASL	14	0.064

N_{ind}: number of individual whiteflies genotyped, Cot: cotton, Tom: tomato, Egg: eggplant, Mar: marrow

[a] Each number refers to a specific locality

Table 2. Frequencies of sodium channel and ace-1 resistance mutations in *B. tabaci* from West Africa per host plant and locality

Acronym[a]	Locality	Host plant	Cytotype	Sodium channel								Ace							
				Males' genotypes			Females' genotypes				Allele frequencies	Males' genotypes			Females' genotypes				Allele frequencies
				n	r1	s	n	r1r1	r1s	ss		n	R	S	n	RR	RS	SS	
Cot4	**Kompienga**	**Cotton**	Q1	6	6	0	10	8	2	0	r1=0.92	6	5	1	10	8	2	0	R=0.88
Tom12	**Bobo/Koko**	**Tomato**	ASL	0	0	0	5	0	0	5	r1=0	0	0	0	5	0	2	3	R=0.20
			Q1	1	1	0	10	10	0	0	r1=1	1	1	0	10	10	0	0	R=1
Tom9	**Léguéma**	**Tomato**	ASL	2	0	2	12	0	0	12	r1=0	2	1	1	12	4	2	6	R=0.42
			Q1	3	3	0	12	12	0	0	r1=1	3	3	0	12	12	0	0	R=1
Mar9		**Marrow**	ASL	0	0	0	5	0	0	5	r1=0	0	0	0	5	1	1	3	R=0.3
			Q1	0	0	0	6	6	0	0	r1=1	0	0	0	6	5	1	0	R=0.92
Tom10	**Toukoro**	**Tomato**	Q1	2	2	0	13	13	0	0	r1=1	2	2	0	13	13	0	0	R=1
Cuc5	**Bobo/Kuinima**	**Cucumber**	Q1	0	0	0	29	29	0	0	r1=1	0	0	0	29	29	0	0	R=1
Cot11	**Bittou**	**Cotton**	Q1	4	4	0	19	19	0	0	r1=1	4	4	0	19	18	1	0	R=0.97
Tom8	**Soumousso**	**Tomato**	ASL	4	1	3	6	0	0	6	r1=0.06	4	4	0	6	1	3	2	R=0.56
			Q1	2	2	0	15	15	0	0	r1=1	2	2	0	15	15	0	0	R=1

r1 and R refer to resistant alleles for the sodium channel and Ace genes, respectively; s: susceptible allele, Cot: cotton, Tom: tomato, Cuc: cucumber, Mar: marrow

[a] Each number refers to a specific locality

Table 3. Summary of genetic variation at the seven microsatellite loci

Locus		Q1	ASL	All samples
	N	86	49	135
BtIS2.3	N_A	12	4	14
	Allele range	102–126	105–109	102–126
	H_E	0.857	0.511	0.669
	H_O	0.726	0.488	0.533
	f	0.154	0.045	0.203
BtIS1.13	N_A	4	3	4
	Allele range	107–117	113–117	107–117
	H_E	0.524	0.279	0.431
	H_O	0.465	0.245	0.333
	f	0.112	0.124	0.228
BtIS1.1	N_A	10	4	11
	Allele range	239–275	241–249	239–275
	H_E	0.562	0.385	0.399
	H_O	0.482	0.121	0.304
	f	0.142	0.689	0.241
BtIS1.2	N_A	29	8	31
	Allele range	274–368	294–348	274–368
	H_E	0.901	0.629	0.622
	H_O	0.724	0.617	0.467
	f	0.198	0.019	0.251
Locus11	N_A	5	5	7
	Allele range	160-178	168-176	160-176
	H_E	0.609	0.512	0.431
	H_O	0.430	0.396	0.296
	f	0.295	0.229	0.314
BT83	N_A	12	10	17
	Allele range	132–174	138–164	132–164
	H_E	0.795	0.691	0.598
	H_O	0.679	0.488	0.482
	f	0.147	0.295	0.196
Locus145	N_A	5	1	6
	Allele range	167–183	173	167–183
	H_E	0.407	0	0.271
	H_O	0.305	0	0.185
	f	0.253	-	0.317
All	Mean N_A	11	5	12.1
	Mean H_E	0.665	0.429	0.670
	Mean H_O	0.544	0.336	0.509
	Multilocus F_{IS}	0.182	0.219	0.187

N: number of samples; N_A: number of alleles; H_E: expected heterozygosity; H_O: observed heterozygosity; *f*: inbreeding coefficient.

Figure 1. Sampling locations in Burkina Faso

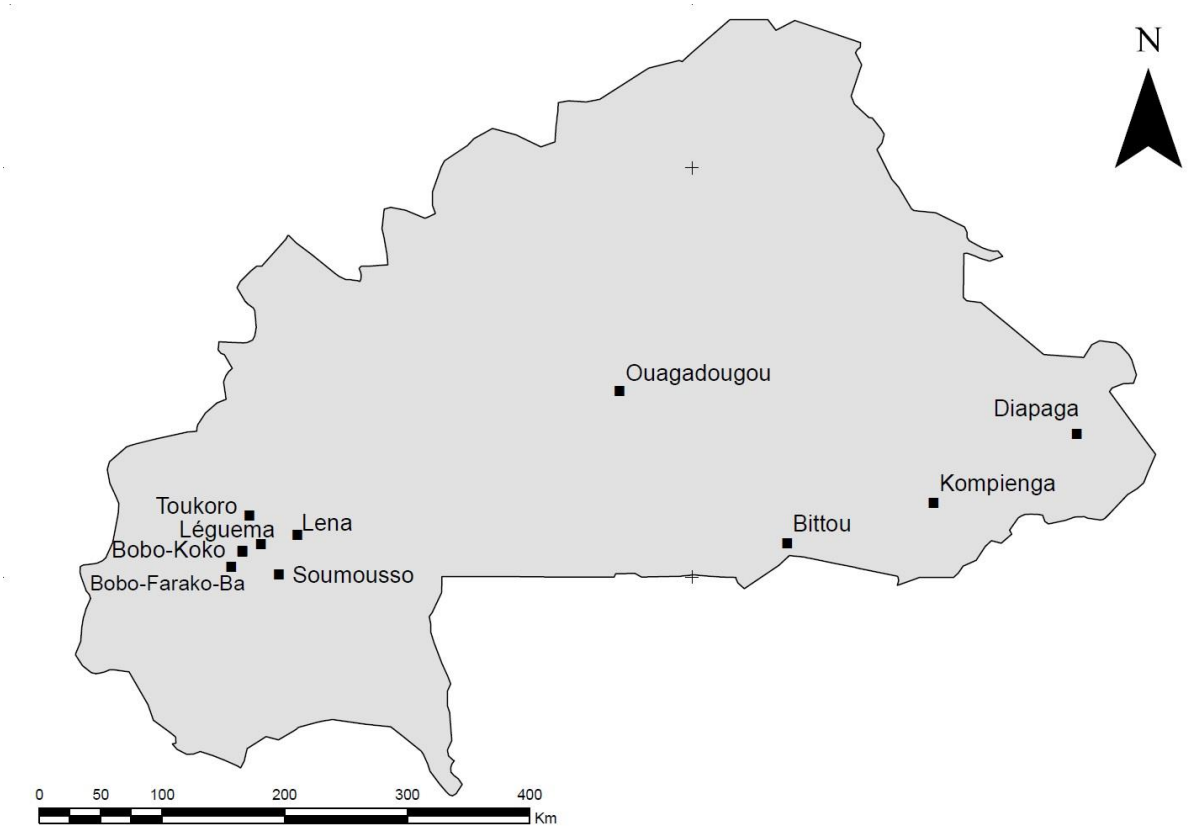
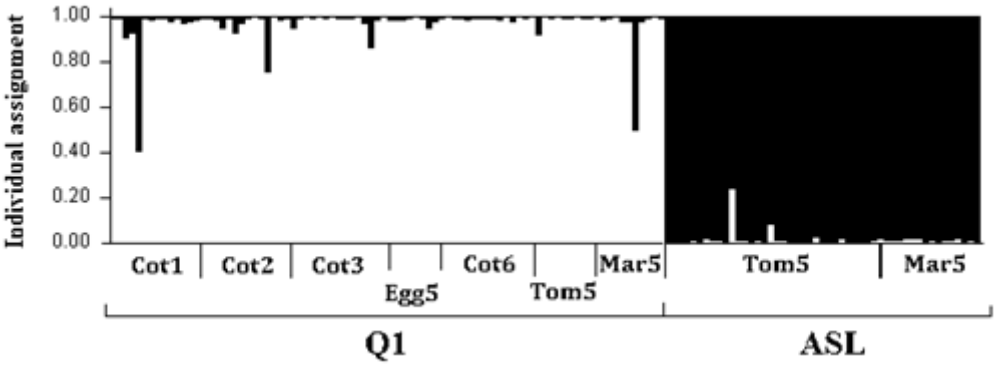


Figure 2. Clustering results for all samples obtained using STRUCTURE.



Cot: cotton, Tom: tomato, Egg: eggplant, Mar: marrow ; Each number refers to a specific locality

REFERENCES

1. Oliveira MRV, Henneberry TJ and Anderson P, History, current status, and collaborative research projects for *Bemisia tabaci*. *Crop Protect* **20**: 709-723 (2001).

2. Brown JK and Czosnek H, Whitefly transmission of plant viruses. *Adv Bot Res* **36**: 65-100 (2002).

3. Boykin LM, Armstrong KF, Kubatko L and De Barro P, Species delimitation and global biosecurity. *Evol Bioinf* **8**: 1-37 (2012).

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

5. Hu J, De Barro P, Zhao H, Wang J, Nardi F and Liu SS, An extensive field survey combined with a phylogenetic analysis reveals rapid and widespread invasion of two alien whiteflies in China. *Plos One* **6**: e16061 (2011).

6. De Barro PJ, Liu SS, Boykin LM and Dinsdale B, *Bemisia tabaci*: a statement of species status. *Ann Rev Entomol* **56**: 1-19 (2011).

7. Shu-Sheng L, Colvin J and De Barro PJ, Species concepts as applied to the whitefly *Bemisia tabaci* systematics: how many species are there? *J Integr Agr* **11**: 176-186 (2012).

8. Lee W, Park J, Lee G-S, Lee S and Akimotot SI, Taxonomic status of the *Bemisia tabaci* complex (Hemiptera: Aleyrodidae) and reassessment of the number of its constituent species. *Plos One* **8**: e63817 (2013).

9. Gueguen G, Vavre F, Gnankiné O, Peterschmitt M, Charif D, Chiel E, Gottlieb Y, Ghanim M, Zchori-Fein E and Fleury F, Endosymbiont metacommunities, mtDNA diversity and the

evolution of the *Bemisia tabaci* (Hemiptera: Aleyrodidae) species complex. *Mol Ecol* **19**: 4365-4378 (2010).

10. Chu D, Hu X, Gao C, Zhao H, Nichols RL and Li X, Use of mitochondrial cytochrome oxidase I Polymerase Chain Reaction-Restriction Fragment Length Polymorphism for identifying subclades of *Bemisia tabaci* Mediterranean group. *J Econ Entomol* **105**: 242-251 (2012).

11. Moya A, Guirao P, Cifuentes D, Beitia F and Cenis JL, Genetic diversity of Iberian populations of *Bemisia tabaci* (Hemiptera: Aleyrodidae) based on random amplified polymorphic DNA-polymerase chain reaction. *Mol Ecol* **10**: 891-897 (2001).

12. Khasdan V, Levin I, Rosner A, Morin S, Kontsedalov S, Maslenin L and Horowitz AR, DNA markers for identifying biotypes B and Q of *Bemisia tabaci* (Hemiptera: Aleyrodidae) and studying population dynamics. *Bull Entomol Res* **95**: 605-613 (2005).

13. Horowitz AR, Kontsedalov S, Khasdan V and Ishaaya I, Biotypes B and Q of *Bemisia tabaci* and their relevance to neonicotinoid and pyriproxyfen resistance. *Arch Insect Biochem Physiol* **58**: 216-225 (2005).

14. Gnankiné O*, Mouton L*, Henri H, Terraz G, Houndeté T, Martin T, Vavre F and Fleury F, Distribution *Bemisia tabaci* biotypes (Homoptera: Aleyrodidae) and their associated symbiotic bacteria on host plants in western Africa. (*1er co-authors). *Ins Cons Div* **6**: 411-421 (2013).

15. Henri H, Terraz G, Gnankiné O, Fleury F and Mouton L. Molecular characterization of genetic diversity within the Africa/Middle East/Asia Minor and Sub-Saharan African groups of the *Bemisia tabaci* species complex. *Int J Pest Manag* 59: 329-338 (2013).

16. Tsagkarakou A and Roditakis N, Isolation and characterization of microsatellite loci in *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Mol Ecol Notes* **3**: 196-198 (2003).

17. Delatte H, David P, Granier M, Lett JM, Goldbach R, Peterschmitt M and Reynaud B, Microsatellites reveal extensive geographical, ecological and genetic contacts between invasive and indigenous whitefly biotypes in an insular environment. *Gen Res* **87**: 109-124 (2006).

18. Gauthier N, Dalleau-Clouet C and Bouvret ME, Twelve new polymorphic microsatellite loci and PCR multiplexing in the whitefly, *Bemisia tabaci*. *Mol Ecol Res* **8**: 1004-1007 (2008).

19. Dalmon A, Halkett F, Granier M, Delatte H and Peterschmitt M, Genetic structure of the invasive pest *Bemisia tabaci*: evidence of limited but persistent genetic differentiation in glasshouse populations. *Heredity* **100**: 316-325 (2008).

20. Ahouangninou C, Fayomi B and Martin T, Évaluation des risques sanitaires et environnementaux des pratiques phytosanitaires des producteurs maraîchers dans la commune rurale de Tori-Bossito (Sud-Bénin). *Cahiers de l'Agriculture* **20**: 216-222 (2011).

21. Morin S, Williamson MS, Goodson SJ, Brown JK, Tabashnik BE and Dennehy TJ, Mutations in the *Bemisia tabaci para*-sodium channel gene associated with resistance to a pyrethroid plus organophosphate mixture. *Insect Biochem Mol Biol* **32**: 1781-1791 (2002).

22. Roditakis E, Tsagkarakou A and Vontas J, Identification of mutations in the para sodium channel of *Bemisia tabaci* from Crete, associated with resistance to pyrethroids. *Pesticide Biochem Physiol* **85**: 161-166 (2006).

23. Alon M, Alon F, Nauen R and Morin S, Organophosphates' resistance in B-biotype of *Bemisia tabaci* (Hemiptera : Aleyrodidae) is associated with a point mutation in an *ace1*-type acetylcholinesterase and overexpression of carboxylesterase. *Insect Bioch Mol Biol* **38**: 940-949 (2008).

24. Alon M, Benting J, Lueke B, Ponge T, Alon F and Morin S, Multiple origins of pyrethroid resistance in sympatric biotypes of *Bemisia tabaci* (Hemipetra: Aleyrodidae). *Insect Bioch Mol Biol* **36**: 71-79 (2006).

25. Palumbo JC, Horowitz AR and Prabhaker N, Insecticidal control and resistance management for *Bemisia tabaci*. *Crop Protect* **20**: 739-765 (2001).

26. Houndete TA, Ketoh GK, Hema OSA, Brevault T, Glitho IA and Martin T, Insecticide resistance in field populations of *Bemisia tabaci* (Hemiptera: Aleyrodidae) in West Africa. *Pest Manag Sci* **66**: 1181-1185 (2010).

27. Gnankiné O, Traoré D, Sanon A, Traoré NS and Ouedraogo AP, Traitements insecticides et dynamique des populations de *Bemisia tabaci* en culture cotonnière au Burkina Faso. *Cahiers d'Agriculture* **16**: 101-109 (2007).

28. Van Oosterhout C, Hutchinson WF, Wills DPM and Shipley O, Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. *Mol Ecol Notes* **4**: 535-538 (2004).

29. Raymond M and Rousset F, Genepop version 2.0: population genetics software for exact tests and ecumenicism. *J Heredity* **86**: 248-249 (1995).

30. Weir BS and Cockerham CC, Estimating F-statistics for the analysis of population structure. *Evolution* **38**: 1358-1370 (1984).

31. Belkhir K, Borsa P, Chikhi L, Raufaste N and Bonhomme F, *GENETIX 4.05, logiciel sous Windows^{TM} pour la génétique des populations.* Laboratoire Génome, Populations, Interactions, CNRS UMR 5171, Université de Montpellier II: Montpellier, France (2001).

32. Pritchard JK, Stephens M and Donnelly P, Inference of population structure using multilocus genotype data. *Genetics* **155**: 945-959 (2000).

33. De Barro PJ, Genetic structure of the whitefly *Bemisia tabaci* in the Asia-Pacific region revealed using microsatellite markers. *Mol Ecol* **14**: 3695-3718 (2005).

34. Evanno G, Regnaut S and Goudet J, Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol Ecol* **14**: 2611-2620 (2005).

35. Piry S, Alapetite A, Cornuet JM, Paetkau D, Baudouin L and Estoup A, GENECLASS 2: a software for genetic assignment and first generation migrant detection. *J Heredity* **95**: 536-539 (2004).

36. Tsagkarakou A, Nikou D, Roditakis E, Sharvit M, Morin S and Vontas J, Molecular diagnostics for detecting pyrethroid and organophosphate resistance mutations in the Q biotype of the whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Pest Biochem Physiol* **94**: 49-54 (2009).

37. Terraz G, Gueguen G, Fleury F and Mouton L, Nuclear and cytoplasmic differentiation among Mediterranean populations of *Bemisia tabaci*: testing the biological relevance of cytotypes. *Pest Manag Sci.* In press. doi: 10.1002/ps.3792.

38. Thierry M, Becker N, Hajri A, Reynaud B, Lett JM and Delatte H, Symbiont diversity and non-random hybridization among indigenous (Ms) and invasive (B) biotypes of *Bemisia tabaci*. *Mol Ecol* **20**: 2172-2187 (2011).

39. Hurst GD and Jiggins FM, Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: the effects of inherited symbionts. *Proc Biol Sci.* **272**: 1525-1534 (2005).

40. Galtier N, Nabholz B, Glémin S and Hurst GD, Mitochondrial DNA as a marker of molecular diversity: a reappraisal. *Mol Ecol.* **18**: 4541-4550 (2009).

41. Cariou M, Duret L and Charlat S, Is RAD-seq suitable for phylogenetic inference? An in silico assessment and optimization. *Ecol Evol.* **3**: 846–852 (2013).

42. Keller I, Wagner CE, Greuter L, Mwaiko S, Selz OM, Sivasundar A, Wittwer S and Seehausen O, Population genomic signatures of divergent adaptation, gene flow and hybrid speciation in the rapid radiation of Lake Victoria cichlid fishes. *Mol Ecol.* **22**: 2848-2863 (2013).

43. Rauch N and Nauen R, Identification of biochemical markers linked to neonicotinoid cross resistance in *Bemisia tabaci* (Hemiptera: Aleyrodidae). *Arch Insect Biochem Physiol* **54**: 165-176 (2003).

44. Ma W, Li X, Dennehy TJ, Lei C, Wang M, Degain BA and Nichols RL, Pyriproxyfen resistance of *Bemisia tabaci* (Homoptera: Aleyrodidae) biotype B: metabolic mechanism. *J Econ Entomol* **103**: 158-165 (2010).