CHAPTER 3.13

Using Molecular Techniques to Analyse Whitefly Species and Biotypes in Latin America

*Lee Calvert**, *Natalia Villarreal** and *Donald Frohlich***

Introduction

When identifying and describing insect taxa, morphology has been used historically to separate species. Among many groups of insects, however, morphological characters can vary with respect to environmental factors within a single species, or be so convergent and cryptic among closely related species as to be of limited usefulness. Under such conditions, studies of their biology and molecular profiles become essential to defining species and characterizing populations. At a molecular level, protein and DNA polymorphisms can be combined with studies of biological characteristics by using one of four experimental or technological approaches: electrophoresis of allozymes, analysis of randomly amplified polymorphic DNAs (RAPDs) and nucleic acid sequence comparisons of nuclear or mitochondrial DNA markers. Here, we review the application of molecular approaches to characterizing whitefly *Bemisia tabaci* (Gennadius) populations and biotypes in Latin America.

Each method has its own characteristic advantages and disadvantages. Isozyme analyses using starch—or polyacrylamide—gel electrophoresis have been in use for several decades and are useful for processing large numbers of samples relatively inexpensively compared to DNA sequencing. However, because the technique relies on detecting enzymes, samples must be kept live or frozen in order to preserve activity. The technique is also less sensitive than DNA approaches because the underlying nucleic acid variability is usually masked.

When, in the early 1980s, changes in whitefly populations and associated begomovirus infections were first noticed, protein polymorphisms were employed to investigate natural populations of *B. tabaci.* Differences in esterase isozyme patterns were used to describe two biotypes in the Americas: a now-known-to-be native form, or biotype A, and a second form, biotype B, which exhibited high population density, wide host plant range, relatively high insecticide resistance and was capable of inducing "silverleaf" symptoms on some plants (Brown et al., 1995). *B. tabaci* biotype A was predominant in most regions of the Americas but many of these populations now have been displaced by *B. tabaci* biotype B. Other reports suggested that there might be additional biotypes in Latin America (Wool et al., 1994). Eighteen other biotypes from throughout the world

* Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia.
** University of St. Thomas, Houston, TX, USA.

have been described based on esterase banding patterns alone (Brown et al., 1995). Reliable morphological markers, which do not vary with ecological parameters (Mound, 1963; David and Ananthakrishnan, 1976; Mohanty and Basu, 1986) and which can distinguish between biotypes, are not known (Rosell et al., 1997).

DNA sequence comparisons can be made between individuals or populations using polymerase chain reaction (PCR) generated sequences from several markers in either the nuclear or mitochondrial genome. The latter offers some advantages because it is maternally inherited and non-recombining and contains known and predictable gene sequences for which general insect primers have been designed. Some sequences vary enough to be useful for population level analyses (e.g., the control region, parts of cytochrome oxidases I-III), while others evolve slowly enough to be more appropriate to analyses above the species level (e.g., conserved parts of cytochrome oxidase I [COI], 12S and parts of the 16S r-DNA). However, as with some nuclear markers, comparisons between mitochondrial sequences may reflect local gene evolution and not population or species evolution. Nuclear data offer a wide range of neutral markers but can be particularly difficult to design PCR primers for specific targets in poorly known or unknown genomes (e.g., introns). Thus, many studies have used variable regions surrounded by relatively conserved sequences (e.g., internal transcribed spacers or ITS of the rDNA genes) for population/species analyses, or more conserved sequences (e.g., 18S rDNA) for higher order studies.

An alternative approach to specific nuclear markers involves the use of short, random sequence PCR primers that anneal randomly in the genome and produce characteristic banding patterns of collections of PCR products when run on an agarose gel (RAPDs). Advantages here include the low cost and capability of processing large numbers of samples without the necessity of cloning or sequencing PCR products as well as no requirement for detailed sequence knowledge about the genome. Disadvantages include the fact that repeatability can be difficult, and the polymorphisms may be hard to distinguish from PCR artefacts. That is, template preparation and amplification conditions must be consistent and tightly controlled. Interpretation of negative data (absence of bands) also may have numerous explanations.

Perring et al. (1993) used RAPD patterns in combination with allozyme frequency data and mating and behavioural studies to elevate the B biotype to new species status, *Bemisia argentifolii* Bellows & Perring. In a detailed study of 20 RAPD primers, however, Gawel and Bartlett (1993) concluded that the technique was not useful for clarifying taxon status in the case of whiteflies. Campbell et al. (1994) were the first to look at a nuclear DNA sequence, albeit highly conserved, and showed that only a single unique nucleotide difference lies between *B. tabaci* biotypes A and B in the 18S gene. More recently, Frohlich et al. (1999) used variable portions of the mitochondrial COI gene and 16S rDNA sequence to evaluate *B. tabaci* populations from four continents, and concluded that biotype B is a recent introduction from the Old World to the Americas. De Barro et al. (2000), using ribosomal ITS 1, reached the same conclusion and cautioned that if the status of the B biotype were to remain species novum, a taxonomic review of all of the biotypes of *B. tabaci* would have to be made.

For this study of the whiteflies affecting crops of economic importance, a variable portion of the 16S mitochondrial ribosomal DNA was chosen for evolutionary analysis. It is more variable than the 18S mt DNA but less so than the COI gene. This allows both a comparison of distinct genera as well as closely related biotypes.

Methods and Materials

PCR, cloning and sequence analysis of a region of the 16S mitochondrial DNA

The primers 4119 (5' CGCCTGTTTAACAAAAACAT) and 4118 (5' CCGGTCTGAACTCAG ATCACGT 3') were used to amplify a region of the 16S mitochondrial DNA (Xiong and Kocher, 1991). The PCR reaction conditions were 30 cycles of 1 min at 95 °C, 50 s at 50 °C and 50 s at 72 °C, and in the last cycle the 72 °C reaction was extended to 10 min. The products were purified using the Wizard™ PCR purification columns (Promega, WI, USA) and were visualized by agarose gel electrophoresis. The PCR products were cloned into the plasmid PCR script amp SK(+)™ (Stratagene, La Joya, CA, USA). Using the ABI dye terminator kit, the sequences were determined in an automated sequencer using the dideoxynucleotide chain termination procedure (Sanger et al., 1977).

Phylogenetic analyses

Phylogenetic analyses were done with multiple individuals within populations. DNA sequences were aligned using the ClustalW algorithm (Thompson et al., 1994) by the ClustalW 1.7 program (BCM Search Launcher at the Human Genome Centre, Baylor College of Medicine, Houston, TX, USA). Because different tree building algorithms make different evolutionary assumptions, data were evaluated by parsimony, neighbour joining and maximum-likelihood. All analyses were performed with PAUP, version 4.0b2, for Macintosh (Swofford, 1999). For parsimony, the branch-and-bound method was used (characters unordered, equal weight). Bootstrapping was performed with the branch-and-bound option for 2000 replicates (stepwise sequence addition, tree-bisection-reconnection [TBR], MulTrees option). For neighbour joining, distances were calculated using the Kimura 2 parameter model. Maximum-likelihood trees were constructed with a transition/transversion ratio of 2.0 by heuristic search (100 replicates, random addition sequence, MulTrees, TBR) (Swofford, 1999).

Whitefly survey

The whiteflies that were identified by RAPD PCR analysis were collected and processed.[1]

RAPD PCR analysis

Total DNA was isolated from individual whiteflies using a method developed for plants (Gilbertson et al., 1991). The DNA was amplified in a PCR. The Operon primers F2 (5'GAGGATCCCT3'), F12 (5'ACGGTACCAG3'), H9 (5'TGTAGCTGGG3') and H16 (5'TCTCAGCTGG3') (De Barro and Driver, 1997) were tested for their efficacy to distinguish whiteflies in Latin America. The reaction conditions for the first cycle were 5 min at 94 °C, 2 min at 40 °C and 3 min at 72 °C. This was followed with 39 cycles of 1 min at 94 °C, 1.5 min at 40 °C, and

1. For further information on specific methodologies used to produce the results published in this book, please contact the information and communication assistant of the Project (www.tropicalwhiteflyipmproject.cgiar.org).

2 min at 72 °C. The PCR products were run in agarose gels stained with ethidium bromide and visualized using UV light.

Results

Analysis of selected whiteflies in Latin America

A region of the 16S mitochondrial DNA of *B. tabaci* biotypes A and biotype B, *B. tuberculata*, *Aleurotrachelus socialis* (Bondar), *Trialeurodes vaporariorum* (Westwood) and *T. variabilis* (Quaintance) were compared by parsimony and distance analysis using Phylip version 3.57 (Felsenstein, 1993). At least two independent clones from each whitefly population of the 3' region of the mitochondrial 16S gene were prepared and sequenced. Included in the comparison were sequence data of the mitochondrial 16S gene from *B. tabaci* biotype A of Arizona (Genbank accession: AF110722), Costa Rica (Genbank accession: AF110715) and Puerto Rico (Genbank accession: AF110719) (Frohlich et al., 1999). In the analysis of various *B. tabaci*, those of biotype A were grouped together and there was at least 97% identity with a maximum mean distance of 0.02 (Table 1) (Calvert et al., 2001). The *B. tabaci* biotype A is widespread throughout the region of the whitefly survey.

In the distance analysis, the Israel *B. tabaci* isolate (Genbank accession: AF110717) was 98.8% identical with the *B. tabaci* biotype B isolates from Colombia (Table 1). Comparisons were also made with individuals from Colombia, Arizona, Israel and Yemen and all were at least 98.2% identical (data not shown). Given the rapid spread of the B biotype, the lack of diversity between populations in Arizona USA and Colombia was expected. This gives additional evidence that *B. tabaci* biotype B was recently introduced into the Americas from the region of the Middle East.

A representative from each species or biotype was used in the analysis to find the most parsimonious tree (Figure 1). *B. tabaci* biotypes A and

Table 1. Mean distances for a 3' region of mitochondrial 16S ribosomal gene in fifteen individual whiteflies representing different species and populations.

Whitefly[a]	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1 *B. tabaci* B Sucre	0.00	0.01	0.01	0.01	0.10	0.10	0.09	0.10	0.10	0.22	0.35	0.35	0.37	0.37	0.36
2 *B. tabaci* B CT cass		0.00	0.00	0.00	0.08	0.09	0.08	0.09	0.09	0.21	0.34	0.34	0.36	0.36	0.35
3 *B. tabaci* B CT bn			0.00	0.00	0.09	0.10	0.09	0.09	0.09	0.21	0.34	0.35	0.37	0.36	0.35
4 *B. tabaci* Israel				0.00	0.08	0.09	0.08	0.09	0.09	0.21	0.34	0.34	0.36	0.36	0.35
5 *B. tabaci* A CT 1					0.00	0.01	0.01	0.02	0.00	0.22	0.34	0.35	0.36	0.35	0.38
6 *B. tabaci* A CR						0.00	0.01	0.02	0.01	0.23	0.35	0.36	0.37	0.36	0.39
7 *B. tabaci* A AZ							0.00	0.02	0.01	0.22	0.34	0.36	0.37	0.36	0.38
8 *B. tabaci* A PR								0.00	0.02	0.22	0.35	0.36	0.36	0.36	0.38
9 *B. tabaci* CT 2									0.00	0.22	0.34	0.35	0.36	0.36	0.38
10 *B. tuberculata* CT										0.00	0.28	0.28	0.37	0.36	0.38
11 *A. socialis* Mon											0.00	0.07	0.40	0.39	0.43
12 *A. socialis* CT												0.00	0.41	0.40	0.41
13 *T. vaporariorum* CT													0.00	0.00	0.39
14 *T. vaporariorum* AZ														0.00	0.38
15 *T. variabilis* CT															0.00

a. *B, Bemisia*; *A, Aleurotrachelus*; *T, Trialeurodes*.

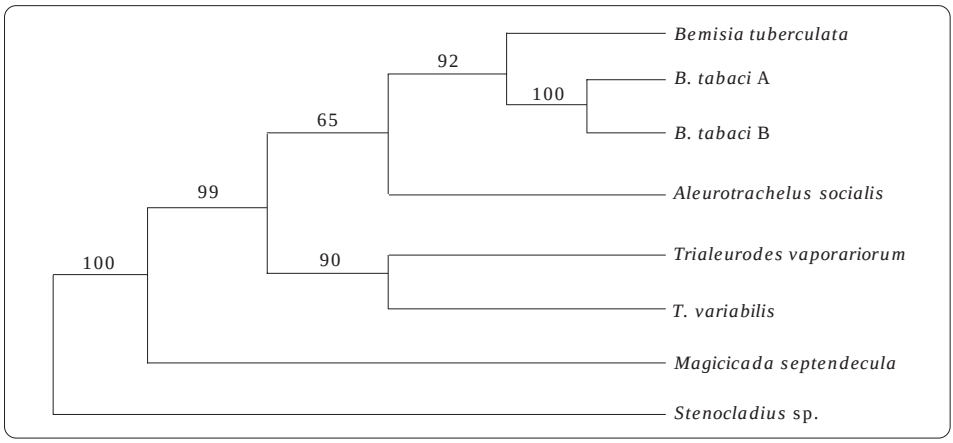

Figure 1. Cladogram showing the relationship between the whiteflies in this study. The cladogram is based on the most parsimonious tree inferred from the analysis of 485 base sites of a region of the mitochondrial 16S gene. Numbers above the branches indicate the level of statistical support for the corresponding node from 10,000 bootstrap replicates.

B were always grouped together. *B. tuberculata* was grouped with the *B. tabaci* 92% of the time and *T. vaporariorum* and *T. variabilis* were grouped together 86% of the time. Using parsimony analysis, the relationship between the three genera of *Bemisia, Trialeurodes* and *Aleurotrachelus* was not clear. The results of the distance analysis (Table 1) show that *Aleurotrachelus* was closer to the genus *Bemisia* than to *Trialeurodes*. An unexpected result was the relatively large mean distance between *T. vaporariorum* and *T. variabilis*.

Gene sequences and phylogenetic studies

The use of DNA sequences has become increasingly more important as a tool to study the evolution, populations and systematics of insects. Since the database for sequence information is expanding exponentially, it is certain that these methods will be even more important in the future. The several advantages to using DNA include the fact that the genotype, and not the phenotype, is examined directly. There is also an expanding base of phylogenetic information on an increasing number of gene sequences that exhibit different rates of change.

This study generated additional evidence that the *B. tabaci* biotype B is highly conserved throughout the Americas. This was expected because it is a recent introduction. The populations of *B. tabaci* biotype A are more conserved than we would have predicted from the studies that used esterase isozyme pattern as the means to detect diversity. Because the expression of esterases can be induced by insecticide applications, their phenotypes are probably not reliable for determining biotypes. Comparing the results of the molecular and esterase analyses, many esterase isozyme patterns are associated with the *B. tabaci* biotype A based on DNA markers.

Even though, in the phylogenetic analysis, *T. vaporariorum* and *T. variabilis* were in the same clade, the absolute distance was fairly high. Since the distance is equally great between *T. vaporariorum, T. variabilis* and the

whiteflies in the other genera, there is a question of how closely related the members of *Trialeurodes* may be. Further studies should determine if these two species are members of the same genera, or whether they should be placed into separate genera.

The application of RAPD for identification of whiteflies

Whenever an increase in whiteflies occurs or they begin to affect additional crops, the introduction of the *B. tabaci* B biotype is suspected. Often, this is the case but whitefly populations are affected by many environmental factors, including the cropping system and the varieties grown. The molecular methods that generate DNA sequence data are time and labour intensive and are not suited to large scale monitoring of populations. Rapid and reliable methods are needed to distinguish between the most common whiteflies. In Latin America, these are *B. tabaci* biotypes A and B, and *T. vaporariorum* on most crops.

Using RAPDs has several advantages. Foreknowledge about any particular gene in the target organism is not needed. More than one primer can be used to increase confidence in the reliability of the method. With whiteflies and many other types of samples, the ability to store them for many months at room temperature in 70% ethanol facilitates shipping them across international borders and allows the analysis of large numbers of samples to be processed in an orderly manner.

RAPD was used (De Barro and Driver, 1997) to distinguish indigenous Australian populations of *B. tabaci* from the introduced B biotype. The authors reported on four oligonucleotide primers that they considered the most useful for identifying the native *B. tabaci* from the B biotype.

Analysis of molecular markers to identify whiteflies in Latin America

Four oligonucleotide primers (De Barro and Driver, 1997) were tested for their utility in distinguishing *B. tabaci* biotype A and biotype B, and *T. vaporariorum*. In the analysis of PCR products using the primer H9 (Operon, USA), there were differences for the range of whitefly species tested (Figure 2). For *B. tabaci* biotype B and *T. vaporariorum*, there are prominent PCR products ca. 600 and 800 bp that can sometimes make distinguishing the two species difficult. The unique PCR product in *B. tabaci* biotype B ca. 950 bp and *T. vaporariorum*'s unique PCR product at ca. 500 bp are important for distinguishing between these two species. Although confusion can occur in the interpretation between some species, the H9 primer was most useful in distinguishing between *B. tabaci* biotypes A and B. At 600 bp, some PCR products are similar in size in both biotypes but the biotype A has several unique PCR products, including doublet bands at 300-350 bp. The

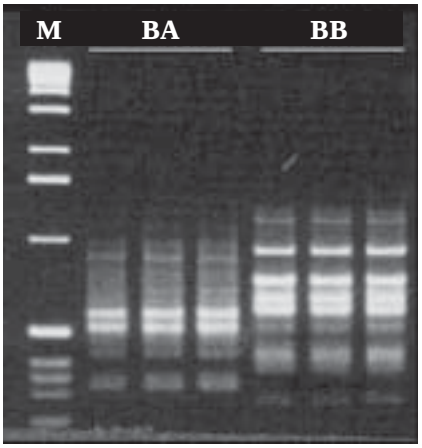

Figure 2. Using Operon primer H9, these are the RAPD-PCR DNA products from individual whiteflies. M:1kb markers (BRL), BA: *Bemisia tabaci* biotype A, BB: *Bemisia tabaci* biotype B.

biotype B has unique PCR products at ca. 600, 700 and 900 bp compared with one product of ca. 850 bp in the biotype A.

The primer H16 (Figure 2) was most useful in distinguishing between the whitefly species. While there can be some common bands in the 500 to 1000 bp range for both *B. tabaci* biotypes, three products in *B. tabaci* biotype B of ca. 350, 450 and 550 bp were consistently useful for identification of the B biotype.

When primers F2 and F12 were used, there were larger numbers of PCR products. These can be used for distinguishing the whitefly species but, because of the large numbers of bands, were generally less useful than H9 and H16. When using RAPDs for the identification of the whiteflies in this study, analysing the individual whiteflies with both the H9 and H16 primers is recommended.

Mapping the Distribution of Whitefly Species and Biotypes in Latin America

This survey was undertaken to map the distribution and range of whiteflies in various countries in Latin America. For this purpose, RAPDs were used to characterize the principal whitefly pests in many countries of Central America and the Caribbean region.

The survey using molecular markers complemented the activities where whitefly specimens were analysed by light microscopy. For the molecular analysis, at least one and often two primers were used and the results were compared to the morphological identification. The use of RAPD data was the only method to distinguish between the biotypes A and B in the *Bemisia* complex.

This survey was extensive but not exhaustive, therefore results should be interpreted as a representation of the predominate whiteflies populations in various regions of Cuba, the Dominican Republic, Guatemala, Honduras, Costa Rica, Panama, Colombia and Venezuela.

Dominican Republic

In the departments of Azua, Barahona, Peravia, Santiago, Montecristo and San Juan, the predominant whitefly was *B. tabaci* biotype B. Plants tested include eggplant (*Solanum melongena* L.), tomato (*Lycopersicon esculentum* Mill.), okra (*Abelmoschus esculentus* [L.] Moench), melon (*Cucumis melo* L.), cucumber (*Cucumis sativus* L. var. *sativus*) and hibiscus (*Hibiscus* spp. L). Only one sample of tomato in Azua was classified a biotype A. In the lowland tropics of the Dominican Republic, the biotype B was introduced nearly a decade ago and has nearly excluded the indigenous biotype A. In the department of Vega, common bean (*Phaseolus vulgaris* L.), tomato and potato (*Solanum tuberosum* L.) were tested and *T. vaporariorum* was the only whitefly found.

Guatemala

In the department of Zacapa, the predominant whitefly was *B. tabaci*. In Zapcapa, the B biotype was present on all the hosts tested, which included okra, melon, watermelon (*Citrullus lanatus* [Thunb.] Matsum. & Nakai) and cucumber. In Japala, both *B. tabaci* biotype B and *T. vaporariorum* were found. The B biotype was found on okra, melon, watermelon, cucumber, tomato, eggplant, tobacco (*Nicotiana tabacum* L.) and weed species. *T. vaporariorum* was the predominate whitefly in several host plants including paw paw (*Carica papaya* L.), "*tomate-manzana*" (*Lycopersicon esculentum* var. beef tomato), cherry tomato (*Lycopersicon esculentum* var.

cerasiforme [Dunal] A. Gray), common bean and some samples of tomato.

Honduras

In Honduras, the predominant whitefly was *B. tabaci* biotype A. Most of the samples were common bean, tomato and cucumber from the Comayagua Department. The paw paw in Comayagua was host to *T. vaporariorum*. On chilli peppers (*Capsicum* spp. L.) in the Francisco Morazan Department, the A biotype was also the only whitefly identified.

Costa Rica

In the lowland tropics of Costa Rica, *B. tabaci* biotype A is still the predominant whitefly. In the departments of Guanacaste, San José, Heredia and Alajuela, most of the samples were biotype A. The A biotype was found on common bean, melon, watermelon, tomato, chilli peppers, cucumber and others. In the departments of Arajuel and Puntarenas, the B biotype was present. It was the only whitefly found in the two samples from Arajuel but in Puntarenas both biotype A and B were present. *T. vaporariorum* was present in the Alajuela Department and was common in Cartago where it was found on chilli peppers and tomato.

El Salvador

Only a limited number of samples (15) have been tested and all were *B. tabaci* biotype B.

Panama

The principal whitefly in the Department of Chiriqui was *T. vaporariorum*. In the other regions of the country, *B. tabaci* biotype B was the predominant whitefly. Populations of *B. tabaci* biotype A are still found and in some areas the populations are mixed. This suggests a relatively recent introduction of *B. tabaci* biotype B in Panama.

Cuba

B. tabaci biotype B was the only whitefly found in the samples from Cuba. The B biotype has been in Cuba for nearly 10 years and clearly has become the predominant whitefly.

Colombia

Most of the samples tested were from the north coastal region of Colombia, where the principal whitefly is *B. tabaci* biotype B. The B biotype is affecting tomato, col (*Brassica oleracea* L. var. *capitata* L.), eggplant, melon and cassava (*Manihot esculenta* Crantz). The A biotype is still present but the introduction of the B biotype is relatively recent.

Venezuela

Samples were collected from the departments of Zamora and Jiménez, where the principal whitefly was the B biotype. In the Urdaneta Department, there was a mixture of the A and B biotypes. Only a few samples were analysed and more are needed before conclusions should be made.

A Regional View of Whitefly Populations

Most of the samples that were determined to be in the *B. tabaci* complex by morphological methods were classified as either biotype A or B (Table 2). In general, about 15% of the samples could not be amplified; only 23% of the samples from Venezuela could be amplified. This may demonstrate the importance of the proper sample handling. About 5% of the samples also had RAPD PCR products that could not be identified. These may well be either different

Table 2. The species of whitefly[a] by country (%) as determined using random amplified polymorphic DNA (RAPD) analysis.

Country	Number of samples	*B. tabaci* biotype A	*B. tabaci* biotype B	*T. vaporariorum*	NI[b]	NB[c]
Guatemala	185	8.1	60.5	26.0	5.4	0
Cuba	44	0	100.0	0	0	0
Dominican Rep.	106	13.2	81.1	0	0	5.7
Colombia	173	0.6	63.6	14.4	5.8	15.6
Venezuela	262	4.6	18.3	0	0	77.1
Costa Rica	160	61.3	6.9	12.5	3.1	16.2
Panama	198	4.6	33.3	40.9	9.1	12.1
El Salvador	15	0	100.0	0	0	0
Honduras	138	88.4	2.9	0	7.3	1.4

a. *Bemisia tabaci, Trialeurodes vaporariorum*.
b. Samples with amplified PCR products but unable to identify.
c. No products were amplified.

species or unique biotypes. This level of uncertainty did not detract from the survey and those unidentified samples are a source of potentially different whitefly populations that merit further study.

B. tabaci biotype A, *B. tabaci* biotype B and *T. vaporariorum* were the principal whiteflies found in the region. Even at the department level, a predominant whitefly usually could be identified. Occasionally, within the same region, mixed populations were present but these were normally separated on different hosts. *T. vaporariorum* was present in many regions but tends to be at higher elevations. Only in the mid-altitudes do *B. tabaci* and *T. vaporariorum* populations overlap. In some countries, including Cuba and the Dominican Republic, the exclusion of the *B. tabaci* biotype A is almost complete, at least on the principal crop species tested for this survey. The pattern in Central America is more complex. In Cuba, Guatemala, Panama and the north cost of Colombia, the process of domination of the B biotype appears to be well advanced. In Colombia, biotype B is a recent introduction (Quintero et al., 1998) and already is the predominant whitefly in the region. This survey documents the expanding range of *B. tabaci* biotype B (Polston and Anderson, 1997) and adds details to the distribution within the countries in the study. In Honduras and Costa Rica, the A biotype is still the major whitefly present. It is probably just a matter of time until the B biotype becomes the principal whitefly in those areas. Nevertheless, these are the zones that need to be monitored closely and where strategies to decrease the probability of introduction of the B biotype should be developed.

Developing a specific marker to identify whiteflies in the Bemisia *complex*

RAPDs are produced by using short oligonucleotides, generally of 10 base pairs. This allows the generation of many amplified PCR products, using just one primer. The lack of specificity of the short oligonucleotides can lead to results that are difficult to interpret because of similar size PCR products. Also, shorter oligonucleotides are even more sensitive to single base changes. RAPD markers can be converted in a sequence characterized amplified region (SCAR). This specific marker utilizes a pair of oligonucleotides generally 20-25 base in length and

amplifies a single region of a genome (Ohmori et al., 1996). This makes interpretation of the results much simpler than with RAPDs. Instead of a group of bands with a degree of variability, SCAR produces single bands in the target organism.

The SCAR for the *B. tabaci* biotype B was developed by cloning the RAPD PCR products of the primer H9. The cDNA clones were analysed and sequenced. The primers were designed and tested. One set of primers only amplified *B. tabaci* biotype B (Figure 3). This set of primers did not amplify any tested population of *B. tabaci* biotype A or *T. vaporariorum*. Further testing is needed to confirm that these primers amplify this PCR product in geographically separated populations of *B. tabaci* biotype B. Given the lack of diversity in the evolutionary studies, this SCAR is expected to be useful for rapidly identifying all populations of biotype B.

The current research is directed to developing SCARs for *B. tabaci* biotype A and *T. vaporariorum*. Each set of primers will amplify bands of different sizes. This will allow the primers to be mixed together and will distinguish between the whiteflies in a single PCR reaction. Thus more extensive surveys possible will be possible and the technique will be more consistent in all the laboratories that need the diagnostic capability to rapidly identify whitefly pests.

Conclusions

Our detailed molecular analysis is helping define both the range and the variability of *B. tabaci* biotypes A and B in Latin America. Similar studies in other regions of the world have been made and will allow a global view of the *Bemisia* complex. The molecular method of RAPDs proved essential in distinguishing between the *B. tabaci* biotype A and B but, because of the nature of the method, comparing results between laboratories is difficult. Therefore we are developing SCARs to simplify the identification of whiteflies.

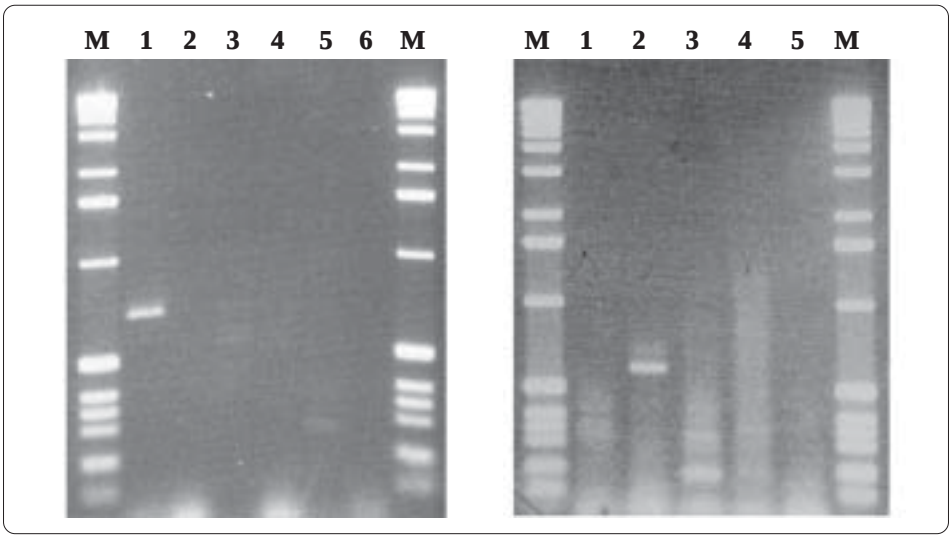

Figure 3. Two SCARs were developed to distinguish *Bemisia tabaci* biotype A (1) and *Bemisia tabaci* biotype B (2). Lane 1 *Bemisia tabaci* biotype A, lane 2 *Bemisia tabaci* biotype B, lane 3 *Bemisia tuberculata*, lane 4 *Trialeurodes vaporariorum*, lane 5 *Aleurotrachelus socialis*, 6:DNA free reaction and M:1kb markers (BRL).

Already we can identify *B. tabaci* biotype B using a SCAR, and we are making progress in the development of SCARs for *B. tabaci* biotype A and *T. vaporarorium*. Specific unambiguous results of one amplified band for each species or biotype of whitefly will simplify the interpretation of results and become a rapid and relatively inexpensive method to use in surveys of whiteflies.

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