Distribution of *Bemisia tabaci* (Hemiptera: Aleyrodidae) Biotypes in Florida-Investigating the Q Invasion

Author(s): C. L. McKenzie, Greg Hodges, Lance S. Osborne, Frank J. Byrne, and Robert G. Shatters, Jr.
Source: Journal of Economic Entomology, 102(2):670-676. 2009.
Published By: Entomological Society of America
DOI: http://dx.doi.org/10.1603/029.102.0227
URL: http://www.bioone.org/doi/full/10.1603/029.102.0227

Distribution of *Bemisia tabaci* (Hemiptera: Aleyrodidae) Biotypes in Florida–Investigating the Q Invasion

C. L. MCKENZIE,[1,2] GREG HODGES,[3] LANCE S. OSBORNE,[4] FRANK J. BYRNE,[5] AND ROBERT G. SHATTERS, JR.[1]

J. Econ. Entomol. 102(2): 670–676 (2009)

ABSTRACT After the 2004 discovery of the *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) Q biotype in the United States, there was an urgent need to determine its distribution. As part of a coordinated country-wide effort, an extensive survey of *B. tabaci* biotypes was conducted in Florida, with the cooperation of growers and state and federal agencies, to monitor the introduction and distribution of both the B and Q biotypes. The biotype status of submitted *B. tabaci* samples was determined by polymerase chain reaction (PCR) amplification and sequencing of a 700–800-bp mitochondrial cytochrome oxidase I small subunit (mtCOI) gene fragment, PCR amplification, and size determination of two unique microsatellite markers and esterase zymogram analysis. One hundred and eighty collections were sampled from 23 counties. Of these samples, 58% were from vegetables, 37% were from ornamentals, and 5% were from peanuts, alfalfa, and weeds. Eighteen percent of all collections were found to be the Q biotype that came from greenhouse grown ornamental and herbs located in six counties. Sequence comparison of the mtCOI gene identified three separate haplotypes within Florida that were defined as Q1, Q2, and Q3. Haplotypes could be used to associate populations known to be related by grower and plant type. For example, collections from five counties were made on hibiscus linked to the same grower and all samples contained only the Q1 haplotype. Other populations contained a mix of the Q2 and Q3 haplotypes, supporting the conclusion that the Q biotype must have entered Florida through at least two separate introductions. Our data also show that two microsatellite markers are a cost-effective diagnostic alternative for biotype identification with 100% concurrence with mtCOI sequence data.

KEY WORDS *Bemisia argentifolii*, silverleaf whitefly, microsatellite markers, mitochondrial cytochrome oxidase I subunit

Worldwide agricultural production losses due to infestations of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) have escalated over the past 25 yr as new and more virulent biotypes have spread to all continents except Antarctica (De Barro et al. 2005). The current count of whitefly biotypes described exceeds 20, with the two most invasive and well known being the B and Q biotypes (Perring 2001). After the introduction of biotype B into the United States ≈1985, unprecedented losses began occurring in the late 1980s in Florida (Hamon and Salguero 1987, Hoelmer et al. 1991, Schuster et al. 1989) and rapidly spread across the southern states to Texas, Arizona, and California where extreme outbreaks occurred during the early 1990s (Perring et al. 1991, 1993; Gonzalez et al. 1992). In addition to having an expanded host range and being a more aggressive colonizer of crops, other traits identified at the morphological levels (Bellows et al. 1994, Costa et al. 1995, Rosell et al. 1997), biochemical levels (Costa and Brown 1991, Perring et al. 1992, Brown et al. 2000), and molecular levels (Gawel and Bartlett 1993, De Barro et al. 2005, Boykin et al. 2007) were considered sufficiently different from the indigenous populations to warrant new species designation. (i.e., *Bemisia argentifolii* Bellows & Perring, the silverleaf whitefly) (Perring et al. 1993, Bellows et al. 1994).

Indistinguishable in appearance from silverleaf whitefly (*B. tabaci* biotype B), biotype Q is extremely problematic to agricultural production because it has a high propensity to develop resistance to insect growth regulators (Horowitz et al. 2003) and neonicotinoid insecticides (Elbert and Nauen 2000, Horowitz et al. 2004). Both classes of insecticides play crucial roles in controlling whiteflies in many different cropping systems, including cotton (Ellsworth and Martinez-Carrillo 2001), vegeta-

Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

[1] USDA–ARS, U.S. Horticultural Research Laboratory, 2001 South Rock Rd., Fort Pierce, FL 34945.

[2] Corresponding author, e-mail: cindy.mckenzie@ars.usda.gov.

[3] Division of Plant Industry, Florida Department of Agriculture and Consumer Sciences, P.O. Box 147100, Gainesville, FL 32614-7100.

[4] Mid-Florida Research & Education Center, University of Florida, 2725 Binion Rd., Apopka, FL 32703-8504.

[5] Department of Entomology, University of California, Riverside, CA 92521.

bles (Palumbo et al. 2001), and ornamentals (http://mrec.ifas.ufl.edu/lso/documents/Export%20Mgmt%20Plan-7-07.pdf). Biotype Q was first detected in the United States in December 2004 on poinsettias from a southwest retail outlet in Arizona during routine resistance monitoring surveys (Dennehy et al. 2005). Determined to be essentially unaffected by pyriproxyfen in egg bioassays, these whiteflies also had noticeably reduced susceptibility to acetamiprid, buprofezin, mixtures of fenpropathirn and acephate, imidacloprid, and thiamethoxam (Dennehy et al. 2005). Other examples of resistance in biotype Q have helped foster a reputation that biotype Q is especially capable of developing resistance under intensive insecticide use conditions (Nauen et al. 2002, Horowitz et al. 2005, Nauen and Denholm 2005). Although there is no definitive evidence that biotype Q is biologically more capable of resisting insecticides than other *B. tabaci* biotypes, its track record in both protected and open agriculture suggests that caution is advisable.

Associated with the appearance of biotype Q in the United States were reports, primarily from ornamental growers, of increasing problems in controlling whitefly infestations. After the discovery of the *B. tabaci* Q biotype in the United States, there was an urgent need to determine its spread. During the past 4 yr, biotype Q has been detected in 25 states across the country, including Florida (http://mrec.ifas.ufl.edu/LSO/BEMISIA/positive_states.htm). As part of an Animal and Plant Health Inspection Service-coordinated multistate, multiagency, and multi-institutional USA Q biotype task force initiative and coordinated whole country survey, an extensive survey of *B. tabaci* biotypes was conducted in Florida. The primary objective of the survey was to monitor the introduction and distribution of both the B and Q biotypes. Following are the results of an extensive survey of *B. tabaci* biotypes in Florida that serves to investigate and document the "Q" invasion into the state.

Materials and Methods

Whitefly Collection. The majority of the samples were provided through cooperation with the Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Glades Crop Care, University of Florida and Agricultural Research Service entomologists, and vegetable and ornamental growers from across the state. Actual sample technique depended on the individual sampler, but once adults were collected they were immediately placed in 95% ethanol for molecular analysis. *B. tabaci* are haplodiploid with 2N females and 1N males (Byrne and Devonshire 1996); therefore, female whiteflies were identified and selected for further analysis to allow microsatellite genotyping of homozygous and heterozygous individuals within populations. At least 12 adult female whiteflies were processed from each sample. If no adults were present, leaves from host plants were collected to obtain whitefly nymphs for mitochondrial cytochrome oxidase I small subunit (mtCOI) sequence analysis but not for microsatellite studies.

Methods Used to Determine Biotype. Molecular techniques used to distinguish whitefly biotypes included esterase zymogram assays (Byrne and Devonshire 1991), analysis of mtCOI DNA sequence (Frohlich et al. 1999, Shatters et al. 2009), and microsatellite fragment analysis (De Barro et al. 2003). Q biotype *Bemisia* can be distinguished from B biotype insects based on the esterase electrophoretic banding patterns (Byrne and Devonshire 1991), and this method was used to routinely confirm biotype status results by using mtCOI sequence and microsatellite data analysis. Preliminary analysis in our laboratory showed that two microsatellite markers identified by De Barro et al. (2003), BEM6 [$(CA)_8$imp] and BEM23 [$(GAA)_{31}$imp], were found to be diagnostic for B and Q biotypes and were used to determine biotype status in conjunction with analysis of mtCOI DNA sequence.

Electrophoresis of Esterase Zymograms. Individual whitefly adults were homogenized in a final volume of 20 μl of 10% sucrose prepared in a 0.1% aqueous solution of Triton X-100. To facilitate the processing of large numbers of insects, individual adults were placed in the wells of a microtiter plate, and then homogenized using a multiple homogenizer (Burkard Scientific, Uxbridge, Middlesex, United Kingdom). Fifteen microliters from each homogenate was then transferred to the wells of a 7.5% polyacrylamide gel (Byrne and Devonshire 1991). Gels were electrophoresed at 200 V for 90 min at 4°C. After electrophoresis, esterases were localized on gels by staining for 30 min with 0.5 mM 1-naphthyl butyrate prepared in 0.2% Fast Blue RR salt at pH 6.0. Esterase bands were fixed by immersing the stained gel in 7% acetic acid. B biotype *B. tabaci* were included on each gel for reference purposes.

Whitefly DNA Extraction. DNA was extracted from individual whiteflies by placing a single whitefly in a 1.5-ml Eppendorf tube, adding 50 μl of DNA lysis buffer (De Barro and Driver 1997), and grinding with a pestle. The pestle was rinsed with an additional 50 μl of DNA lysis buffer and collected in the same tube. Tubes were placed in a metal boiling rack and boiled at 95°C for 5 min and then placed directly in ice for 5 min. Tubes were then centrifuged at $8000 \times g$ for 30 s, and the supernatant (crude DNA lysate) was transferred to another tube and stored at −80°C for future processing. Aliquots from the same individual whitefly DNA extract were used for both mtCOI and microsatellite marker analysis so that both methods could be directly compared.

Mitochondrial Cytochrome Oxidase I Sequence Analysis. Polymerase chain reaction (PCR) amplifications for the mtCOI gene were performed using either the universal COI primers C1-J-2195 and L2-N-3014 (Simon et al. 1994) as originally used by Frohlich et al. (1999) for *B. tabaci* or amplified with the Btab-Uni primer set described by Shatters et al. (2009). Mitochondrial COI sequence analysis was performed first by PCR amplification of an ≈700–800 bp mtCOI DNA fragment (depending on the primer set)

Table 1. *B. tabaci* survey sites and host plants in Florida agricultural ecosystems, 2005–2008

Host plant common name[a]	Host plant scientific name[a]	Florida county (no. collection sites)
Vegetables		
Bean	*Phaseolus vulgaris* L.	Lee (1)
Bell pepper	*Capsicum annuum* L.	Collier (4), Hendry (1), Lee (2)
Cabbage	*Brassica oleracea* L. var. *capitata* L	Okeechobee (1)
Cantaloupe	*Cucumis melo* L. var. *cantalupensis* Naudin	Alachua (1), Hendry (1), Lee (1)
Collard greens	*Brassica oleracea* L. var. *Acephala* DC	Leon (2), Orange (1)
Cucumber	*Cucumis sativus* L.	Orange (1)
Eggplant	*Solanum melongena* L.	Collier (1), Hillsborough (1), Orange (1), Seminole (1)
Okra	*Abelmoschus esculentus* (L.) Moench	Dade (2)
Potato		Lee (3), Manatee (1)
Summer squash (zucchini squash)	*Cucurbita pepo* L.	Collier (3)
Sweet potato	*Ipomoea batatas* (L.) Lam.	Dade (6)
Tomato	*Lycopersicon esculentum* Mill.	Collier (29), Dade (3), Hendry (9), Hillsborough (4), Jackson (1), Lee (2), Manatee (9), Orange (2), St Lucie (4), Suwanee (1)
Watermelon	*Citrullus lanatus* (Thunb.) Matsum. & Nakai	Collier (1), Hendry (1), Lee (3)
Ornamentals and herbs		
Asparagus Fern	*Asparagus densiflorus* (Kunth) Jessop	Suwannee (1)
Azaleas	*Rhododendron* L. spp.	Manatee (1)
Desert rose	*Adenium obesum* (Forssk.) Roem. & Schult.	Orange (1)
Gerbera Daisy	*Gerbera jamesonii* Bolus ex Hook. f.	St Lucie (1)
Hibiscus	*Hibiscus mosheutos* L.	Dade (2), Hillsborough (1), Lee (18), Manatee (3), Martin (1), Orange (2), Palm Beach (1), Pasco (1), Sarasota (1), St Lucie (1)
Hydrangea	*Hydrangea* L. spp.	Suwannee (1)
Lantana	*Lantana camara* L.	Alachua (1), Dade (1), St Lucie (1), Suwannee (1)
Lisianthus	*Eustoma russellianum* (Hook.) G. Don	Manatee (1)
Mandevilla	*Mandevilla spp.*	Lee (1)
Mum	*Chrysanthemum xmorifolium* Ramat.	Lee (1)
Garden nasturtium	*Tropaeolum majus* L.	Marion (1)
Poinsettia	*Euphorbia pulcherrima* Willd. ex Klotzsch	Alachua (1), Citrus (1), Collier (1), Dade (1), Lee (2), Okeechobee (1), Orange (3), Suwannee (2)
Sage	*Salvia* L. spp.	Orange (1)
Zinnia	*Zinnia violacea* Cav.	Gadsden (2), Suwannee (1)
Basil	*Ocimum basilicum* L.	Hendry (1), Suwannee (2)
Mint	*Mentha* L. spp.	Orange (1), Suwannee (2)
Spearmint	*Mentha spicata* L.	Suwannee (1)
Miscellaneous hosts		
Peanut	*Arachis hypogaea* L.	Sumpter (1)
Alfalfa	*Medicago sativa*	St. Lucie (1)
Sow-thistle	*Sonchus oleraceus* L.	Dade (1)
Ruellia	*Ruellia strepens*	Dade (1)
Empty field with weeds or roadside sample	N.A.	Dade (5)

N.A., not applicable.
[a] Host plant common and scientific names according to Brako et al. (1995).

and then sequencing the PCR-amplified DNA. The 30-μl PCR reactions were run using the conditions described by Shatters et al. (2009) and a PTC-200 Peltier thermal cycler (MJ Research, Watertown, MA). Conditions were the same for both primer sets with the exception of the annealing temperatures. Before sequencing, the amplified products were cleaned using Montage PCR cleanup filters (Millipore, Billerica, MA). Fifty nanograms of total whitefly genomic DNA was used in BigDye sequencing reactions. All sequencing was performed bidirectionally with the amplification primers and BigDye Terminator cycle sequencing kits (Applied Biosystems, Foster City, CA). Sequence reactions were analyzed on an Applied Biosystems 3730XL DNA sequence analyzer and were then compared and edited using Sequencher software (Gene Codes, Ann Arbor, MI). Biotype determination was based on direct sequence comparisons using the web-based National Center for Biotechnology Information BLAST sequence comparison application (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and when necessary by inference from neighbor-joining methods of phylogenetic analysis of the mtCOI sequences by using CLUSTALW alignments of each sequence type (Boykin et al. 2007).

Microsatellite Marker Analysis. PCR amplifications for microsatellite primers developed by De Barro et al. (2003), BEM6 [$(CA)_8$imp], and BEM23 [$(GAA)_{31}$imp] were conducted in 13-μl reactions composed of 6.5 μl of Immomix (catalog no. BIO-25019, Bioline, Taunton, MA), 5.0 μl of sterile water, 0.25 μl of forward primer (10 pmol), 0.25 μl of reverse primer (10 pmol), and 1 μl of DNA template. The forward primer was labeled with the fluorescein derivative 5-carboxyfluorescein (FAM) (MWG Biotech, High Point, NC) for microsatellite scoring, and the 13 μl of PCR reactions were run with the following thermal regime of 94°C for 7 min followed by 35 cycles of 1 min at 94°C (denaturation), 1 min at 47°C

(annealing), 1 min at 73°C (extension), and a final step of 72°C for 1 h. One microliter of the FAM-labeled PCR product was added to a mix of 13.75 μl of formamide (code size K295-100 ml, Amresco, Solon, OH) and 0.25 μl of GENESCAN 500 ROX Size Standard (part no. 401734) and loaded onto an Applied Biosystems 3730XL DNA analyzer. Whiteflies were genotyped and their amplicons were sized and characterized using GENEMAPPER 4.0 (Applied Biosystems).

Results and Discussion

Extensive whitefly surveys were conducted from 2005 to 2008 from multiple locations across Florida representing 26 different counties and 34 different host plants (Table 1). Whiteflies sampled from three counties (Brevard, Lake, and Monroe) were determined not to be *B. tabaci* and are not included in this survey. Sample hosts were split between ornamental and herb (37%) and vegetable (58%) commodities but also included alfalfa, peanut, and some weeds (Table 1). The same crops were surveyed across multiple locations, when possible, and many counties were sampled multiple times. Tomato was the most extensively sampled host, with collections from 10 counties representing 35% of all the samples collected. Hibiscus was also sampled from 10 counties but not as extensively as tomato (31 samples or 17% of all samples collected).

The biotype status of submitted *B. tabaci* samples was determined using a mtCOI small subunit sequence, unique microsatellite markers, and esterase zymogram analysis. In total, 2,372 individual whiteflies from 180 different collections were analyzed by mtCOI and microsatellite markers. Of those individuals, 1,944 (82%) individuals from 168 collections were biotype B and 428 (18%) individuals from 32 collections were biotype Q. When biotype Q was detected, 34% of the samples were from collections containing a mix of both biotypes. Biotype B was detected in 23 counties and on all hosts sampled except hydrangea; however, hydrangea was only sampled on one occasion. Biotype Q was detected in six counties, all of which were on ornamentals and herbs in greenhouses (Table 2). Some counties had more than one positive Q sample, but in no case, did the Q biotype continue to spread and all populations were managed with no new finds since summer 2006. Samples were routinely split and sent to California for esterase zymogram analysis. In all cases, esterase comparisons concurred with mtCOI and microsatellite results.

Sequence comparison of the mtCOI gene identified three separate haplotypes for biotype Q within Florida that were defined as Q1, Q2, and Q3 (Fig. 1; Table 3). There were 38 single-nucleotide polymorphisms between biotype B and the biotype Q haplotype Q1 (the biotype Q haplotype most similar to biotype B). Among the biotype Q haplotypes, Q2 and Q3 were the most similar with only a single G/C polymorphism at position 109. The Q1 haplotype was the most divergent with six and seven polymorphisms between Q2 and Q3, respectively. In contrast, all biotype B whitefly individuals analyzed from Florida had identical mtCOI sequence in the region amplified and sequenced. Within the Q biotype, haplotypes could be used to associate populations known to be related by plant host and plant source (Table 2). For example, collections from five counties were made on hibiscus linked to the same grower and all samples contained only the Q1 haplotype. Biotype Q was detected on five different host plants in Suwannee County and these populations contained a mix of the Q2 and Q3 haplotypes. Four of the five plant hosts were all located at the same nursery and the other plant host (mint) was being grown within a two mile radius of this nursery. These data support the conclusion that the Q biotype must have entered Florida through at least two separate introductions.

Table 2. Biotype Q detection in Florida counties by crop, haplotypes, and GenBank accession numbers

County	Crop	Haplotype	GenBank accession no.
Dade	Hibiscus	Q^1	FJ188582
Hillsborough	Hibiscus	Q^1	FJ188483
Lee	Hibiscus	Q^1	FJ188480, FJ188481, FJ188482, FJ188541, FJ188543, FJ188558, FJ188559, FJ188569, FJ188597, FJ188614, FJ188617, FJ188618, FJ188619, FJ188620, FJ188622, FJ188623, FJ188686
Manatee	Hibiscus	Q^1	FJ188539
Orange	Hibiscus	Qv^1	FJ188567, FJ188600, FJ188628, FJ188629
Suwannee	Asparagus fern	Q^2	FJ188573
		Q^3	FJ188572
	Hydrangea	Q^2	FJ188524
	Mint	Q^2	FJ188508
		Q^3	FJ188507
	Poinsettia	Q^2	FJ188574, FJ188553
		Q^3	FJ188552
	Zinnia	Q^2	FJ188571
		Q^3	FJ188570

Our data also show that two microsatellite markers are a cost-effective diagnostic alternative for biotype B and Q identification, providing 100% concurrence with mtCOI sequence data (Table 4). The two markers must be used simultaneously and provide confirmatory results. When comparing microsatellite markers (indicated in Table 4 as size in bases of the fragment that was amplified), all populations containing the Q1 mitochondrial COI haplotype had only two BEM6 markers, 210 and 217, and three BEM23 markers, 407, 410, and 224, with >98% of the alleles being 210 and 407 for the BEM6 and BEM23 microsatellites, respectively. The Q2 population had only the 210 BEM6 marker and a 410 BEM 23 marker, whereas the Q3 had a unique single 204 marker for BEM6, and 410, 407, and 230 markers for BEM 23. The BEM23 marker distinguished the Q1 from the Q2 and Q3 populations and within Q2 and Q3, BEM6 was diagnostic. It was surprising to find distinctions among the nuclear microsatellite markers between Q2 and Q3 because they were often collected in the same populations. This

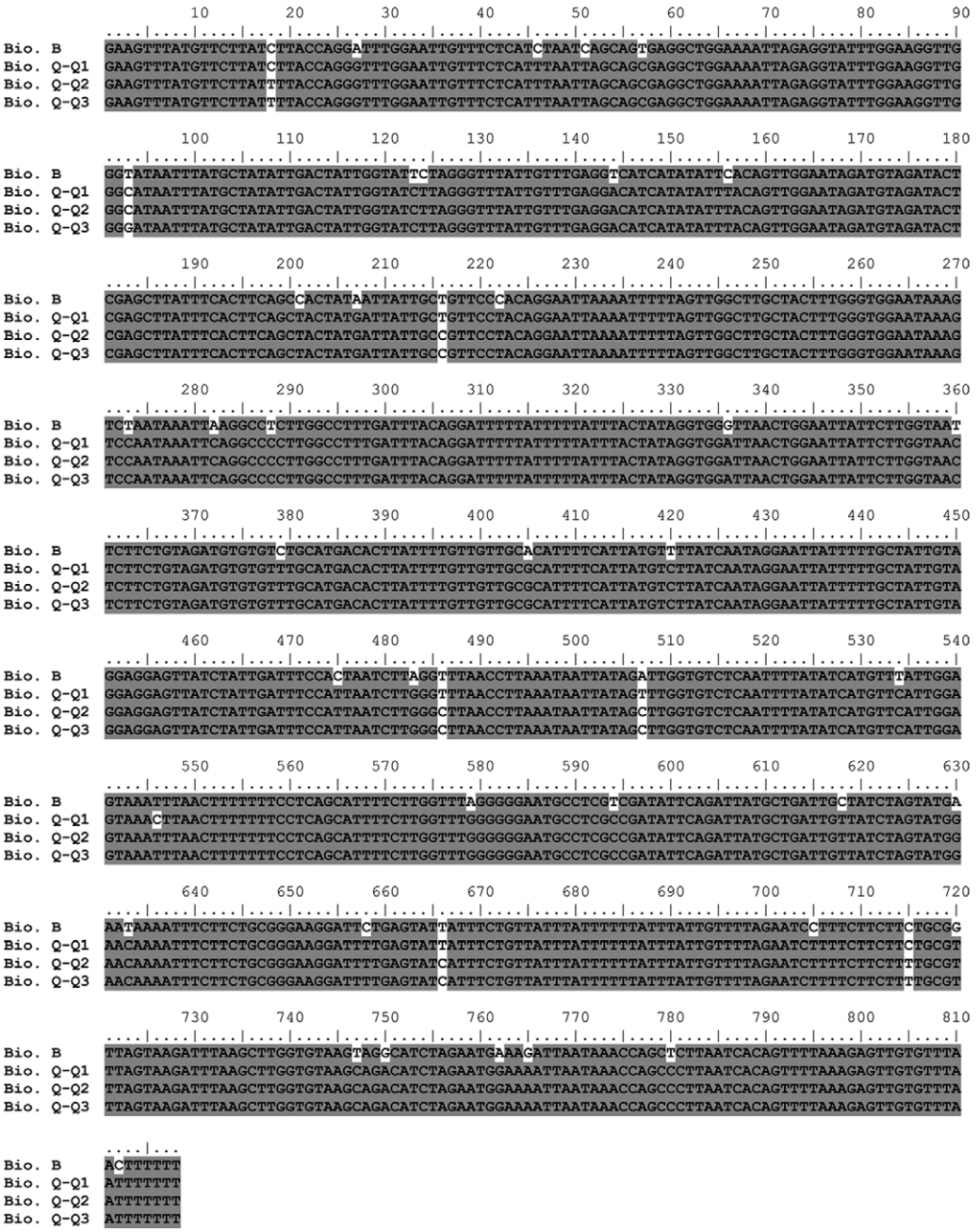

Fig. 1. ClustalW alignment of *B. tabaci* biotype B and three haplotypes of biotype Q mtCOI PCR fragment indicating polymorphisms.

Table 3. Polymorphisms between three Q haplotypes collected in Florida

Haplotype designation	Base no. on the amplified mtCOI fragment[a]						
	109	232	502	523	562	682	731
Q^1	C	T	T	T	C	T	C
Q^2	C	C	C	C	T	C	T
Q^3	G	C	C	C	T	C	T

[a] GenBank accession EU427719.

suggests that they may not have existed together for a long period and only had limited opportunity to interbreed. Comparison of the B and Q microsatellite markers showed no sharing of markers between the two biotypes, even when B and Q whiteflies were collected from the same host plant. Because the microsatellite markers were imperfect, we were able to sequence the products and determine exactly where the addition or deletion occurred relative to the im-

Table 4. *B. tabaci* biotype mtCOI haplotypes correlated to diagnostic microsatellite primers BEM6 and BEM23 in Florida

mtCOI	Size in bases of the amplified fragment (%)	
Haplotype	BEM6 $(CA)_8$ imp	BEM23 $(GAA)_{31}$ imp
Q^1	210 (99), 217 (1)	224 (1), 407 (98), 410 (1)
Q^2	210	410
Q^3	204	230 (5), 407 (2), 410 (93)
Biotype B	217 (98), 224 (2)	224

perfection. Although alleles seemed to be shared (same size amplicon) 1% of the time between biotype B and haplotype Q1, in all cases, these additions/deletions were the result of independent events (they occurred in different places relative to the imperfection). Consequently, we could find no evidence of hybridization between the two whitefly biotypes in Florida.

Earlier surveys of *B. tabaci* populations in Florida (McKenzie et al. 2004) by using random amplification of polymorphic DNA PCR techniques indicated the presence of only the B biotype of *B. tabaci.* However, in that study, herbs and ornamental hosts were not surveyed. In this survey, 17 herb and ornamental hosts were surveyed from 18 counties, with biotype Q being detected on five different ornamental hosts and one herb (Table 2). There was great concern among growers and researchers alike that biotype Q would make the jump from protected ornamental greenhouse production to open agriculture (Dalton 2006). In Florida, tomato transplants for field production can be grown in the same greenhouses that grow a variety of ornamental plants, so there were opportunities for biotype Q to infest tomato transplants destined for the field. We surveyed 13 preferred whitefly field-grown vegetable hosts in 14 counties and did not detect biotype Q in any of the samples. In fact, the last biotype Q detection was in August 2006 on hibiscus and sample submission from growers experiencing control problems has drastically declined. The reduced number of submissions for whitefly biotype determination during the past 2 yr may be an indication that growers took note of the extent of the problem and were diligent in their efforts to implement best management practices for the control of this pest. A Management Program for Whiteflies on Propagated Ornamentals with an Emphasis on the Q-Biotype was developed in 2006 (Bethke et al. 2006) and continues to be distributed to >10,000 ornamental growers and propagators (http://www.mrec.ifas.ufl.edu/LSO/bemisia/bemisia.htm).

In-depth analysis of insecticide resistance profiles of different biotype Q populations indicates that different populations have different insecticide resistance profiles (Nauen et al. 2002, Dennehy et al. 2005, Horowitz et al. 2005, Nauen and Denholm 2005); therefore, the ability to identify the Q haplotype is of practical importance to growers. However, currently published taxonomic comparisons of the *B. tabaci* populations worldwide are not sensitive enough to allow statistically supported distinctions of unique Q biotype classes (Boykin et al. 2007). Using a genotyping method referred to as microsatellite analysis or simple sequence repeats to characterize individuals from Q biotype infestations throughout Florida, in combination with mtCOI markers, we have been able to identify three distinct haplotypes of the Q biotype. The Q biotype has much greater mtCOI and microsatellite diversity than observed for the B biotype in the United States. The genetic diversity of the Q biotype is similar to that reported for the indigenous Asia–Pacific genotypes (De Barro 2005). The mtCOI and microsatellite results show that these are powerful genotyping methods that could be used to provide information that will improve management decision making with respect to pesticide applications. Future work coordinating the mtCOI and microsatellite genotyping with insecticide resistance profiles will be conducted to determine whether these genotyping methods can be used as a predictor of insecticide resistance profiles. Furthermore, the use of these molecular tools will allow investigators to track the likely origin(s) of whitefly biotypes allowing for the implementation of management efforts against the insects before they arrive on U.S. shores.

Nucleotide Sequence Accession Numbers. The GenBank accession numbers for the mtCOI fragment amplified from biotype B and Q whiteflies from 23 counties and various host plants across Florida are FJ188480 through FJ188501, FJ188503, FJ188505 through FJ188609, FJ188611 through FJ188642, FJ188644 through FJ188678, and FJ188680 through FJ188687. Biotype Q specific accession numbers also are listed in Table 2.

Acknowledgments

We thank Dave Schuster; Jim Price; Gary Leibee; Phil Stansly; Glades Crop Care; KAC Agricultural Research, Inc.; Epcot Science; Valent USA Corporation; Farm-OP, Inc.; and Division of Plant Industry staff for whitefly submissions for identification. We also thank John Prokop, Laurie Keidong, and Ankush Zutshi (USDA–ARS, U.S. Horticultural Research Laboratory, Fort Pierce, FL) for dedicated technical laboratory assistance. This research was partially funded by the Ornamental Research Initiative.

References Cited

Bellows, T. S., Jr., T. M. Perring, R. J. Gill, and D. H. Headrich. 1994. Description of a species of *Bemisia* (Homoptera: Aleyrodidae). Ann. Entomol. Soc. Am. 76: 310–313.

Bethke, J., L. Canas, J. Chamberlin, R. Cloyd, J. Dobbs, R. Fletcher, D. Fujino, D. Gilrein, R. Lindquist, S. Ludwig, C. McKenzie, R. Oetting, L. Osborne, C. Palmer, and J. Sanderson. 2006. Management Program for Whiteflies on Propagated Ornamentals with an Emphasis on the Q-Biotype. (http://www.mrec.ifas.ufl.edu/LSO/bemisia/bemisia.htm).

Boykin, L. M., R. G. Shatters, Jr., R. C. Rosell, C. L. McKenzie, R. A. Bagnall, P. De Barro, and D. R. Frohlich. 2007. Global relationships of *Bemisia tabaci* (Hemiptera: Aleyrodidae) revealed using Bayesian analysis of mitochondrial COI DNA sequences. Mol. Phylogenet. Evol. 44: 1306–1319.

Brako, L., A. Y. Rossman, and D. F. Farr. 1995. Scientific and common names of 7,000 vascular plants in the United States. APS Press, St. Paul, MN.

Brown, J. K., T. M. Perring, A. D. Cooper, I. D. Bedford, and P. G. Markham. 2000. Genetic analysis of *Bemisia tabaci* (Hemiptera: Aleyrodidae) populations by isoelectric focusing electrophoresis. Biochem. Genet. 38: 13–25.

Byrne, F. J., and A. L. Devonshire. 1991. *In vivo* inhibition of esterase and acetylcholinesterase activities by profenofos treatments in the tobacco whitefly *Bemisia tabaci* (Genn.): implications for the routine biochemical monitoring of these enzymes. Pestic. Biochem. Physiol. 40: 198–204.

Byrne, F. J., and A. L. Devonshire. 1996. Biochemical evidence of haplodiploidy in the whitefly *Bemisia tabaci.* Biochem. Genet. 34: 93–107.

Costa, H. S., and J. K. Brown. 1991. Variation in biological characteristics and esterase patterns among populations of *Bemisia tabaci,* and the association of one population with silverleaf symptom induction. Entomol. Exp. Appl. 61: 211–219.

Costa, H. S., D. M. Westcot, D. E. Ullman, R. Rosell, J. K. Brown, and M. W. Johnson. 1995. Morphological variation in *Bemisia* endosymbionts. Protoplasma 189: 194–202.

Dalton, R. 2006. The Christmas invasion. Nature (Lond.) 443: 898–900.

De Barro, P. J., and F. Driver. 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.

De Barro, P. J., K. D. Scott, G. C. Graham, C. L. Lange, and M. K. Schutze. 2003. Isolation and characterization of microsatellite loci in *Bemisia tabaci.* Mol. Ecol. Notes 3: 40–43.

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

De Barro, P. J., J.W.H. Rueman, and D. R. Frohlich. 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.

Dennehy, T. J., B. A. Degain, V. S. Harpold, J. K. Brown, S. Morin, J. A. Fabrick, F. J. Byrne, and R. L. Nichols. 2005. New challenges to management of whitefly resistance to insecticides in Arizona. The University of Arizona-Cooperative Extension Report, Tucson, AZ.

Elbert, A., and R. Nauen. 2000. Resistance of *Bemisia tabaci* (Homoptera: Aleyrodidae) to insecticides in southern Spain with special reference to neonicotinoids. Pest Manag. Sci. 56: 60–64.

Ellsworth, P. C., and J. L. Martinez-Carrillo. 2001. IPM for *Bemisia tabaci:* a case study from North America. Crop Prot. 20: 853–869.

Frohlich, D., I. Torres-Jerez, I. D. Bedford, P. G. Markham, and J. K. Brown. 1999. A phylogeographic analysis of the *Bemisia tabaci* species complex based on mitochondrial DNA markers. Mol. Ecol. 8: 1593–1602.

Gawel, N. J., and A. C. Bartlett. 1993. Characterization of differences between whiteflies using RAPD-PCR. Insect Mol. Biol. 2: 33–38.

Gonzalez, R. A., J. I. Grieshop, G. E. Goldman, S. R. Sutter, E. T. Natwick, T. Funakoshi, H. R. Rosenberg, and S. Davila-Garcia. 1992. Whitefly invasion in Imperial Valley costs growers, workers millions in losses. Calif Agric. 46: 7–8.

Hamon, A. B., and V. Salguero. 1987. *Bemisia tabaci,* sweetpotato whitefly, in Florida (Homoptera: Aleyrodidae: Aleyrodinae). Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Entomology Circular 292.

Hoelmer, K. A., L. S. Osborne, and R. K. Yokomi. 1991. Foliage disorders in Florida associated with feeding by sweetpotato whitefly, *Bemisia tabaci.* Fla. Entomol. 74: 162–166.

Horowitz, A. R., K. Gorman, G. Ross, and I. Denholm. 2003. Inheritance of pyriproxyfen resistance in the whitefly, *Bemisia tabaci* (Q biotype). Arch. Insect Biochem. Physiol. 54: 177–186.

Horowitz, A. R., S. Kontsedalov, and I. Ishaaya. 2004. Dynamics of resistance to the neonicotinoids acetamiprid and thiamethoxam in *Bemisia tabaci* (Homoptera: Aleyrodidae). J. Econ. Entomol. 97: 2051–2056.

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

McKenzie, C. L., P. K. Anderson, and N. Villreal. 2004. Survey of *Bemisia tabaci* Homoptera: Aleyrodidae) in agricultural ecosystems in Florida. Fla. Entomol. 87: 403–407.

Nauen, R., N. Stumpf, and A. Elbert. 2002. Toxicological and mechanistic studies on neonicotinoid cross resistance in Q-type *Bemisia tabaci* (Hemiptera: Aleyrodidae). Pest Manag. Sci. 58: 868–875.

Nauen, R., and I. Denholm. 2005. Resistance of insect pests to neonicotinoid insecticides: current status and future prospects. Arch. Insect Biochem. Physiol. 58: 200–215.

Palumbo, J., A. R. Horowitz, and N. Prabhaker. 2001. Insecticidal control and resistance management for *Bemisia tabaci.* Crop Prot. 20: 739–765.

Perring, T. M. 2001. The *Bemisia tabaci* species complex. Crop Prot. 20: 725–737.

Perring, T. M., A. Cooper, D. J. Kazmer, C. Shields, and J. Shields. 1991. New strain of sweetpotato whitefly invades California vegetables. Calif Agric. 45: 10–12.

Perring, T. M., A. Cooper, and D. J. Kazmer. 1992. Identification of the poinsettia strain of *Bemisia tabaci* (Homoptera: Aleyrodidae) on broccoli by electrophoresis. J. Econ. Entomol. 85: 1278–1285.

Perring, T. M., A. D. Cooper, R. J. Russell, C. A. Farrar, and T. S. Bellows, Jr. 1993. Identification of a whitefly species by genomic and behavioral studies. Science (Wash., D.C.) 259: 74–77.

Rosell, R. C., I. D. Bedford, D. R. Frohlich, R. J. Gill, P. G. Markham, and J. K. Brown. 1997. Analyses of morphological variation in distinct populations of *Bemisia tabaci.* Ann. Entomol. Soc. Am. 90: 575–589.

Schuster, D. J., J. F. Price, J. B. Kring, and P. H. Everett. 1989. Integrated management of the sweetpotato whitefly on commercial tomato. Univ. Fla. IFAS, Bradenton GCREC Res. Rep. BRA1989-12.

Shatters, Jr., R. G., C. A. Powell, L. M. Boykin, H. Liansheng, and C. L. McKenzie. 2009. Improved DNA barcoding method for *Bemisia tabaci* and related Aleyrodidae: development of universal and *Bemisa tabaci* biotype-specific mitochondrial cytochrome c oxidase I polymerase chain reaction primers. J. Econ. Entomol. 102: 750–758.

Simon, C., F. Frati, A. Beckenbach, B. Crespi, H. Liu, and P. Flook. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Entomol. Soc. Am. 87: 651–701.

Received 17 September 2008; accepted 17 December 2008.