Mitochondrial DNA Variability and Development of a PCR Diagnostic Test for Populations of the Whitefly *Bemisia afer* (Priesner and Hosny)

M. N. Maruthi, A. R. Rekha, P. Sseruwagi, and R. J. Hillocks*

Abstract

The whitefly, *Bemisia afer* (Hemiptera; Aleyrodidae), is emerging as a major agricultural pest. The current identification methods based on adult and pupal morphology are laborious and unreliable. A diagnostic polymerase chain reaction (PCR) protocol was developed for the first time in this study to discriminate *B. afer* from other whitefly species. Primers specific to mitochondrial cytochrome oxidase I gene (mtCOI) were designed to amplify a band of approx 650 bp. The PCR products were sequenced from *B. afer* samples collected from Malawi, Tanzania, Uganda, Zanzibar, and the United Kingdom. Phylogenetic analyses of mtCOI sequences and those of reference *B. afer* sequences clustered the African *B. afer* separately from the UK and Chinese populations and from other whitefly species. The African cluster was divided into two clades by parsimony and neighbor-joining methods. This indicates the existence of at least two genotypic clusters of *B. afer*, which are diverged by 0.8 to 3.2% nucleotide (nt) identities. Analysis of molecular variance indicated that these differences were the result of within population variation but were insufficient to identify discrete populations. Among the whitefly species used in the analysis, *B. afer* was equally dissimilar to *Bemisia tabaci* and *Bemisia tuberculata* (21.3–26.2% nt identities). As is the case for *B. tabaci*, these data show that mtCOI sequences are informative also for identifying *B. afer* variants, which lack distinguishing morphological features.

Index Entries: Aleyrodidae; cytochromoe oxidase I gene; genetic diversity; genotypes; molecular markers.

1. Introduction

The whitefly, *Bemisia afer* (Priesner and Hosny) (Aleyrodidae; Sternorrhyncha; Hemiptera), is an emerging pest whose geographical range is increasing ***(1,2)***. It is an apparently polyphagous pest of a variety of crop plants belonging to 20 families ***(3,4)***. High numbers of *B. afer* adults and nymphs cause extensive damage by direct feeding on cassava in Malawi ***(5)*** and South Africa (J. P. Legg, personal communication), on soybean and citrus in China and Italy ***(2)***. The first outbreak of *B. afer s.l.* in the New World occurred in Peru on sweet potato ***(1)***. More recently, *B. afer* has been found colonizing ornamental plants in the United Kingdom in unheated polytunnels with temperatures near freezing levels.

The taxonomy of whiteflies has long been problematic because of similarities in the morphology of adults. Precise identification of *B. afer* and *Bemisia tabaci* (Gennadius), which occur together on cassava in Africa ***(6)***, is difficult and presents a practical problem for field ecologists. In addition, nymphs and pupae of *Bemisia* species exhibit phenotypic variation in response to differences in leaf surface topology ***(7)*** and to environmental and physical factors ***(8)***. For these reasons, whitefly species have often been synonymized ***(3,9)***. *B. afer*, for example, was described originally in the genus

*Author to whom all correspondence and reprint requests should be addressed. Natural Resources Institute, University of Greenwich, Central Avenue, Chatham Maritime, Kent ME4 4TB, UK. E-mail: M.N.Maruthi@greenwich.ac.uk.

 ISSN: 1073–6085/Online ISSN: 1559–0305/2007/35:1/031–040/$30.00

Dialeurodes ***(10)***, and then later transferred to the genus *Bemisia* ***(11)***. *Bemisia hancocki* ***(3,12)*** was then synonymized with *B. afer* ***(13)***. This synonymy, however, may have been premature, because it was based on the examination of one badly damaged syntype of *B. afer* ***(1)***. Moreover, the basis of synonymization remains questionable because it relied on the morphology of the fourth instar nymph. Examination of puparial morphology of *B. hancocki* in Africa ***(14)*** and of *B. afer* from Europe and USA indicated that the *B. afer* group actually displayed much greater morphological variation than does the much studied *B. tabaci* and its variants ***(1)***. These observations have stimulated the need for molecular studies to resolve the systematic questions.

Molecular markers, such as the mitochondrial cytochrome oxidase I (mtCOI) gene ***(15–19)*** and internal transcribed spacer 1 (ITS1) region ***(20–22)*** sequences, have been used extensively to identify *B. tabaci* variants that exhibit rich biological differences, but lack distinguishing morphological features. Using these two markers, a strong biogeographical pattern has been established for the world's *B. tabaci* populations, whereas only one *B. afer* population has been characterized at the molecular level to date ***(6)***.

In this study we were unable to amplify the mtCOI gene of *B. afer* reliably using the primer set (C1-J-2195 and L2-N-3014) ***(15)*** that has been used extensively on *B. tabaci*. For this reason and the lack of reliable alternative techniques, experiments were undertaken to design new set of primers to amplify *B. afer* mtCOI gene and, subsequently, to develop a polymerase chain reaction (PCR)-based diagnostic technique for nymphs and adults. This will facilitate future studies on *B. afer* genetic diversity and thus contribute to our understanding of the systematic status of populations currently grouped under *B. afer*.

2. Materials and Methods

2.1. Collection of B. afer Samples

Samples of *B. afer* adults were collected from cassava fields at Entebbe and Namulonge in Uganda; Chenika, Kibaha, Zigowale, and Zanzibar Island in Tanzania, and at Nkhata Bay (Mkondezi Reasearch Station) in Malawi (**Table 1**). Depending on their numbers, 5 to 20 adults were collected from each location using an aspirator and stored in 70% ethanol. Samples of UK *B. afer* were collected as nymphs on the ornamental plant sweet bay (*Laurus nobilis*) in the gardens of Hampton Court, Surrey.

About 50 to 100 live adult *B. afer* were collected from Zanzibar and placed into plastic boxes containing fresh cassava leaves. The insects were transported to the quarantine insectary of the Natural Resources Institute and colonies were established on a Colombian cassava variety as described previously ***(6,23)***. The colonies were maintained in the insectary at 25 ± 1ºC, 50% relative humidity, and 12-h light and dark cycles. Pupae and adults were identified as *B. afer* on the basis of pupal and adult morphology at the Natural History Museum, London.

2.2. DNA Extraction and Primer Design

The method used for DNA extraction from individual whitefly was described previously ***(24)***. DNA extracted from two *B. afer* female adults or nymphs were used initially in separate PCR to amplify partial mtCOI gene (~860 bp) using the primers C1-J-2195 and TL2-N-3014 ***(15,25)***, which have been used extensively on *B. tabaci*. These primers, however, did not reliably amplify products of the expected size. A new set of primers specific to *B. afer* mtCOI gene was designed (Ba1F 5'-GGGGGCATCACATGTT TACTG-3' and Ba1R 5' –TGTGTCTGACGC TGGGCTT-3') by aligning (CLUSTAL W 1.7) ***(26)*** the reference sequences of *B. afer* from Uganda (EMBL accession number AF418673) and China (AJ784260); *B. tabaci* from Uganda (AF418669) and Tanzania (AF418667); *Trialeurodes vaporariorum* from South America (AF110708) and Reunion (AJ550183); *T. abutilonea* (AY057221) and *B. tuberculata* (AY057220). Ba1F was designed to anneal about 130 bases downstream of the primer C1-J-2195 and Ba1R about 30 bases upstream of TL2-N-3014 ***(25)***. Primers were designed at these nucleotide positions because they were conserved

Table 1
Details of *B. afer* Sample Collections and Their mtCOI Gene Sequences Used in the Study

Location	Host plant	Sample abbreviations[a]	EMBL accession no.	Date of collection	Collector/ supplier
Namulonge, Uganda	Cassava	UgCas-Nam1 and UgCas-Nam2	AJ842022 & AJ842023	May 2003	J P Legg
Kibaha, Tanzania	Cassava	TzCas-Kib1 to TzCas-Kib11	AJ842024 to AJ842034	April 2003	M N Maruthi
Zanzibar, Tanzania	Cassava	TzCas-Zan1 to TzCas-Zan9	AJ842035 to AJ842043	April 2003	M N Maruthi
Chenika, Tanzania	Cassava	TzCas-Che1 and TzCas-Che2	AJ842044 & AJ842045	April 2003	M N Maruthi
Zigowale, Tanzania	Cassava	TzCas-Zig	AJ842046	April 2003	M N Maruthi
Nkhata Bay, Malawi	Cassava	MaCas-Nkh1 to MaCas-Nkh5	AJ842047 to AJ842051	July 2003	R J Hillocks
Hampton Court, Surrey, UK	Sweet bay	UKSwe-Sur	AJ842052	November 2002	D Collins
Entebbe, Uganda[b]	Cassava	UgCas-Ent	AF418673	February 1999	J Colvin

[a]Abbreviation for the *B. afer* samples follows the standard given for *B. tabaci* in our earlier publication ***(18)***, i.e., using the trinomial system of country–host–location.

[b]Described in Maruthi et al. ***(6)***.

Table 2
Whitefly Species Used in the PCR Amplification of mtCOI Gene Using the *B. afer*-Specific Primers

Whitefly species/ biotype	Geographical origin	Host–plant origin
Aleurodicus dispersus	Bangalore, India	Cassava
Aleurotrachilus socialis	Cali, Colombia	Cassava
Bemisia afer s.l.	Valle de Caunete, Peru	Sweet potato
Bemisia tabaci	Mtwara, Tanzania	Cassava
	Zanzibar, Tanzania	Cassava
	Entebbe, Uganda	Cassava
	Mukono, Uganda	Cassava
	Tamale, Ghana	Cassava
	Bangalore, India	Sunflower
	Bangalore, India	Okra
	Bangalore, India	Pumpkin
	Midatharahally, India	Field bean
	Bangalore, India	Eggplant
	Jessore, Bangladesh	Eggplant
	Bogra, Bangladesh	Chilli
	Philippines	Cassava
B-biotype	Tel Aviv, Israel	Cabbage
Bemisia spp.	Las Cuevas, Belize	*Bocconia* spp.
Trialeurodes vaporariorum	Bangalore, India	*Phyllanthus emblica*

within *B. afer* and diverged in other whitefly species. The Ba1F anneals the nucleotides between positions 2100 and 2400 in the most conserved region of mtCOI gene, and Ba1R on the most divergent $tRNA^{leu}$(UUR) gene ***(25)***.

PCR was performed in 25-µL reaction mix containing 2 µL of template DNA, 0.5 U of Red hot polymerase (Abgene, UK), 2.0 m*M* $MgCl_2$, 0.15 m*M* each of dNTP, 0.6 pmol of each primer in 1X reaction buffer (20 m*M* NH_4SO_4, 75 m*M* Tris-HCl pH 9.0, 0.01% v/v Tween). Reactions were carried out in a Gene Amp PCR System 9700 thermal cycler (Applied Bio-Systems, UK) programmed for 94ºC for 2 min, followed by 35 cycles at 94ºC for 45 sec, 52ºC for 30 sec, and 72ºC for 1 min, and ending with 72ºC for 10 min. The PCR products were separated through 1% agarose gels and visualized under ultraviolet light after staining in 0.5 µg/mL ethidium bromide solution.

Using these protocols, total DNA extracted from five other whitefly species (**Table 2**) were used in separate PCR reactions to test the specificity of primers to *B. afer*. Mock DNA extractions (without insect) and negative (water) PCR reactions were included as controls.

Several attempts to amplify ITS1 by PCR using polymerases from different suppliers and universal primers ***(22)*** were unsuccessful. This prevented the characterization of *B. afer* by one other molecular marker.

2.3. mtCOI Gene Sequencing and Phylogenetic Analysis

The PCR products generated using the *B. afer*-specific primers were cloned into the pGEMT-Easy vector system (Promega, UK) following manufacturers' instructions. For each *B. afer,* two clones were sequenced and each clone was sequenced in its entirety, in both orientations. Sequencing reactions were carried out at the John Innes Centre, Norwich, UK. A consensus sequence was obtained by editing the sequences using the software package Editseq (DNAStar Inc., USA).

A sequence of length 602 bases was used in the phylogenetic analyses and compared with the ref-

Table 3
Analysis of Molecular Variance of African *B. afer* Populations Using the Software Program Arlequin (version 2.0)

Source of variation	Degrees of freedom (d.f.)	Sum of squares	Variance components	Percentage variation
Between populations	3	1.50	0.00 Va	0.00
Within populations	28	14.00	0.50 Vb	100
Total	31	15.50	0.50	

Abbr: Va: variation not detected between African *B. afer* populations due to geographical isolation in Malawi, Tanzania, Uganda, and Zanzibar.
Vb: all the diversity in *B. afer* was the result of variation within populations that are present in the same geographical locations.

erence mtCOI sequences of *B. afer*, *B. tuberculata*, *T. abutilonea,* and *T. vaporariorum* that were used initially while designing the primers (*see* **Subheading 2.2.**). Multiple sequence alignments were carried out using the software package CLUSTAL W 1.7 ***(26)***. Phylogenetic analyses were performed by parsimony and distance methods using the software PAUP version 4.0 b10 for Mac ***(27)***, as described previously ***(18)***. The heuristic and neighbor-joining methods were used to generate phylogenetic trees with 70% confidence levels. Bootstrapping for 1000 replications was performed by stepwise sequence addition and tree-bisection-reconnection. A distance matrix was calculated with a Kimura 2-parameter model. We were unable to carry out the maximum likelihood analysis using this software package may be because of the high numbers (42) of sequences used in the analysis.

2.4. Analysis of Molecular Variance

Analysis of molecular variance (AMOVA) ***(28)*** was performed using the software program ARLEQUIN, version 2.0 ***(29)*** (**Table 3**). AMOVA estimates the variance in gene frequencies by calculating the mutational changes in the sequences. Sequences of African *B. afer* were used in the analysis, which were classified based on their geographical origin into Uganda (n = 4), mainland Tanzania (n = 14), Zanzibar island (n = 9), and Malawi (n = 5) (**Table 1**). Variance was calculated on populations between countries, within locations, and between the two genotype clusters (I and II) identified in the phylogenetics analyses (discussed later). The significance of the variance components was tested using 1023 permutations.

3. Results

3.1. Development of a Diagnostic Test for B. afer

Figure 1 shows the PCR products generated from the DNA of several whitefly species using Ba1F and Ba1R primers. An expected, PCR product of size approx 650 bp was amplified in all reactions containing DNA of *B. afer* nymphs or adults from Malawi, Tanzania, Uganda, and UK. No specific PCR products were obtained from the DNAs of *Aleurodicus dispersus*, *Aleurotrachilus socialis*, *Bemisia* spp., and *T. vaporariorum,* and 13 samples of *B. tabaci* from Asia (Bangladesh, India, Israel, and Philippines) and Africa (Ghana, Tanzania, and Uganda) (**Table 2**). However, a faint band of size approx 600 bp was obtained from DNA of *T. vaporariorum* from India (**Fig. 1**), the sequence of which is not determined. No PCR products were obtained from the DNAs of *B. afer s.l.* from Peru and in reactions that contained blank DNA extractions and negative (water) controls.

3.2. mtCOI Sequence Diversity in B. afer

A total of 31 *B. afer* mtCOI gene sequences of length 650 bases were obtained from populations of Malawi, Tanzania, Uganda, and UK. Unambiguous sequences of 602 bases (after removing the primer sequence) were analyzed that consisted of 276 invariable, 69 variable, and 259 parsimony informative characters. The phylogeny from parsimony and neighbor-joining methods generated trees with similar topology and the consensus most parsimonious tree is shown herein (**Fig. 2**). The most parsimonious tree generated by heuris-

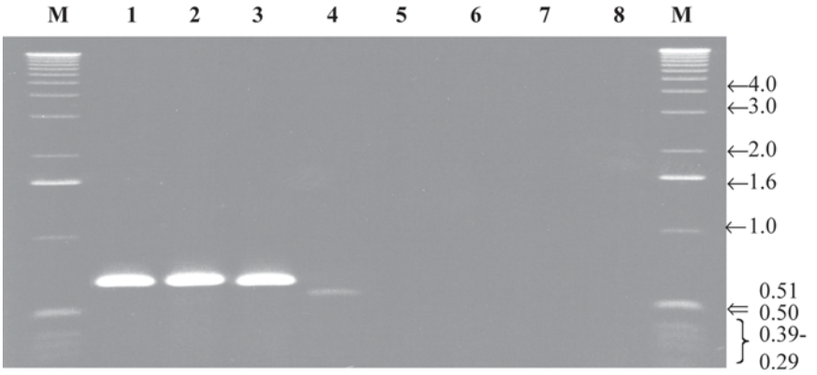
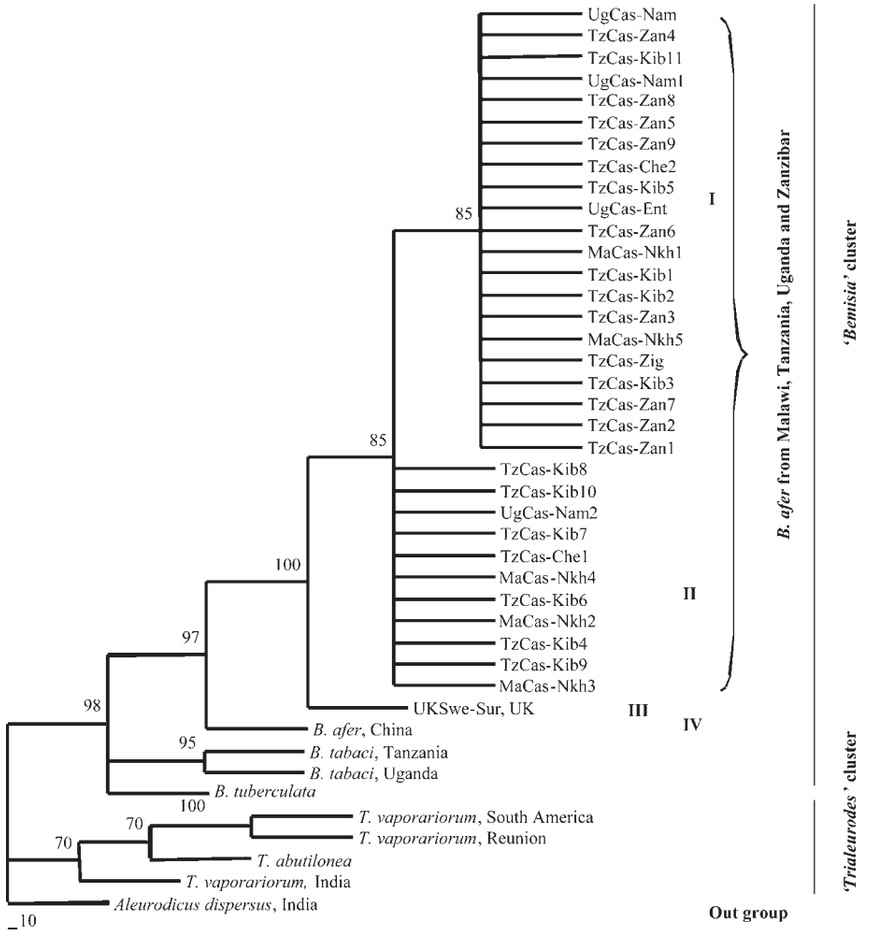


Fig. 1. A photograph of gel electrophoresis of typical PCR products generated using the *B. afer*-specific primers Ba1F and Ba1R. *B. afer* (lanes 1–3); *T. vaporariorum* (lane 4); *B. tabaci* (lane 5); *A. disperses* (lane 6); *A. socialis* (lane 7); negative (water) control (lane 8); M: 1 kb molecular weight marker, the sizes of which are shown on the right.



tic search with 70% confidence levels had a score of 856.

B. afer, *B. tabaci,* and *B. tuberculata* formed a single large "*Bemisia*" cluster, whereas the two species of *Trialeurodes* (*T. abutilonea* and *T. vaporariorum*) formed a separate cluster, and the *A. dispersus* formed the out-group. *B. afer* grouped into four distinct clades with bootstrap support ≥85% for each clade. *B. afer* from Malawi, Tanzania, and Uganda formed two clades (I and II), indicating the existence of at least two genotypic clusters of *B. afer* on cassava in Africa. A single sample each from UK and China formed clade III and IV, respectively.

The two *B. afer* genotypes were distributed in the three African countries. The sample UgCas-Nam1 grouped in clade I with two other samples from Uganda (UgCas-Nam and UgCas-Ent), whereas the UgCas-Nam2, which originated from the same field as UgCas-Nam1, grouped in clade II. Similarly, samples from Chenika, Tanzania, were distributed in both the clades. Two of the Malawi samples grouped in clade I and the remaining three in clade II, and five samples from Kibaha, Tanzania, grouped in clade I and the remaining six in clade II. All of the nine samples from Zanzibar Island, however, grouped in a single clade I (**Fig. 2**).

Percentage nucleotide identities of mtCOI sequences of whiteflies are summarized in **Table 4**. The mtCOI sequence variation within African *B. afer* was 0.8 to 3.2%. The UK *B. afer* was closest to African *B. afer* by 92.5 to 94.3% nt identities. The Chinese *B. afer* was most divergent and, equally to the African and UK *B. afer* (77.6–78.2% similar). *B. afer* mtCOI sequences were equally similar to *B. tabaci* and *B. tuberculata* in the ranges of 73.8 to 78.7% nt identities. Among the whitefly species, *B. afer* was most similar to *B. tuberculata* and dissimilar to *T. abutilonea* (**Table 4**).

Analysis of molecular variance performed to estimate the genetic variation of *B. afer* revealed no significant difference between populations collected from different countries in Africa. All the variation (100%) found in African *B. afer* populations was the result of differences within populations in the same geographical area.

4. Discussion

We report a simple, PCR-based technique for the reliable identification of *B. afer* using a new primer pair designed to their mtCOI gene. The results were further extended by sequencing of PCR products, which confirmed the specificity of the new primers to *B. afer*. The new primers were developed after a lack of a reliable detection test for an increasingly important pest, *B. afer*, and the inability of the most commonly used primers C1-J-2195 and TL2-N-3014 ***(15)*** to produce PCR products reliably.

Failures in PCR can be the result of mutational changes on the DNA of the target organism at the annealing sites of primers. The inability of the new primers Ba1F and Ba1R to produce PCR products from *B. afer s.l.* from Peru may be the result of such mutations. Zhang and Hewitt ***(25)***, for example, designed several sets of primers to amplify mtCOI sequences of arthropods because of mutations. Obtaining additional mtCOI sequences of *B. afer* populations from a wide range of countries and host plants may facilitate designing more general primers.

This is the first study on the genetic diversity of *B. afer* using the mtCOI gene. As recorded for *B. tabaci* ***(15–18,20)***, the mtCOI gene sequences were informative in identifying variability in *B. afer* for a species rich in biological variants but lacking distinguishing morphological characters ***(1,2)***. Phylogenetic analysis grouped the world's *B. afer* populations into four distinct clades based on their geographical origin and indicated the existence of at least two closely related *B. afer*

Fig. 2. *(opposite page)* Parsimonious tree showing the relationship of *B. afer* populations with other whitefly species based on 602 nt fragment of mtCOI gene sequences. Vertical distances are arbitrary and the horizontal distances are proportional to mutational changes in sequences. Numbers at nodes indicate bootstrap scores using 1000 replicates. Abbreviations are as described in **Table 1**.

Table 4
Percentage mtCOI Gene Nucleotide Sequence Identities Between and Within Clades of Different Whitefly Species

	B. afer								
	Clade I (Africa)	Clade II (Africa)	Clade III (UK)	Clade IV (China)	*B. tabaci*[a]	*B. tuberculata*	*T. abutilonea*	*T. vaporariorum*	*A. dispersus*
B. afer Clade I	99.3–100								
B. afer Clade II	97.7–99.2	96.8–100							
B. afer Clade III	92.7–93.0	92.5–94.3	100						
B. afer Clade IV	77.6–77.9	77.7–78.3	78.2	100					
B. tabaci[a]	74.7–75.6	73.8–75.5	74.7–74.8	78.2–78.6	83.9				
B. tuberculata	76.2–76.5	75.8–76.5	76.0	78.7	79.9–80.5	100			
T. abutilonea	67.1–67.5	66.6–67.5	64.9	69.7	69.9–70.2	72.0	100		
T. vaporariorum	69.3–71.4	69.3–71.7	69.4–70.4	70.6–71.2	69.7–74.9	72.6–73.7	72.8–73.3	72.6–98.7	
A. dispersus	68.4–68.8	68.0–69.7	68.1	73.6	74.1–75.6	77.4	75.6	71.8–74.4	100

[a]mtCOI sequences of two *B. tabaci* from Uganda (AF418669) and Tanzania (AF418667) that co-exist with *B. afer* on cassava were compared.

genotypes on cassava in Africa. AMOVA indicated that all the diversity in the African *B. afer* populations was the result of within population variation, implying that populations of both clusters had a common ancestor and that each group may have evolved separately only recently. Within population variation was highest (3.2%) for *B. afer* from Zanzibar. Variation was recorded in the pupal morphology of *B. afer* ***(1)***, and for the first time in this study *B. afer* was shown to exhibit large genetic variation.

The UK *B. afer* cluster was supported by 100% bootstrap score and diverged from African *B. afer* by 5.7 to 7.5% nt identities. This was twice the level of variation seen within African *B. afer* (3.2%) and far too high for a highly conserved marker such as mtCOI ***(25)***. These results thus support the conclusion that this particular genotype may not have been introduced to UK from Africa in the recent past. However, *B. afer* is being constantly intercepted by the UK quarantine authorities on ornamental plants imported from many countries ***(2)***. Therefore, it continues to pose a constant threat to the UK horticultural industry, especially in polytunnels and glass house conditions.

B. afer affects a large number of plant species. In this study, however, samples were collected on cassava in Africa and on ornamental plants in the UK. Similar to *B. tabaci*, analyses of samples from more locations and different host plant species will almost certainly result in the detection of higher genetic diversity and identification of more variants of *B. afer*. Compared to African cassava *B. tabaci,* the detected genetic diversity of cassava *B. afer* was low, which may be because of limited data available on the latter species. Examination of mtCOI sequences of *B. tabaci*, however, indicated the existence of at least five cassava-associated genotypes, some of which diverged by 8% ***(17,19,30)***. Analyses of 30 *B. afer* mtCOI sequences from Africa showed the highest divergence at 3.2%. Whether this is the result of a low evolutionary change in *B. afer* compared to *B. tabaci* requires investigation. Because of its importance as an agricultural pest, *B. tabaci* has been subjected to changes in cropping practices, exposed to wide range of insecticides and other control methods thus forcing the species to change constantly to adapt to new environments. In contrast, until recently *B. afer* has been considered a minor pest on crops and seldom being subjected to selection pressures of insecticides, which may partially explain why *B. afer* genetic diversity was not as high as *B. tabaci*.

Acknowledgments

We thank Jon Martin for supplying whitefly samples from Belize and Peru, and John Colvin for reading of the manuscript. This publication is an output from a research project funded by the United Kingdom Department for International Development (DFID) for the benefit of developing countries [Crop Protection Programme]. The live *B. afer* colonies were held under the DEFRA license number PHL 176/4251.

References

1. Anderson, P. K., Martin, J. H., Hernandez, P., and Lagnaout, A. (2001) *Bemisia afer sens. lat.* (Homoptera: Aleyrodidae) outbreak in the Americas. *Fla. Entomol.* **84,** 316–317.
2. Malumphy, C. (2003) The status of *Bemisia afer* (Priesner and Hosny) in Britain (Homoptera: Aleyrodidae). *Entomol. Gazette* **54,** 191–196.
3. Mound, L. A. and Halsey, S. H. (1978) *Whitefly of the World*. John Wiley and Sons, Chichester.
4. Martin, J. H. (1987) An identification to common whitefly pest species of the world (Homoptera, Aleyrodidae). *Trop. Pest Manage.* **33,** 298–322.
5. Munthali, D. C. (1992) Effect of cassava variety on the biology of *Bemisia afer* (Priesner and Hosny) (Hemiptera: Aleyrodidae). *Insect Sci. Applic.* **13,** 459–465.
6. Maruthi, M. N., Navaneethan, S., Colvin, J., and Hillocks, R. J. (2004) Bionomics, morphometrics and molecular characterisation of a cassava *Bemisia afer* (Priesner and Hosny) population. *Int. J. Trop. Insect Sci.* **24,** 323–329.
7. Mound, L. A. (1963) Host correlated variation in *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae). *Proc. R. Entomol. Soc. London (A)* **38,** 171–180.
8. Mohanty, A. K. and Basu, A. N. (1986) Effect of host plant and seasonal factors on intraspecific variations on pupal morphology of the whitefly vector, *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodidae). *J. Entomol. Res.* **10,** 19–26.

9. Russell, L. M. (1957) Synonyms of *Bemisia tabaci* (Gennadius) (Homoptera, Aleyrodidae). *Bull. Brok. Entomol. Soc.* **52,** 122–123.
10. Priesner, H. and Hosny, M. (1934) Contribution to a knowledge of the whiteflies (Aleurodidae) of Egypt (III). *Bull. Min. Agric. Egypt, Technical and Scientific Service* **145,** 1–11.
11. Habib, A. and Fang, F. A. (1970) Studies on nine common aleurodids of Egypt. *Bull. Soc. Entomol. Egypt* **54,** 1–41.
12. Corbett, G. H. (1936) New Aleurodidae (Hem.). *Proc. R. Entomol. Soc. London (B)* **5,** 18–22.
13. Bink-Moenen, R. M. (1983) *Revision of the African Whiteflies (Aleyrodidae) Mainly Based on a Collection From Tchad.* Monografieu van de Nederlanse Entomologisch Vereniging No. 10. 211 pp. Amsterdam.
14. Mound, L. A. (1965) An introduction to the Aleyrodidae of Western Africa (Homoptera). *Bull. Br. Mus. (Natural History)* **17,** 115–160.
15. Frohlich, D. R., Torres-Jerez, I., Bedford, I. D., Markham, P. G., and Brown, J. K. (1999) A phylogeographical analysis of Bemisia tabaci species complex based on mitochondrial DNA markers. *Mol. Ecol.* **8,** 1683–1691.
16. Brown, J. K. (2001) Molecular markers for the identification and global tracking of whitefly vector–begomovirus complexes. *Virus Res.* **71,** 233–260.
17. Berry, S. D., Fondong, V. N., Rey, C., Rogan, D., Fauquet, C. M., and Brown, J. K. (2004) Molecular evidence for five distinct Bemisia tabaci (Homoptera: Aleyrodidae) geographic haplotypes associated with cassava plants in sub-Saharan Africa. *Ann. Entomol. Soc. Am.* **97,** 852–859.
18. Maruthi, M. N., Colvin, J., Thwaites, R. M., Banks, G. K., Gibson, G. and Seal, S. E. (2004) Reproductive incompatibility and cytochrome oxidase I gene sequence variability amongst host-adapted and geographically separate *Bemisia tabaci* populations (Hemiptera: Aleyrodidae). *Syst. Entomol.* **29,** 560–568.
19. Sseruwagi, P., Maruthi M. N., Colvin, J., Rey, M. E. C., Brown, J. K., and Legg, J. P. (2006) Colonization of non-cassva plant species by cassava whiteflies (*Bemisia tabaci*) in Uganda. *Entomol. Expt. Appl.* **119,** 145–153.
20. De Barro, P. J., Driver, F., Trueman, J. W. H., and Curran, J. (2000) Phylogenetic relationship of world populations of *Bemisia tabaci* (Gennadius) using ribosomal ITS1. *Mol. Phylogenet. Evol.* **16,** 29–36.
21. De Barro, P. J., Trueman, J. W. H., and Frohlich, D. R. (2005) *Bemisia argentifolii* is a race of *B. tabaci* (Hemiptera: Aleyrodidae): the molecular genetic differentiation of *B. tabaci* populations around the world. *Bull. Entomol. Res.* **95,** 193–203.
22. Abdullahi, I., Winter, S., Atiri, G. I., and Thottappilly, G. (2003) Molecular characterisation of whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae) populations infesting cassava. *Bull. Entomol. Res.* **93,** 97–106.
23. Maruthi, M. N., Colvin, J., and Seal, S. (2001) Mating compatibility, life-history traits and RAPD-PCR variation in *Bemisia tabaci* associated with the cassava mosaic disease pandemic in East Africa. *Entomol. Expt. Appl.* **99,** 13–23.
24. De Barro, P. J. and Driver, F. (1997) Use of RAPD PCR to distinguish the B biotype from other biotypes of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae). *Aust. J. Entomol.* **36,** 149–152.
25. Zhang, D.-X. and Hewitt, G. M. (1996) Assessment of the universality and utility of a set of conserved mitochondrial COI primers in insects. *Insect Mol. Biol.* **6,** 143–150.
26. Higgins, D. G., Thompson, J. D., and Gibson, T. J. (1996) Using CLUSTAL for multiple sequence alignments. *Method Enzymol.* **266,** 383–402.
27. Swofford, D. L. (2002) *PAUP4.0b10 Phylogenetic Analysis Using Parsimony (and other methods).* Version 4. 0. Beta. Sinauer Associates, Inc., Sunderland, MA.
28. Excoffier, L., Smouse, P., and Quattro, J. (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* **131,** 479–491.
29. Schneider, S., Roessli, D., and Excoffier, L. (2000) *Arlequin ver. 2.000: A Software for Population Genetics Data Analysis*. Genetics and Biometry Laboratory, University of Geneva, Switzerland.
30. Legg, J. P., French, R., Rogan, D., Okao-Okuja, G., and Brown, J. K. (2002) A distinct *Bemisia tabaci* (Gennadius) (Hemiptera: Sternorrhyncha: Aleyrodidae) genotype cluster is associated with the epidemic of severe cassava mosaic virus disease in Uganda. *Mol. Ecol.* **11,** 1219–1229.