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THE INTRODUCTION OF THE EXOTIC Q BIOTYPE OF *BEMISIA TABACI* FROM THE MEDITERRANEAN REGION INTO CHINA ON ORNAMENTAL CROPS

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ABSTRACT

The Q biotype of *Bemisia tabaci* (Gennadius), which has been described from the Mediterranean/North African region, was identified for the first time infesting ornamental crop species in several locations in China. Identification and partial distributions of the exotic B biotype and the recently introduced Q biotype in China were established by using the mitochondrial cytochrome oxidase I gene (mtCOI) as a molecular marker. Collections of *B. tabaci* were made from representative geographical locations and plant hosts in different provinces of China. MtCOI sequence analysis revealed that collections from Beijing [AY582872, AY589499], Yunnan [AY518189, AY587516], and Henan [AY587514] shared >99.6% sequence identity with the Q biotype from Spain [AY587513, AY562216, AY596950]. The Q type from China shared 98.9-99.4% nucleotide sequence identity with Q-like relatives of *B. tabaci* described from Israel [AY518191, AY582869]. Phylogenetic analyses indicated that certain *B. tabaci* populations that are present in China are the Q biotype, and that the Q biotype now in China may have originated from Spain or other nearby locations where the Q biotype has been identified. This is the first report of the introduction of the Q biotype from the Mediterranean region into China. The specific outcomes of the Q biotype as an invasive species in Asia are presently unknown. Certain Q biotype populations from Spain have been reported to exhibit resistant to neonicotinoid insecticides, which are commonly used for controlling this pest and virus vector in ornamental and field crops. Thus, the close monitoring of the Q biotype in China and elsewhere, particularly where commercial plants are grown for export or received for importation, respectively, is essential to avoid the further geographical expansion of the habitat of the Q biotype.

Key Words: *Bemisia tabaci* biotype Q, geographical origin, introduced species, mitochondrial COI gene, phylogenetic analysis

RESUMEN

El biotipo Q de *Bemisia tabaci* (Genn.), el cual fue descrito de la región Mediterráneo/Norte de África, fue identificado por primera vez infestando especies de cultivos ornamentales en varios lugares de China. La identificación y la distribución parcial del exótico biotipo B y el recién introducido biotipo Q en China fueron determinados usando el gen citocromo oxidase I mitocondrial (mtCOI) como un marcador molecular. Se realizaron colecciones de *Bemisia tabaci* de lugares geográficos representativos y hospederos de plantas en diferentes provincias de China. El análisis de la secuencia de mtCOI reveló que las colecciones de Beijing [AY582872, AY589499], Yunnan [AY518189, AY587516] y Henan [AY587514] compartieron >99.6% de la identidad de la secuencia con el biotipo Q de España. [AY587513, AY562216, AY596950]. El tipo Q de China compartieron 98.9-99.4% de la identidad de la secuencia de nucleótidos con los relacionados de clase como del tipo Q de *B. tabaci* descritos de Israel [AY518191, AY582869]. El análisis filogenético indica que ciertas poblaciones de *B. tabaci* que están presentes en China son de biotipo Q biotipo, y el biotipo Q que ahora está presente en China puede haberse originado en España u otros lugares cercanos donde el biotipo Q ha sido identificado. Este es el primer informe de la introducción de biotipo Q de la región Mediterránea a China. Los resultados específicos de biotipo Q como una especie invasora en Asia son en estos momentos desconocidos. Ciertas poblaciones del biotipo Q de España han sido reportadas que muestran resistencia a los insecticidas neonicotinoides que se usa regularmente para controlar esta plaga y vector de virus en cultivos ornamentales y de campo. Por esto, es esencial realizar un monitoreo extensivo del biotipo Q en China y en otros lugares, particularmente donde se siembra plantas comerciales para exportación o recibidas para importación para evitar una mayor expansión geográfica del habitat del biotipo Q.

The *Bemisia tabaci* (Genn.) complex (Brown et al. 1995b) is a hemipteran (Aleyrodidae) pest that feeds on plant phloem. It also is the most important vector worldwide of several genera of plant virus (Brown 2000, 2001; Brown & Bird 1992).

Bemisia tabaci is best described as a species complex that comprises an unexpectedly large number of genetically variable populations, some of which are discernible owing to distinct phenotypes (Brown et al. 1995a,b). Well-studied *B. tabaci* populations that have been differentiated are referred to as races (Brown & Bird 1992) or biotypes (Brown et al. 1995a; Costa & Brown 1991). The B biotype (Costa & Brown 1991) is a particularly aggressive *B. tabaci* variant. It has an extremely broad host range, is highly fecund, and disperses relatively long distances (Brown 2000; Brown et al. 1995b), and has become established in many locations beginning approximately in 1988-present (Costa & Brown 1991; Costa et al. 1993). Since that time, it has been of considerable concern as a pest and virus vector in subtropical and temperate, mild climate zones where the majority of the world's vegetable and fiber crops are produced (Brown et al. 1995a,b; Brown 2000). Population genetics studies have shown that the B biotype probably originated from the Middle Eastern/North African region (Frohlich et al. 1999).

In China *B. tabaci* has become an important agricultural pest in the late 1990s (Chu et al. 2004; Zhang 2000), and the introduction of the B biotype into China was first reported in 2002 (Luo et al. 2002). The B biotype is now known to be widespread in a number of provinces in China where vegetables, cotton, and ornamentals are produced (Chu et al. 2004; Wu et al. 2003; this report).

In China and elsewhere, the understanding that *B. tabaci* is a polymorphic, cryptic species that can upsurge without warning and cause great damage to crop and ornamental species is lacking. This has often resulted in the delayed recognition of upsurges in local whitefly populations and/or of exotic introductions (Reitz & Trumble 2002), such as the B biotype. This realization has prompted an accelerated interest in practicing routine monitoring of *B. tabaci* populations to detect early the potentially invasive *B. tabaci*, or otherwise upsurgent haplotypes, with particular emphasis on those that are associated with the global commercial plant industry.

The purpose of this study was to determine if the Q biotype (Costa et al. 1993; Guirao et al. 1997) was present in ornamentals and/or annual flowering or bedding plants in China. Such knowledge is important because the Q biotype has only recently been recognized as a potentially invasive pest species in the vegetable and ornamentals industries in the Mediterranean/Middle Eastern region (Guirao et al. 1997; Horowitz et al. 2003). The introduction and establishment of the Q biotype is

anticipated, or was possibly expected to already have occurred, in at least certain locations, and is expected to have important and far-reaching economic relevance. The potential for damage will further be exacerbated if Q biotype populations exhibit resistance to a well-known neonicotinoid (Nauen et al. 2002; Rauche & Nauen 2003), upon which the industry presently relies to control *B. tabaci*. Resistance to this compound has already been reported in Spain and Israel (Ebert & Nauen 2000; Rauche & Nauen 2003).

Recent studies have demonstrated that the mitochondrial cytochrome oxidase I (mtCOI) gene (Brown 2001; Brown et al. 1995b; Frohlich et al. 1999) is a highly informative coding sequence for differentiating populations and haplotypes/biotypes in the *B. tabaci* complex. In this study, the mtCOI was used as a population genetics marker to detect the presence of and identify the Q biotype, and subsequently to determine its partial distribution in a number of provinces in China, which routinely produce ornamental and bedding plants. Phylogenetic analysis of the mitochondrial COI sequence for *B. tabaci* collections from China revealed for the first time that the Q biotype is distributed in multiple locations throughout the country.

MATERIALS AND METHODS

Whitefly Collections

Adult whiteflies (*B. tabaci*) were collected live and placed into tubes containing 95% ethanol. Populations were collected from representative locations and plant species throughout select provinces of China (Table 1).

Whiteflies DNA Extraction, the Polymerase Chain Reaction, and Sequencing

Individual whiteflies were subjected to lysis and DNA extraction following the procedure of Frohlich et al. (1999). Polymerase chain reaction (PCR) (Saiki et al. 1988) primers were employed to amplify a fragment of the *B. tabaci* mitochondrial COI gene (800-820 bp), using parameters and PCR primers, as described by Frohlich et al. (1999).

PCR assays were conducted with 2 μ L of each template DNA in a total reaction volume of 25 μ L. The PCR reaction mix and PCR conditions followed Frohlich et al. (1999) with a little modification, and 1 unit of Taq DNA polymerase was contained in the PCR reaction mix. PCR products were separated on 1.0% agarose gels, and bands were visualized by ethidium bromide staining and viewed with a UV light source. PCR products were purified with a kit (EZ Spin Column DNA Gel Extraction Kit purchased from Sangon Technology Company, Shanghai) according to the man-

TABLE 1. BIOTYPE, GEOGRAPHICAL SOURCE, HOST PLANT, AND GENBANK ACCESSION NUMBER FOR *B.TABACI* MITOCHONDRIAL CYTOCHROME OXIDASE DNA (MTDNA COI) SEQUENCES.

Geographical location and year	Host plant	GenBank Accession number	Acronym	Whitefly haplotype or biotype
Zhengzhou 2003	<i>Brassica oleracea</i> L.	AY582870 AY518186	HeNanBoleAY582870	B
Zhengzhou 2003	<i>Gossypium hirsutum</i> L.	AY587515	Zhengzhou	B
Zhengzhou 2003	<i>Cucurbita moschata</i> L.	AY596949	Zhengzhou	B
Zhengzhou 2003	<i>Brassica oleracea</i> L. <i>var. capitata</i> L.	AY582873	Zhengzhou	B
Zhengzhou 2003	<i>Ipomoea batatas</i> L.	AY589497	Zhengzhou	B
Zhengzhou 2 003	<i>Solanun melongena</i> L.	AY587514	HeNanSmelAY587514	Q
Beijing 2003	<i>Cucumis sativus</i> L.	AY587519	BJCsatAY587519	B
Beijing 2003	Unknown	AY582867	BJAY582867	B
Beijing 2003	<i>Capsicum annuum</i> L.	AY596953 AY582871	BJ	B
Beijing 2003	<i>Cucumis sativus</i> L.	AY589498	BJ	B
Beijing 2003	<i>Heliantus annuus</i> L.	AY582872 AY589499	BJInilAY582872	Q
Zaozhuang, Shandong 2003	<i>Cucumis sativus</i> L.	AY587518	SDZZAY587518	B
Taian, Shandong 2003	Unknown	AY587517	SDTAAAY587517	B
Nanjing, Jiangsu 2003	<i>Gossypium hirsutum</i> L.	AY518185	JSNJAY518185	B
Zhejiang 20003	Unknown	AY566182	ZJAY566182	B
Zhejiang 2003	Unknown	AY596952	ZJNBAY596952	(non-B/Q) Asian clade
Shanghai 2003	<i>Euphorbia pulcherrima</i> Willd.	AY550274	ShHaiEpulAY550274	B
Shanghai 2003	<i>Brassica albo-glabra</i> Bail.	AY550273	Shanghai	B
Kunming, Yunnan 2003	<i>Euphorbia pulcherrima</i> Willd.	AY587516 AY518189	YN2AY587516 YNAY518189	Q
Haikou, Hainan 2003	<i>Solanun melongena</i> L.	AY518187	HaiNAY518187	B
Tulufan, Xinjiang 2003	<i>Euphorbia pulcherrima</i> Willd.	AY582868	XJAY582868	B
Israel 2003	<i>Gossypium hirsutum</i> L.	AY518190	IBAY518190	B
Israel 2003	<i>Gossypium hirsutum</i> L.	AY518191 AY582869	IQAY518191	Q-like
Spain 2003	<i>Solanum lycopersicum</i> L.	AY596951	SB AY596951	B
Spain 2003	<i>Solanum lycopersicum</i> L.	AY596950 AY587513 AY562216	SQWAY596950 SQ1AY587513 SQ2	Q
Arizona, USA 2003	<i>Hibiscus rosa-sinensis</i> L.	AY518194	AZAY518194	B

TABLE 1. (CONTINUED) BIOTYPE, GEOGRAPHICAL SOURCE, HOST PLANT, AND GENBANK ACCESSION NUMBER FOR *B. TABACI* MITOCHONDRIAL CYTOCHROME OXIDASE DNA (MTDNA COI) SEQUENCES.

Geographical location and year	Host plant	GenBank Accession number	Acronym	Whitefly haplotype or biotype
California, USA 2003	<i>Solanun melongena</i> L.	AY589496	California	B
California, USA 2003	<i>Euphorbia pulcherrima</i> Willd.	AY550272	CLAY550272	B
Texas, USA 2003	<i>Brassica oleracea</i> L. <i>var. capitata</i> L.	AY518192	TXAY518192	B

ufacturer's instructions. The DNA sequence for each PCR product was determined from the 5' end at the Sangon Technology Company, Shanghai.

Phylogenetic Analyses and Identification of *B. tabaci* Haplotypes in China

The mtCOI sequences for select, representative whiteflies (geographical and host or origin) were determined, and reference mtCOI sequences were obtained from the GenBank database. The DNA sequence was obtained for one to three individuals from each of 30 whitefly collections (Table 1). The mtCOI sequences were aligned with the CLUSTAL W algorithm (Thompson et al. 1994). Distances were calculated with the Kimura 2-parameter model of MEGA2.1 (Kumar et al. 2001). The NJ (Neighbour-Joining) and UPGMA (Unweighted Pair Group Method with Arithmetic Mean) algorithms available in MEGA2.1 (Kumar et al. 2001) were used to infer phylogenetic relationships, respectively. Two thousand bootstrap replicates were performed for each analysis.

RESULTS

Phylogenetic Analysis of the *B. tabaci* MtCOI

The mtCOI sequence was edited to remove PCR primer sequences, which yielded a ~470-bp fragment for each *B. tabaci* mtCOI sequence. The mtCOI sequences have been deposited in GenBank and the Accession Number for each is shown parenthetically (Table 1). Because the same haplotype was typically observed in field populations, only one mtCOI sequence was included for each representative haplotype per field collection.

The mtCOI sequence was used to identify biotypes and haplotypes, based on phylogenetic relationships. The mtCOI NJ (Fig. 1) and UPGMA (not shown) trees revealed similar results and four main clades were supported, each by robust bootstrap value. Three distinct clades were revealed with two major nodes strongly supported by 100% bootstrap values.

One major clade (I) contained sequences for the B biotype, and this identification was based on a high degree of shared nucleotide identity with reference sequences for the B biotype (Costa & Brown 1991). These collections were from Zhengzhou in Henan Province [AY582870, AY518186, AY587515, AY596949, AY582873, AY589497], Beijing [AY587519, AY582867, AY596953, AY582871, AY589498], Shandong [AY587518, AY587517], Jiangsu [AY518185], Zhejiang [AY566182], Shanghai [AY550274, AY550273], Hainan [AY518187], and Xinjiang [AY582868]. The latter haplotypes grouped closely with the reference sequences for the B biotype previously identified in Israel [AY518190], Spain [AY596951], and the U.S.A., including Arizona [AY518194], California [AY589496], and Texas [AY518192].

A second major clade (II) contained *B. tabaci* identified as the Q biotype (Guirao et al. 1997; Moya et al. 2001) or variants of the Q haplotype sequence. The Q-like haplotype was identified in collections from Yunnan [AY587516, AY518189], from Zhengzhou in Henan [AY587514], and from Beijing [AY582872, AY589499]. The Q haplotype has been identified previously in Spain [SQ1AY587513, SQWAY596950], where it was subsequently characterized in biological terms as the Q biotype (Guirao et al. 1997; Moya et al. 2001), and in Israel [IQAY518191] (Horowitz et al. 2003). The latter population/haplotype, which is a very close relative of the Spanish Q biotype, is probably indigenous to Israel, because *B. tabaci* is composed of several genetically distinct groups with a strong geographical association between more closely related biotypes (Frohlich et al. 1999; De Barro et al. 2000).

The third clade (III) was represented by a haplotype for a population collected from Zhejiang, China [ZJNBAY596952], which appears to be of Asian origin.

Nucleotide Divergence Estimates

Within and between nucleotide sequence divergence were calculated for field collections from China and reference population sequences for the

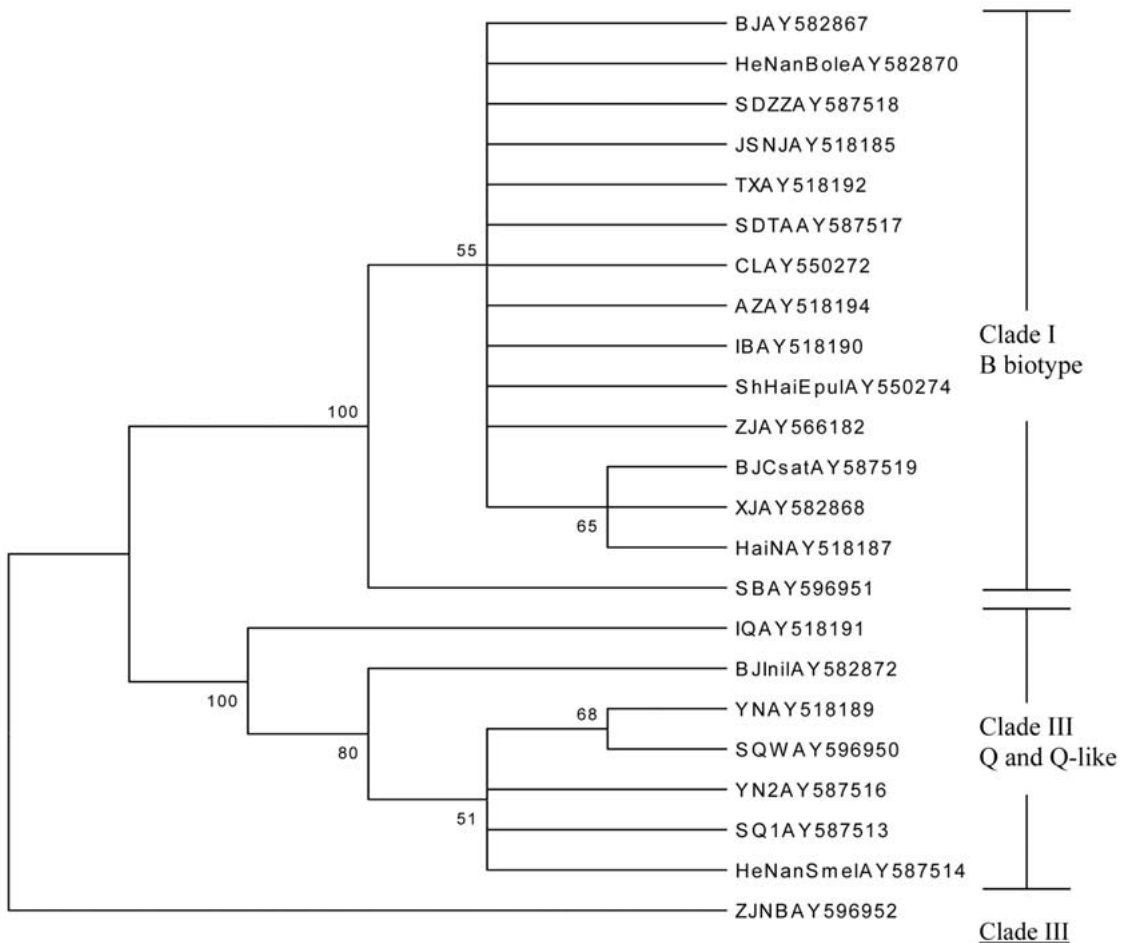


Figure 1. Phylogenetic tree for *B. tabaci* based on a fragment (~450 bases) of the mitochondrial cytochrome oxidase I gene. The tree was inferred by using the UPGMA method and 2000 bootstrap replicates. Abbreviations for whitefly collections are shown in Table 1.

B and Q biotypes of *B. tabaci*. Populations from Beijing [BJAY582867, BJCAY587519], Xinjiang [XJAY582868], Shandong [SDZZAY587518, SDTAAAY587517], Henan [HeNanBoleAY582870], Jiangsu [JSNJAY518185], Shanghai [ShHaiEpuAY550274], Zhejiang [ZJAY566182], Hainan [HaiNAY518187] from China shared more than 99.6% nucleotide sequence identity with *B. tabaci* B biotype identified from Spain [SBAY596951], Israel [IBAY518190], Arizona [AZAY518194], California [CLAY550272], and Texas [TXAY518192]. These mtCOI sequences were 100% identical with B biotype sequences for populations collected from various other locations, worldwide.

The nucleotide divergence estimates indicated that accessions from Beijing [BJlniAY582872], Yunnan [YN1YNAY518189, YN2AY587516] and Henan [HeNanSmelAY587514] shared >99.6%

nucleotide sequence identity with the Q biotype from Spain [SQ1AY587513, SQWAY596950]. The mtCOI sequences from the latter collections from China likewise shared 98.9%-99.4% nucleotide sequence identity with one population of *B. tabaci* from Israel [IQAY518191].

Sequence comparisons collectively suggest certain collections of *B. tabaci* from China are the Q biotype, and the *B. tabaci* population from Israel [IQAY518191] also is Q-like. The populations from Israel and China were slightly more divergent from one another than collections from China were from sequences obtained from *B. tabaci* Q biotype from Spain. The mtCOI DNA sequence (~470 bp) for whiteflies identified as the Q biotype from China was highly invariant (99-100% nucleotide identity) (data not shown), suggesting that they originated recently from a single or a very few introductions and/or original source(s).

The collection from Zhejiang [ZJNBAY596952] shared only 82.3%-83.5% nucleotide sequence identity with reference *B. tabaci* sequences included here, indicating that the Zhejiang haplotype was neither B nor Q biotype, and likely represents a divergent *B. tabaci* population that originated 'locally' and is indigenous to Asia.

DISCUSSION

Recently, the Q biotype of *B. tabaci*, which had been a relatively benign pest in the Mediterranean region (Simón et al. 1999), has been recognized as a serious pest and virus vector, owing to its ability to reach high population densities (Moya et al. 2001; Simón et al. 1999) and to develop resistance to at least one neonicotinoid (Rauch & Nauen 2003). These characteristics have been noted together with an increase in Q biotype infestations in southern Spain, where the B biotype is now almost absent, despite its introduction there in the mid-1990s (Simón et al. 1999). What is now recognized as the Q biotype has been identified in the Iberian Peninsula, in Sardinia and Sicily, and in Morocco (Brown 2000; Moya et al. 2001), the general region (Mediterranean/Middle East/North Africa) to which Q-like haplotypes are thought to be indigenous (Brown 2000).

Significant differences in host suitability (Muniz 2000) and developmental parameters (Muniz & Nombela 2001) for the B and Q biotypes, with respect to four weed species that occur in the winter months, were determined in no-choice assays. Except for *Lactuca serriola* L., the mean reproductive parameters for the Q biotype were significantly greater than those for the B biotype (Muniz 2000). The Q biotype showed higher daily infestation rates than the B biotype on most tomato varieties tested (Nombela et al. 2001). On sweet pepper, the generation time for the Q biotype was found to be shorter than that of B biotype at 33 and 17 d, respectively. The number of cumulative generations of Q biotype also was somewhat greater than for the B biotype (Muniz & Nombela 2001). Furthermore, the resistance of the Q biotype to pesticides has been shown to be more resilient than observed for the B biotype (Anthony et al. 1995; Costa et al. 1993; Rauch & Nauen 2003). These collective results are likely linked to the increased pest status of the Q biotype in the Mediterranean region during the past several years.

Prior to this study, the Q biotype had not been reported in China. The high intra-population homogeneity suggests that a recent introduction of this biotype has occurred in China. Knowledge of insecticide resistant-Q haplotype populations in Spain (Ebert & Nauen 2000) and Israel (Nauen et al. 2002), and also that Spain and the Canary Islands are important producers of ornamentals crops that could have made their way to China in commercial trade, suggests a link between the

movement of ornamentals species from the Mediterranean region and the recent presence of the Q type in China. Importation records have revealed that poinsettia and other ornamental plants were imported to China from Spain for the International Horticultural Exposition held in 1999 in Kunming, Yunnan Province. Such plants could have provided one possible route of entry into China for the Q biotype.

Herein, we report that the Q-biotype of *B. tabaci* was identified for the first time on infested ornamentals plants in several different regions of China. It is now important to closely monitor the potential establishment and spread of the Q biotype in the country to avoid its further dissemination, which could be devastating to vegetable and ornamental production. We have identified the Q biotype in distant locations in China, suggesting that multiple introductions may have occurred, or that plants from a single introduction were moved long distances from the original sources. Additional studies will be required to test this hypothesis and to determine if the Q biotype is sufficiently adapted to conditions in China to establish as an invasive pest and vector of plant viruses in ornamental, vegetable, and fiber crops.

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