Evidence from Molecular Markers and Population Genetic Analyses Suggests Recent Invasions of the Western North Pacific Region by Biotypes B and Q of *Bemisia tabaci* (Gennadius)

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ABSTRACT Invasive events by *Bemisia tabaci* (Gennadius) biotypes in various parts of the world are of continuing interest. The most famous is biotype B that has caused great economic losses globally. In addition, biotype Q has also recently been reported to be a new invasive pest. These two biotypes have been monitored for some time in the Western North Pacific region, but the invasive events and population genetic structures of these two biotypes are still not clear in this region. In this study, the mitochondrial *cytochrome oxidase I* (COI) gene was used to reconstruct a phylogenetic tree for identifying biotypes B and Q and to study the relationships between invasive events and ornamental plants. Population genetic analyses of mtCOI sequences were also used to study the genetic relationships within and between populations. A combination of a phylogenetic tree and haplotype analysis suggested the recent invasion of biotype Q in this region is related to the international ornamental trade from the Mediterranean region. Low levels of haplotype diversity and nucleotide diversity indicate that the presence of biotypes B and Q in the Western North Pacific region are caused by multiple invasions. Hierarchical analysis of molecular variance supports the hypothesis of multiple invasions. In addition, high sequence identities and low genetic distances within and between populations of the two biotypes revealed that these invasive events occurred recently. The low levels of genetic differentiation revealed by pairwise F_{ST} values between populations also suggests the invasions were recent. Therefore, results of this study suggested that biotypes B and Q entered this region through multiple recent invasions. A quarantine of agricultural crops may be necessary to prevent further invasions.

KEY WORDS whitefly, molecular marker, phylogeny, population genetics, pest

The whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae), was originally described as *Aleyrodes tabaci* by Gennadius in 1889 on tobacco in Greece (Perring 2001). This species is an important agricultural pest and causes great economic losses (Brown et al. 1995, De Barro 1995, Perring 2001). *B. tabaci* is widely distributed throughout tropical and subtropical areas of the world (Brown et al. 1995). The concept of biotypes of *B. tabaci*, proposed in the 1950s, indicates that morphologically indistinguishable populations exhibit different biological traits (Brown et al. 1995). Variations in biotypes exist in terms of host range, dispersal behavior, fecundity, insecticide resistance, and transmission competency for begomoviruses (Berry et al. 2004).

More than 24 biotypes of *B. tabaci* have been identified by multiple techniques (Perring 2001), the most well-known being the biotype B superbug. It is polyphagous with a broad host range and causes damage through feeding, excretion of honeydew, and virus transmission (De Barro 1995). Biotype B was de-

scribed previously as *B. argentifolii* Bellows and Perring, with the proposed common name of silverleaf whitefly (Perring 2001). Biotype Q was originally thought to be restricted to the Iberian Peninsula but recently has been widely reported in the Mediterranean Basin (Horowitz et al. 2005, De la Rúa et al. 2006). Horowitz et al. (2005) indicated that biotype Q has a high level of resistance to insecticides and also causes economic damage. Furthermore, biotypes B and Q of *B. tabaci* have different inherent levels of resistance to insecticides, and insecticide applications affect the proportion of both biotypes (Horowitz et al. 2005). Molecular markers have been developed to identify biotypes B and Q and to study their population dynamics (Khasdan et al. 2005).

Molecular markers are useful tools for distinguishing biotypes and include esterase, random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR), amplified fragment length polymorphism (AFLP), and mitochondrial, ribosomal, and microsatellite DNA markers (De Barro and Driver 1997, Frohlich et al. 1999, De Barro et al. 2000, De Barro 2005, Zhang et al. 2005). Based on the genetic diversity

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revealed by RAPD-PCR analysis, biotype B is likely an invader, and biotype Q may be endemic to the Iberian Peninsula (Moya et al. 2001). In addition, many reports have used mitochondrial and ribosomal markers to reconstruct phylogenetic trees and distinguish biotypes based on tree topology (Frohlich et al. 1999, De Barro et al. 2000, Abdullahi et al. 2003, De la Rúa et al. 2006, Hsieh et al. 2006, Ueda and Brown 2006). Accordingly, biotype B presumably originated in the Middle East, and evidence supports its having spread worldwide because of human trade activities (Frohlich et al. 1999, De Barro et al. 2000).

The phylogenetic tree of the mitochondrial *cytochrome oxidase I* (mtCOI) gene indicated four biotypes of *B. tabaci* in East Asia: B, Q, Nauru, and An (Hsieh et al. 2006). The Nauru and An biotypes are indigenous to East Asia (Hsieh et al. 2006). Biotype B as an invader is the most widely distributed biotype in the Western North Pacific region, and it has already caused huge economic losses in the region (Zhang et al. 2005, Hsieh et al. 2006, Ueda and Brown 2006). Biotype Q was previously found only in China, but now has also been reported from Japan (Zhang et al. 2005, Ueda and Brown 2006). Otherwise, biotype Q has been reported only in a local region of China and Japan (Zhang et al. 2005, Ueda and Brown 2006). Some reports have hypothesized that ornamental plants such as the poinsettia may have been a possible vector of entry for biotypes B and Q into China and Japan (Zhang et al. 2005, Ueda and Brown 2006). However, the relationships between invasive events and ornamental plants are indefinite, and genetic relationships within and between populations also are not clear in the Western North Pacific region.

Mitochondrial DNA is haploid and particularly useful for population studies because it undergoes no recombination, is maternally inherited, and has a simple sequence organization (Harrison 1989). In addition, the mtCOI gene has been a popular marker for studying relationships among *B. tabaci* biotypes (Frohlich et al. 1999, De la Rúa et al. 2006, Hsieh et al. 2006, Ueda and Brown 2006). In this study, we applied the mtCOI gene to reconstruct a phylogenetic tree for identifying biotypes B and Q in the Western North Pacific region, and we discuss the relationships between invasive events and ornamental plants. Furthermore, we used genetic analyses of the mtCOI gene to determine the population genetic structure of biotypes B and Q in this region. We attempted to study the genetic diversity within and between populations and determine whether or not biotypes B and Q are invasive agents in this region. We also tried to determine the genetic relationships within and between populations separated by natural barriers. This study provides information for understanding genetic variation of the invasive biotypes of *B. tabaci* in the Western North Pacific region.

Materials and Methods

Whitefly Samples. Whitefly samples of *B. tabaci* of the Western North Pacific region were from China,

Japan, Korea, and Taiwan (Table 1; Fig. 1). Specimens of *B. tabaci* were collected from weeds, vegetables, and ornamental plants (from the wild, farms, and greenhouses, respectively). We selected fourth instars and used classical taxonomic criteria to identify the whitefly species. Adult whiteflies were preserved in 95% ethanol and stored at -20°C . Samples from Cyprus, Israel, the Netherlands, and Spain of the Mediterranean Basin also were analyzed.

COI Gene Sequencing. Genomic DNA was extracted from individual adult whiteflies according to the method suggested by De Barro and Driver (1997). The mitochondrial partial COI gene sequence (816 bp) was amplified by PCR with the primers C1-J-2195 (5'-TTGATTTTTTGGTCATC CAGAAGT-3') and L2-N-3014 (5'-TCCAATGCAC TAATCTGCCATATTA-3') (Frohlich et al. 1999). The PCR reaction program was initialized at 94°C for 2 min, followed by 35 cycles of 94°C for 1 min, 52°C for 1 min, and 72°C for 1 min, with a final extension of 5 min at 72°C . The PCR products were subsequently gel-purified using the Micro-Elute DNA Clean/Extraction Kit (GeneMark, Taipei, Taiwan) and sequenced in one direction on an ABI 3730 DNA Analyzer (Applied Biosystems, Foster, CA) using an ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit, V3.1 (Applied Biosystems). Sequences obtained in this study were submitted to GenBank, and some samples from the Western North Pacific region in GenBank also were analyzed (Table 1).

Phylogenetic Analysis. Phylogenetic analysis included the B, Q, and other biotypes of *B. tabaci* (Tables 1 and 2), with *Lipaleyrodes emiliae* Chen and Ko (DQ989555) as an outgroup. All sequences were aligned using the Clustal $\times$ 1.18 program (Thompson et al. 1997). The resulting alignment was manually edited using the GeneDoc program (Nicholas et al. 1997), and the phylogenetic analysis was performed using Bayesian inference (Yang and Rannala 1997). The best-fitting model of DNA substitution was selected by the Akaike information criterion (AIC) using MrMODELTEST version 2.2 (Nylander 2004). The phylogenetic tree was constructed by using the GTR + G model. The Bayesian analysis was performed using MrBayes version 3.1.2 (Huelsenbeck and Ronquist 2001). Metropolis-coupled Markov chain Monte Carlo analyses were run with four chains (one cold chain and three heated chains). Analyses were initiated with random starting trees and run for 1×10^6 generations and were sampled every 100 generations. For the burn-in period, we discarded 100,000 generations. Posterior clade probabilities were used to assess the levels of nodal support. Branch lengths were saved and are presented on the 50% majority-rule consensus trees.

Analysis of Genetic Variations and Population Genetics. After our test, we found that samples from the same site revealed the same mtCOI sequences. Therefore, we only selected one individual to represent all specimens from the same site (Table 1; Fig. 1). The mtCOI sequence identities within and among popu-

Table 1. Samples of biotypes B and Q of *B. tabaci* from the Western North Pacific region

No.	Acronym	Biotype	Haplotype	GenBank accession no.	Host plant	Year	Location	Environment
1	ChinaShanghaiB1	B	BH6	AY550274	<i>Euphorbia pulcherrima</i>	2003	China, Shanghai	Field
2	ChinaNanjingB2	B	BH6	AY518185	<i>Gossypium hirsutum</i>	2003	China, Nanjing	Field
3	ChinaBeijingB3	B	BH6	AY582867	<i>Brassica oleraceae</i>	2003	China, Beijing	Field
4	ChinaHeNanB4	B	BH6	AY582873	<i>Lycopersicon esculentum</i>	2003	China, Henan	Field
5	ChinaZhejiangB5	B	BH6	AJ867555	<i>Brassica oleraceae</i>	2003	China, Zhejiang	Field
6	ChinaHainanB6	B	BH1	AY518187	<i>Solanum melongena</i>	2003	China, Hainan	Field
7	ChinaXinJiangB7	B	BH1	AY582868	<i>Euphorbia pulcherrima</i>	2003	China, Xinjiang	Field
8	ChinaZhejiangB8	B	BH6	DQ989520	<i>Brassica oleraceae</i>	2005	China, Zhejiang	Field
9	ChinaShandongB9	B	BH6	DQ989521	<i>Gossypium hirsutum</i>	2004	China, Shandong	Field
10	ChinaShandongB10	B	BH6	DQ989522	<i>Brassica oleraceae</i>	2004	China, Shandong	Field
11	ChinaBeijingB11	B	BH6	DQ989523	<i>Brassica oleracea</i>	2005	China, Beijing	Field
12	ChinaBeijingB12	B	BH6	DQ989524	<i>Cucumis sativus</i>	2005	China, Beijing	Field
13	ChinaShanghaiB13	B	BH6	DQ989525	<i>Cucumis sativus</i>	2005	China, Shanghai	Field
14	ChinaGuangdongB14	B	BH6	DQ989526	<i>Luffa aegyptiaca</i>	2005	China, Guangdong	Field
15	ChinaHubeiB15	B	BH6	DQ989527	<i>Brassica oleraceae</i>	2005	China, Hubei	Field
16	ChinaGuangdongB16	B	BH6	DQ989528	<i>Cucurbita moschata</i>	2005	China, Guangdong	Field
17	ChinaFujianB17	B	BH6	DQ989529	<i>Brassica oleraceae</i>	2005	China, Fujian	Field
18	ChinaShanxiB18	B	BH6	DQ989530	<i>Lycopersicon esculentum</i>	2005	China, Shanxi	Field
19	KoreaJincheonB1	B	BH6	DQ462587	—	2006	Korea, Jincheon	—
20	KoreaGoyangB2	B	BH6	DQ989531	<i>Rosa hybrida</i>	2003	Korea, Goyang	Field
21	KoreaGoyangB3	B	BH6	DQ174538	<i>Rosa hybrida</i>	2003	Korea, Goyang	Field
22	JapanAIB1	B	BH6	AB204577	<i>Lycopersicon esculentum</i>	2004	Japan, Ehime	Field
23	JapanKOSB2	B	BH4	AB204578	cucurbit plant	1991	Japan, Kumamoto	Field
24	JapanKAKB3	B	BH3	AB204580	<i>Lycopersicon esculentum</i>	2004	Japan, Kagoshima	Field
25	JapanHARB4	B	BH2	AB204581	Melon	2004	Japan, Kochi	Field
26	JapanMATB5	B	BH6	AB204582	<i>Capsicum annuum</i>	2004	Japan, Chiba	Field
27	JapanASAB6	B	BH6	AB204583	<i>Solanum melongena</i>	2004	Japan, Shizuoka	Field
28	JapanKURB7	B	BH6	AB204584	<i>Lycopersicon esculentum</i>	2004	Japan, Okayama	Field
29	JapanTOSB8	B	BH6	AB204585	Melon	2004	Japan, Kochi	Field
30	JapanHonshuB9	B	BH6	DQ989532	<i>Euphorbia pulcherrima</i>	2005	Japan, Chiba	Field
31	JapanShikokuB10	B	BH6	DQ989533	<i>Capsicum annuum</i>	2005	Japan, Kochi	Field
32	TaiwanTPB1	B	BH6	DQ989534	<i>Euphorbia pulcherrima</i>	2006	Taiwan, Taipei	Field
33	TaiwanCYB2	B	BH6	DQ989535	<i>Lycopersicon esculentum</i>	2006	Taiwan, Chiayi	Field
34	TaiwanYLB3	B	BH6	DQ989536	<i>Lycopersicon esculentum</i>	2006	Taiwan, Yunlin	Field
35	TaiwanTNB4	B	BH6	DQ989537	<i>Euphorbia hirta</i>	2006	Taiwan, Tainan	Field
36	TaiwanKHB5	B	BH6	DQ989538	<i>Lycopersicon esculentum</i>	2006	Taiwan, Kaohsiung	Field
37	TaiwanHLB6	B	BH5	DQ989539	<i>Brassica oleraceae</i>	2006	Taiwan, Hualien	Field
38	TaiwanYLB7	B	BH5	DQ989540	<i>Lycopersicon esculentum</i>	2006	Taiwan, Ilan	Field
39	TaiwanTYB8	B	BH6	DQ989541	<i>Synedrella nodiflora</i>	2006	Taiwan, Taoyuan	Field
40	TaiwanMLB9	B	BH6	DQ989542	<i>Euphorbia pulcherrima</i>	2006	Taiwan, Miaoli	Greenhouse
41	TaiwanNTB10	B	BH6	DQ989543	<i>Synedrella nodiflora</i>	2006	Taiwan, Nantou	Field
42	TaiwanCHB11	B	BH6	DQ989544	<i>Erigeron bonariensis</i>	2006	Taiwan, Changhua	Field
43	TaiwanTHB12	B	BH6	DQ989545	<i>Erigeron bonariensis</i>	2006	Taiwan, Taichung	Field
44	ChinaZhejiangQ1	Q	QH4	DQ473394	<i>Cucumis sativus</i>	2006	China, Zhejiang	Field
45	ChinaYunNanQ2	Q	QH3	AY587516	<i>Euphorbia pulcherrima</i>	2003	China, Yunnan	Field
46	ChinaHeNanQ3	Q	QH3	AY587514	<i>Solanum melongena</i>	2003	China, Henan	Field
47	ChinaBeijingQ4	Q	QH1	AY589499	<i>Ipomoea nil</i>	2003	China, Beijing	Field
48	ChinaYunnanQ5	Q	QH4	AY518189	<i>Euphorbia pulcherrima</i>	2003	China, Yunnan	Field
49	ChinaBeijingQ6	Q	QH1	AY582872	<i>Ipomoea nil</i>	2003	China, Beijing	Field
50	KoreaWhaseongQ1	Q	QH4	DQ462586	—	2006	Korea, Whaseong	—
51	KoreaGeojeQ2	Q	QH3	DQ462585	—	2006	Korea, Geoje	—
52	KoreaJinjuQ3	Q	QH3	DQ462584	—	2006	Korea, Jinju	—
53	KoreaBuyeoQ4	Q	QH3	DQ462583	—	2006	Korea, Buyeo	—
54	JapanMIHQ1	Q	QH3	AB204588	<i>Lycopersicon esculentum</i>	2004	Japan, Hiroshima	Field
55	JapanOKCQ2	Q	QH3	AB204587	<i>Lycopersicon esculentum</i>	2004	Japan, Kagoshima	Field
56	JapanMYJQ3	Q	QH3	AB204586	Melon	2004	Japan, Kagoshima	Field
57	JapanNSCQ4	Q	QH4	AB204579	<i>Cucurbita maxima</i>	2004	Japan, Kumamoto	Field
58	JapanKyushuQ5	Q	QH2	DQ989546	<i>Capsicum annuum</i>	2005	Japan, Miyazaki	Field
59	TaiwanTNQ1	Q	QH3	DQ989547	<i>Euphorbia pulcherrima</i>	2006	Taiwan, Tainan	Potting
60	TaiwanMLQ2	Q	QH3	DQ989548	<i>Euphorbia pulcherrima</i>	2006	Taiwan, Miaoli	Greenhouse
61	TaiwanMLQ3	Q	QH3	DQ989549	<i>Euphorbia pulcherrima</i>	2006	Taiwan, Miaoli	Greenhouse
62	TaiwanMLQ4	Q	QH3	DQ989550	<i>Euphorbia pulcherrima</i>	2006	Taiwan, Miaoli	Greenhouse

lations were estimated using GeneDoc (Nicholas et al. 1997). In addition, the genetic distances within and among populations were estimated using Kimura's two-parameter model in MEGA version 3.1 (Kumar et al. 2004). Values of haplotype diversity (h) and nucleotide diversity (π) were evaluated with DnaSP

version 4.10.8 (Rozas et al. 2003). Analysis of molecular variance (AMOVA) was used to characterize patterns of genetic variation and estimate variance components at different hierarchical levels (among biotypes [Va], among populations within biotypes [Vb], and within populations [Vc]) using Arlequin

Fig. 1. Locations of samples of *B. tabaci* biotypes B and Q in the Western North Pacific region. Numbers refer to specimens in Table 1.

version 3.01 (Excoffier et al. 2005). The fixation index of the Φ statistics was estimated from the pairwise genetic distances among populations (Wright 1951). These distances were also used for the hierarchical analysis of population differentiation (among biotypes [Φ_{CT}], among populations within biotypes [Φ_{SC}], and within populations [Φ_{ST}]) (Excoffier et al. 1992). In addition, the statistical significance of the Φ statistics at different hierarchical levels was tested by 1,000 permutations. The value of F_{ST} was used to estimate the degree of genetic differentiation among populations (Hudson et al. 1992) using DnaSP 4.10.8.

Results

Phylogenetic Analysis of *B. tabaci* Biotypes. Approximately 816 bp of the mtCOI gene was amplified from individual whiteflies using PCR. In addition, we also downloaded sequences from GenBank, and 78 samples were used to reconstruct a phylogenetic tree that included an outgroup (Table 2). After alignment, 441 bp of the mtCOI sequence was used to reconstruct the phylogenetic tree. There were 256 invariable sites, 40 singleton variable sites, and 145 parsimoniously informative sites. The phylogenetic tree based on the Bayesian inference divided *B. tabaci* into eight biotypes (Fig. 2). Biotype Q, B, and Ms were clustered into a monophyletic clade with high posterior probability (99%) support. The results revealed that these three biotypes are sister groups and also indicated that

they could be clearly clustered by the three biotypes. Within this group, there was high posterior probability (100%) support for biotype Ms as a monophyletic group. However, biotypes B and Q clearly belong to different monophyletic subgroups with high posterior probability (99 and 83%, respectively). Therefore, biotypes B and Q are sister groups.

We compared the distributions and host-plant records of biotypes B and Q in the Western North Pacific region (Table 1; Fig. 1) (China, Korea, Japan, and Taiwan) relative to the phylogenetic tree (Fig. 2). Biotype Q collected from different host plants of the Western North Pacific region were clustered in the same clade which also included samples from the Mediterranean Basin. Samples of biotype B from the Western North Pacific region clustered in the same clade as a polytomy, along with samples from the Mediterranean Basin and around the world. The branch lengths of samples of biotype B were similar, revealing that their relationships were close. Biotype Q branch lengths also were similar, indicating close relationships of samples from the Western North Pacific region.

Genetic Variation and Population Genetics of Biotype B and Q in the Western North Pacific Region. After sequence alignment, 473 bp of the mtCOI sequence was used to analyze genetic variation and population genetics of biotypes B and Q in the Western North Pacific region. Comparison of 43 samples of biotype B from this region revealed 468 sequence-

Table 2. Samples of *B. tabaci* whose mtCOI sequences were used to construct the phylogenetic tree in Fig. 2

Acronym	Biotype	GenBank accession no.	Host plant	Year	Location
AusBF5	B	DQ174535	—	—	Australia
ArizonaB	B	AY057123	<i>Euphorbia pulcherrima</i>	2001	USA, Arizona
SouthAfricaB	B	AY057140	<i>Lycopersicon esculentum</i>	2001	South Africa
FranceB	B	AJ550170	<i>Solanum melongena</i>	2003	France, Antibes
IndiaB	B	AF321927	<i>Lycopersicon esculentum</i>	2000	India, Kolar
ArgentinaB	B	AF340215	—	2001	Argentina, Buenos Aires
ReunionB	B	AJ550177	<i>Solanum melongena</i>	2003	Reunion
UgandaB	B	AY903569	<i>Hibiscus esculentus</i>	2005	Uganda, Namulonge
SpainF32B	B	DQ989551	<i>Lycopersicon esculentum</i>	2003	Spain
IsraelF60B	B	DQ989552	—	2006	Israel
TurkeyQ	Q	AF342776	<i>Gossypium arboreum</i>	2001	Turkey
FranceQ	Q	AM180063	—	2005	France, Roussillon
AlgeriaQ	Q	AM176575	<i>Cucurbita</i> sp.	2005	Algeria, Biskra
MoroccoQ	Q	AM176573	<i>Lycopersicon esculentum</i>	2005	Morocco, Biougra
TurkeyQ	Q	AF342776	<i>Gossypium arboreum</i>	2001	Turkey
Cameroon Q	Q	AF344258	<i>Hibiscus esculentus</i>	2001	Cameroon, Banga-Bakundu
ZimbabweQ	Q	AF344285	—	2001	Zimbabwe, Mazowe
SudanQ	Q	AY827613	<i>Gossypium arboreum</i>	2004	Sudan
NetherlandsQF13	Q	DQ174541	<i>Hibiscus</i> sp.	2002	Netherlands
SpainQF7	Q	DQ174539	—	2002	Spain
CyprusF42Q	Q	DQ989553	—	2004	Cyprus
IsraelF61Q	Q	DQ989554	—	2006	Israel
ArgentinaA	A	AF340212	<i>Gossypium arboreum</i>	2001	Argentina, Santiago
ArizonaA	A	AY057122	<i>Gossypium arboreum</i>	1983	Arizona
BoliviaA	A	AF342768	<i>Lycopersicon esculentum</i>	1999	Bolivia
MexicoA	A	AY057125	<i>Lycopersicon esculentum</i>	1989	Mexico
ColombiaA	A	AJ550168	<i>Chromolaena odorata</i>	2003	Colombia, Cali
HondurasA	A	AF342770	Melon	1998	Honduras
TanzaniaC	Cassava	AF418667	<i>Manihot esculenta</i>	2004	Tanzania, Mtwara
GhanaC	Cassava	AF418668	<i>Manihot esculenta</i>	2004	Ghana, Accra
MalawiC	Cassava	AY057215	<i>Manihot esculenta</i>	2001	Malawi
UgandaBMC	Cassava	AY057142	<i>Manihot esculenta</i>	1997	Uganda
UgandaBC	Cassava	AY057141	<i>Manihot esculenta</i>	1997	Uganda
ZambiaC	Cassava	AF344281	—	2001	Zambia
SwazilandC	Cassava	AF344269	—	2001	Swaziland
SouthAfricaC	Cassava	AF344260	—	2001	South Africa
MozambiqueC	Cassava	AF344278	—	2001	Mozambique
MadagascarMs	Ms	AJ550171	<i>Lycopersicon esculentum</i>	2003	Madagascar, Tulear
MauritiusMs	Ms	AJ550172	<i>Phaseolus</i> sp.	2003	Mauritius
SeychellesMs	Ms	AJ550182	<i>Jatropha</i> sp.	2003	Seychelles, Mahe
ReunionMs	Ms	AJ550178	<i>Euphorbia pulcherrima</i>	2003	Reunion
UgandaMs	Ms	AY903524	Bean	2005	Uganda
ItalyPT1	T	AY827595	<i>Euphorbia characias</i>	2004	Italy, Puglia
ItalyPT2	T	AY827597	<i>Euphorbia characias</i>	2004	Italy, Puglia
ItalyST1	T	AY827600	<i>Euphorbia characias</i>	2004	Italy, Sicily
ItalyST2	T	AY827601	<i>Euphorbia characias</i>	2004	Italy
ItalyST3	T	AY827602	<i>Euphorbia characias</i>	2004	Italy
ItalyST4	T	AY827603	<i>Euphorbia characias</i>	2004	Italy
India2	Nauru	AJ748374	<i>Solenidiopsis peruvianum</i>	2004	India, Karnataka
Nepal	Nauau	AF342779	<i>Citrullus lanatus</i>	2001	Nepal
Pakistan2	Nauau	AY057582	<i>Gossypium arboreum</i>	2001	Pakistan
TaiwanNauru1	Nauru	DQ174518	<i>Boehmeria nivea</i>	2003	Taiwan, Hsinchu
TaiwanNauru2	Nauru	DQ174519	<i>Humulus scandens</i>	2002	Taiwan, Chiayi
TaiwanNauru3	Nauru	DQ174520	<i>Ipomoea acuminata</i>	2003	Taiwan, Taitung
TaiwanNauru4	Nauru	DQ174521	<i>Euphorbia pulcherrima</i>	2002	Taiwan, Kaohsiung
ChinaNauru1	Nauru	DQ174522	<i>Gossypium hirsutum</i>	2004	China, Jiangsu
ChinaNauru2	Nauru	DQ174523	<i>Codiaeum variegatum</i>	2004	China, Guangdong
Indonesia	Nauru	DQ174524	<i>Capsicum annuum</i>	2003	Indonesia
AustraliaAn	An	DQ174529	—	—	Australia
TaiwanAn1	An	DQ174525	<i>Momordica ochinchinensis</i>	2002	Taiwan, Chiayi
TaiwanAn2	An	DQ174526	<i>Achyranthes obtusifolia</i>	2003	Taiwan, Kaohsiung
TaiwanAn3	An	DQ174527	<i>Mesona chinensis</i>	2003	Taiwan, Hualien
TaiwanAn4	An	DQ174528	Solanaceae	2003	Taiwan, Hsinchu
ChinaAn	An	AF342777	<i>Gossypium arboreum</i>	2001	China
Malaysia	An	AY057137	—	2001	Malaysia
India1	An	AJ748365	Field bean	2004	India, Karnataka
India3	An	AJ748360	<i>Solanum melongena</i>	2004	India, Karnataka
Pakistan1	An	AF164669	<i>Cucurbita moschata</i>	1999	Pakistan
Thailand	An	AF164670	<i>Gossypium arboreum</i>	1999	Thailand

Fig. 2. Phylogenetic tree of mtCOI sequences for *B. tabaci* based on Bayesian inferences. Numbers at the nodes are the posterior probabilities as support values. The outgroup was *L. emiliae*.

invariable sites, 3 singleton-variable sites, and 2 parsimoniously informative sites. Therefore, overall sequences of these samples revealed six haplotypes in this region (Table 3), and BH6 was the most common

haplotype in all four populations. Furthermore, BH1 was found only in China, BH5 was found only in Taiwan, and BH2 and BH3 were found only in Japan. The highest haplotype ($h = 0.53333$) and nucleotide

Table 3. Distribution of haplotypes, haplotype diversity (h), and nucleotide diversity (π) based on mtCOI sequences within populations of *B. tabaci* B and Q biotypes from Western North Pacific region

Biotype	Population (n)	Haplotype						Haplotype diversity (h)	Nucleotide diversity (π)
		BH1	BH2	BH3	BH4	BH5	BH6		
B	ChinaB (18)	2					16	0.20915	0.00044
	KoreaB (3)						3	—	—
	JapanB (10)		1	1	1		7	0.53333	0.00127
	TaiwanB (12)					2	10	0.30303	0.00064
	Total	2	1	1	1	2	36	0.30011	0.00068
Q		QH1	QH2	QH3	QH4				
	ChinaQ (6)	2		2	2			0.8	0.00226
	KoreaQ (4)			3	1			0.5	0.00106
	JapanQ (5)		1	3	1			0.7	0.00169
	TaiwanQ (4)			4				—	—
	Total	2	1	12	4			0.57310	0.00138

diversities ($\pi = 0.00127$) were found among individuals of the Japanese population. In addition, the lowest haplotype ($h = 0.20915$) and nucleotide diversities ($\pi = 0.00044$) were found among individuals of the Chinese population. Across all samples of this region, haplotype diversity (h) was 0.30011 and nucleotide diversity (π) was 0.00068. However, we could not calculate the haplotype or nucleotide diversity for the Korean population because there was only one haplotype.

A comparison of 19 samples of biotype Q from the Western North Pacific region revealed 470 sequence-invariable sites, 1 singleton-variable site, and 2 parsimoniously informative sites. There were four haplotypes in this region (Table 3), with QH3 being the most common. The highest haplotype ($h = 0.8$) and nucleotide diversities ($\pi = 0.00226$) were found among individuals of the Chinese population. The lowest haplotype ($h = 0.5$) and nucleotide diversities ($\pi = 0.00106$) were found among individuals of the Korean population. Across all samples of this region, haplotype diversity (h) was 0.57310 and nucleotide diversity (π) was 0.00138. We could not calculate the haplotype or nucleotide diversity for the Taiwanese population because there was only one haplotype.

Hierarchical analysis by AMOVA detected that the great majority of variation was among biotypes B and Q (98%; Table 4). The variation among populations within biotypes was only 0.05%. In addition, the variation within populations (1.94%) was higher than that among populations within biotypes. Population differentiation of the Φ statistics accounted for three sources of variation (Table 4). A significant Φ_{ST} value was detected within populations ($\Phi_{ST} = 0.98056$, $P < 0.001$). A significant Φ_{CT} value was detected among

biotypes ($\Phi_{CT} = 0.98004$, $P < 0.05$). The results indicated that genetic differences among biotypes were responsible for the differences within populations. No significant Φ_{SC} value was observed among populations within biotypes ($\Phi_{SC} = 0.02565$).

Sequence identity and genetic distance (of Kimura's two-parameter model) were used to compare populations. Sequence identities between populations of biotype B in the Western North Pacific region were all 99–100%, and those within populations were also 99–100%. The average genetic distance between populations was 0.00024–0.00099. The maximum distance was 0.00099 between the Japanese and Taiwanese populations. The minimum distance was 0.00024 between the Chinese and Korean populations. The mean distance within populations was 0–0.00127. Sequences from different individuals within the Korean population of biotype B were identical. Sequence identities among populations of biotype Q in the Western North Pacific region were 99–100%, whereas those within populations were 99–100%. Mean genetic distances between populations were 0.00053–0.00198. Average distances within populations were 0–0.00226. Sequences from different individuals within the Taiwanese population of biotype Q were identical.

Genetic differentiation between populations was estimated by pairwise F_{ST} (Table 5). Comparisons among populations of biotype B in the Western North Pacific region resulted in low F_{ST} values, suggesting no genetic differentiation among populations of biotype B in this region. Likewise, pairwise comparisons among populations of biotype Q in the Western North Pacific region also resulted in low F_{ST} values, indicating no genetic differentiation among populations of biotype Q in this region.

Table 4. Hierarchical analysis of molecular variance and Φ statistics of genetic differences for *B. tabaci* biotypes B and Q from the Western North Pacific region

Source of variation	df	Sum of squares	Variance components	Percent of variation	Φ statistics
Among biotypes	1	274.797	10.41741 Va	98	Φ_{CT} :0.98004 ^a
Among populations within biotypes	6	1.478	0.00544 Vb	0.05	Φ_{SC} :0.02565
Within populations	54	11.161	0.20669 Vc	1.94	Φ_{ST} :0.98056 ^b
Total	61	287.435	10.62953		

Significant population structure is tested among biotypes, among populations within biotypes, and within populations.

^a $p < 0.05$; ^b $p < 0.001$.

Table 5. Pairwise estimates of F_{ST} among populations of *B. tabaci* biotypes B and Q from the Western North Pacific region

		1	2	3
B biotype (n)	1 ChinaB (18)			
	2 KoreaB (3)	0.05882		
	3 JapanB (10)	0.0159	<0.00001	
	4 TaiwanB (12)	0.07807	0.09091	0.03247
Q biotype (n)	1 ChinaQ (6)			
	2 KoreaQ (4)	-0.04444		
	3 JapanQ (5)	<0.00001	-0.18182	
	4 TaiwanQ (4)	0.2	<0.00001	<0.00001

Discussion

The phylogenetic tree based on Bayesian inferences revealed that biotypes B and Q of *B. tabaci* are both present in China, Korea, Japan, and Taiwan of the Western North Pacific region. Biotype B has already been reported from these four countries, but biotype Q was known only from China until a recent report from Japan (Zhang et al. 2005, Hsieh et al. 2006, Ueda and Brown 2006). Biotype Q was detected for the first time in Taiwan in this study. Biotype B is an invader worldwide and biotype Q is a recent invader of China, Japan, and the United States (Perring 2001, Zhang et al. 2005, Ueda and Brown 2006). Therefore, we hypothesize that biotype Q is a new invader of Taiwan.

Results of the phylogenetic tree provide some information on the invasive events of biotypes B and Q in the Western North Pacific region. Biotype B of this region clustered with the worldwide samples, forming a clade that was a polytomy. This result supports previous studies that found that biotype B has been spread rapidly across the globe by human trade activities (Frohlich et al. 1999, De Barro et al. 2000, Perring 2001). Therefore, biotype B in this region undoubtedly represents an invasive event, as suggested in previous reports (Zhang et al. 2005, Hsieh et al. 2006, Ueda and Brown 2006). The biotype Q clade was divided into three branches and appeared more genetically polymorphic. The tree topology revealed that biotype Q samples from this region clustered with Mediterranean countries. Although biotype Q was thought to be restricted originally to the Iberian Peninsula, it has recently been found in various countries around the Mediterranean basin (Brown et al. 2000, Horowitz et al. 2005). Consequently, we hypothesize that the invasion by biotype Q in the Western North Pacific region originated recently from one of several possible Mediterranean countries.

The routes of invasive biotypes of *B. tabaci* are believed to be related to the international trade in ornamentals (Brown et al. 1995, De Barro 1995). Imported poinsettias were probably the source of biotype B, which dispersed from ornamental greenhouses into the field in the United States (Brown et al. 1995). Invasions of biotype Q were hypothesized to be associated with ornamental crops in China and Japan (Zhang et al. 2005, Ueda and Brown 2006). Fortunately, we found direct evidence to support this hy-

pothesis. There was no evidence of biotype Q in Taiwan before 2005 (Hsieh et al. 2006); in 2006 we recorded biotype Q only in one greenhouse containing poinsettias. The poinsettia plantlets had been obtained from Italy in 2006. Furthermore, we did not find any biotype Q whiteflies outside the greenhouse or in the field in Taiwan in 2006. Thus, we consider this to be powerful evidence supporting the hypothesis that routes of invasion by biotypes of *B. tabaci* are related to the international ornamentals trade.

Population genetic analyses were used to study genetic variation of invasive biotypes and to reconstruct the invasive events. According to haplotype analyses, BH6 was the only haplotype shared among countries, whereas the other five haplotypes were unique to a given country. In addition, haplotype and nucleotide diversities within each country were very low. Six haplotypes with low levels of genetic variability were observed, which suggests that multiple invasive events or rapid divergence have occurred. However, the molecular clock hypothesis based on Brower's estimates for mitochondrial DNA indicated 2.3% pairwise sequence divergence per million years (Brower 1994). Therefore, we hypothesize that the invasive biotype B in this region exists because of multiple invasive events. However, QH3 was found to be the most common haplotype within each country. Haplotype QH4 also was shared among countries besides Taiwan, while the other two haplotypes were unique to individual countries. Haplotype and nucleotide diversities also revealed low genetic variability within each country. Based on our results, we hypothesize that the invasive event of biotype Q in Taiwan was a single event, because only the QH4 haplotype was found in one poinsettia greenhouse. Although the ocean provides a natural barrier between China and Japan and between Korea and Japan, they all share two haplotypes. This suggests that the invasion of biotype Q in this region may have occurred through multiple invasive events. A recently introduced population that has expanded in size from a low number of founders is expected to have a common haplotype which is shared by a majority of individuals and many rarer haplotypes with a few independent mutations (Avice 2000). Based on the genetic variability of the population genetics analyses, we hypothesize that the invasions of biotypes B and Q in the Western North Pacific region occurred through multiple invasive events.

Hierarchical analysis with AMOVA was used to characterize patterns of genetic variations and to estimate variance components. The results revealed that the great majority of variation was among biotypes B and Q, whereas there were also significant genetic differences among B and Q biotypes. This supports biotypes B and Q being genetically different, as previously reported (Moya et al. 2001). The fixation index of the Φ statistics was used for the hierarchical analysis of population differentiation. We noted that there was relatively large differentiation among biotypes, which was responsible for differences within populations. The results revealed higher levels of genetic differ-

ences within populations than among populations as a whole, a result that is unreasonable given the ocean as a natural barrier. Thus, the combined results of AMOVA analyses supported the hypothesis of multiple invasive events of biotypes B and Q in the Western North Pacific region.

The population genetic structure was studied to determine the genetic relationships within populations and between populations of invasive biotypes of *B. tabaci* in this region. Similar results of low genetic distances and high sequence identities between and within populations of biotype B revealed no differences between populations. Low F_{ST} values between populations of biotype B also revealed low levels of genetic differentiation. These results indicate that few differences exist among populations. However, it seems impossible that no differences would exist between long-resident natural populations with the ocean serving as a natural barrier. Instead, one would expect to observe isolation by distance, where genetic similarity among populations decreases as the geographic distance between them increases (Jensen et al. 2005). We found that biotypes B and Q in this region do not exhibit. Consequently, the population structure analyses support hypothesis of recent multiple invasions of biotypes B and Q in this region.

Actually, biotypes B and Q are known invaders of the Western North Pacific region. Biotype B has already become an important pest and has transmitted begomoviruses that have caused economic losses in this region (Zhang et al. 2005, Hsieh et al. 2006, Ueda and Brown 2006). Therefore, biotype B has already passed through the three stages of invasion—i.e., importation, introduction, and establishment (Williamson and Fitter 1996)—to become a pest species. Furthermore, biotype Q in China, Korea, and Japan has also passed through the three stages to become a pest. However, we found biotype Q in Taiwan only in a poinsettia greenhouse, revealing that it is only in the importation stage, and has not yet been introduced (or released) to the environment.

Whether biotype B of *B. tabaci* is an invader has long been controversial. Molecular markers and viral disease are used to show incursions of biotype B and have indicated that it was transported on ornamental plants (Brown et al. 1995, Frohlich et al. 1999, De Barro et al. 2000). Furthermore, this study supports the invasion of biotype Q being a recent event related to ornamental plants, and it can be expected that it will soon spread worldwide as did biotype B through human trade activities. However, inherent levels of resistance to insecticides differ in biotypes B and Q (Khasdan et al. 2005). Insecticide applications have affected the dynamics and distribution of biotypes B and Q elsewhere (Khasdan et al. 2005). Therefore, ornamental crops should be quarantined to prevent the future invasion of these biotypes, and better ways to eliminate and control these two pests should be sought. Molecular markers will play an important role in monitoring and preventing their further invasion.

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