Evidence For Rapid Spatiotemporal Changes in Genetic Structure of an Alien Whitefly During Initial Invasion

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The sweetpotato whitefly Bemisia tabaci Q species is a recent invader and important pest of agricultural crops in China. This research tested the hypothesis that the Q populations that establish in agricultural fields in northern China each year are derived from multiple secondary introductions and/or local populations that overwinter in greenhouses (the pest cannot survive winters in the field in northern China). Here, we report the evidence that the Q populations in agricultural fields mainly derive from multiple secondary introductions. In addition, the common use of greenhouses during the winter in certain locations in northern China helps increase the genetic diversity and the genetic structure of the pest. The genetic structure information generated from this long-term and large-scale field analysis increases our understanding of B. tabaci Q as an invasive pest and has important implications for B. tabaci Q management.

Various factors, such as invasion history, pest dispersal ability, founder numbers, and passive dispersal by human activity¹⁴, affect the genetic structure and diversity of invasive pests. Introduced populations often experience rapid genetic differentiation and microevolution as they adapt to the new environmental conditions⁴⁻⁵. Knowledge of the population genetics can be used for predicting invasiveness and the efficacy of alternative control efforts and improving management of invasive species⁵. Although a substantial body of research exists describing the structure and diversity of an alien species after establishment, data regarding diversification during initial invasion are limited. An important reason for this is that the introduction often remains unrecognised until the alien population becomes very large⁶. Population genetic structure and diversity can also be readily affected by spatial and temporal factors⁷⁻¹¹, and several studies have highlighted the need for long-term and large-scale analyses¹²⁻¹⁴.

The sweetpotato whitefly, Bemisia tabaci (Gennadius), is an important agricultural pest worldwide and is regarded as a species complex comprising at least 28 cryptic species¹⁵⁻¹⁷. Some of these cryptic species have become invasive where they have been introduced. One species complex member, designated MED and commonly known as B. tabaci biotype Q (hereafter referred to as B. tabaci Q or Q), has been introduced to many countries from its original Mediterranean distribution, including China, over the past decade¹⁶. However, the various introduced B. tabaci Q populations have differed in invasive ability¹⁸⁻²¹ and other biological traits such as those related to virus transmission²²⁻²³. Genetic analysis of the introduced populations is a promising strategy to discern the microevolutionary and ecological adaptations that underlie the ability of this insect pest to invade agro-ecosystems. Such knowledge is important for the design and optimisation of sustainable pest management strategies³⁻⁴.

In China, B. tabaci Q was first detected in Yunnan, Beijing, and Henan provinces in 2003¹⁸. During subsequent years, B. tabaci Q has gradually displaced the previously well-established populations of MEAM1 (commonly known as B. tabaci biotype B) and has been the dominant species in most regions of China since 2008¹⁸⁻²⁰. Since 2005, the invasion process of B. tabaci Q has been well documented in many locations in Shandong Province in northern China²⁴⁻²⁶, and since this initial detection, we have continuously sampled whitefly populations from numerous Shandong locations²⁴. As is the case for other areas of China, the percentage of Q in B. tabaci populations has gradually increased, and Q has been the dominant species in most locations in Shandong Province since 2008²⁷⁻²⁸. B. tabaci Q cannot survive the winter in the field in Shandong²⁷, but can survive in greenhouses, which are commonly used in the area of Shouguang. Despite elimination by low
winter temperatures, Q has been present and abundant in the field during every growing season since its initial appearance in the province.

To explain how Q can appear in the field year after year in spite of its inability to overwinter in the field, we hypothesise that the initial populations detected in the field (i.e., from 2006 to 2012) derived from multiple, secondary introductions and/or from populations that overwintered in local greenhouses in Shouguang. The purpose of the present study is to test this hypothesis.

**Results**

*Changes in genetic diversity and in microsatellite allele frequencies.* From 2006 to 2012 in Shandong Province, the genetic diversity of *B. tabaci* Q (i.e. the value of *He*) increased over time only in one location (Shouguang) (Fig. 1) (*R*² = 0.673, *P* = 0.024), which is the location where greenhouses are widely used during winter. However, the values of *He* in the six other locations were not significantly correlated with time (*P* > 0.05). In addition, the relationship between average *He* and time was not statistically significant (*R*² = 0.486, *P* = 0.082) (Fig. S1).

**Analysis of genetic structure.** Of the 45 collections, 44 exhibited significant departures from Hardy-Weinberg equilibrium and had high and positive mean values of *Fis* (0.287–0.552), indicating the existence of heterozygote deficiencies (Table 1). With respect to pairwise comparisons between collections, 841 of 990 values of *Fst* (84.8%) were associated with a significant exact test (Table S1).

The results of a hierarchical analysis of molecular variance (AMOVA) revealed significant differences according to sample time (Table 2) and indicated no significant genetic difference between locations (Table 2). Further investment showed a significant difference in the genetic structure of *B. tabaci* Q as time progressed from 2006 to 2012. Of the 21 values of FCT (difference among groups), only seven were not significant (Table S2).

Analyses using BAPS software identified ten genetic clusters within the 45 collections (Figs. 2 and S2). In each year, 2–4 genetic clusters were observed with samples from the different locations. In 2006, three distinct genetic clusters were present for all four collections that were sampled in that year, but in 2007, the 2006 clustering pattern was replaced by new cluster formations for all four locations. In 2008, the 2007 clusters were replaced by novel clustering patterns in three of six locations, and in 2009, the genetic clusters from 2008 persisted in all locations except Linyi. In 2010, the 2009 clusters were replaced by other clusters at all locations, and in 2011, the 2010 clusters had, once again, been replaced by other clusters at all locations. In 2012, the genetic clusters from 2011 had been replaced by other clusters in four of the seven locations.

**Discussion**

The specimens we collected during 2006–2012 provide an excellent opportunity to examine the changes in *B. tabaci* Q genetic diversity and structure. Because *B. tabaci* Q cannot overwinter in the field in northern China, we hypothesised that the initial populations detected in the field each year derived from multiple, secondary introductions and/or from populations that overwintered in local greenhouses. To test this hypothesis, we analysed the spatiotemporal genetic changes of field populations using 7 years of microsatellite data from samples collected annually at seven locations in Shandong Province, China.

**Rapid spatiotemporal changes in genetic diversity.** The microsatellite DNA data describing genetic diversity and allele frequency presented here reveal a significant genetic shift at the seven field locations during the initial invasion of the whitefly *B. tabaci* Q in Shandong Province, China. At most locations, the genetic diversity (*He*) remained constant from 2006 to 2009, increased substantially from 2010 to 2011, and decreased slightly in 2012 (Table 1). The values of microsatellite allele frequencies and average number of alleles per locus (*Na*) were consistent with the trends in *He*.

The changes in genetic variation in *B. tabaci* Q populations were unexpected. We expected that shortly after populations became established, those in different locations in Shandong Province would experience selection due to insecticide exposure and wintering in the greenhouse, and that these selective forces would reduce genetic diversity. In support of this expectation, Chu et al. (2008) found that selection by the insecticide thiamethoxam decreased the genetic diversity of *B. tabaci* under laboratory conditions. Franklin et al. (2010) reported that heterozygosity levels in greenhouse populations of *Trichoplusia ni* were slightly lower in the spring than in the summer and fall, perhaps as a result of winter cleanup operations.

The increases in the genetic diversity indices may be associated with the repeated yearly introduction of alien whiteflies from other regions as a consequence of natural dispersal or human activities. Our results also suggest that the number of alien whiteflies introduced was highly variable over time, i.e., the number introduced was small during 2006–2009 and in 2012, but was large during 2010–2011. An increase in genetic diversity caused by new introductions of alien whiteflies, however, could be countered by winter clean up in greenhouses.

An interesting finding in this study was that the location with abundant greenhouses was the only location in which genetic diversity was significantly correlated with time (expressed as years). We infer that populations overwintering in greenhouses may play an important role in increasing the genetic diversity of *B. tabaci* Q in Shouguang, northern China.

**Rapid spatiotemporal changes in genetic structure.** The *Fst* values reveal substantial genetic structure in the collections of *B. tabaci* Q at the seven locations. These results indicate that gene flow was low between locations and that inbreeding was prevalent at most locations, which is consistent with Tsagkarakou et al. (2007), who documented low gene flow between *B. tabaci* Q populations in Greece that were separated by only a few kilometres. These data suggest that gene flow between whitefly populations may be minimal and that dispersal by *B. tabaci* Q is usually limited. Low gene flow between populations may contribute to the genetic differentiation of the populations across the region. BAPS analysis revealed the existence of at least ten genetic clusters of *B. tabaci* Q in Shandong Province.

The most interesting result in our study was the rapid change in population genetic structure, which was significantly associated with
Table 1 | Characteristics of *B. tabaci* Q in seven locations in Shandong Province from 2006 to 2012. Note that only four and six of the seven locations were sampled in 2006 and 2007, respectively.

| Location Code | Year | Host Plant                          | N   | Na  | Ne  | Ho  | He  | Nei | Fis  |
|---------------|------|-------------------------------------|-----|-----|-----|-----|-----|-----|------|
| Liaocheng     | 06–LC| Eggplant                            | 15  | 3.60| 2.134| 0.413| 0.439| 0.424| 0.413|
| Zibo          | 06–ZB| Eggplant, cotton                    | 30  | 4.60| 2.733| 0.373| 0.529| 0.520| 0.373|
| Dezhou        | 06–DZ| Cotton                              | 15  | 3.20| 2.061| 0.480| 0.607| 0.480| 0.388|
| Shouguang     | 06–SG| Eggplant                            | 14  | 2.60| 1.931| 0.389| 0.443| 0.426| 0.438|
| Jinan         | 07–JN| Eggplant                            | 15  | 3.40| 2.174| 0.453| 0.443| 0.438| 0.428|
| Liaocheng     | 07–LC| Cotton                              | 30  | 4.40| 2.472| 0.490| 0.443| 0.438| 0.457|
| Zibo          | 07–ZB| Eggplant                            | 30  | 3.00| 2.052| 0.473| 0.433| 0.426| 0.473|
| Linyi         | 07–LY| Eggplant, cucumber                  | 30  | 3.40| 1.866| 0.287| 0.290| 0.285| 0.287|
| Dezhou        | 07–DZ| Eggplant, cotton                    | 30  | 3.20| 2.026| 0.383| 0.408| 0.402| 0.387|
| Shouguang     | 07–SG| Cotton                              | 15  | 4.20| 2.319| 0.388| 0.476| 0.459| 0.388|
| Jinan         | 08–JN| Cotton, Japanese hop                | 30  | 3.00| 1.964| 0.473| 0.385| 0.447| 0.491|
| Zaozhuang     | 08–ZZ| Eggplant, cotton                    | 30  | 4.00| 2.453| 0.527| 0.493| 0.493| 0.493|
| Liaocheng     | 08–LC| Cotton                              | 30  | 3.80| 2.007| 0.440| 0.351| 0.345| 0.440|
| Zibo          | 08–ZB| Eggplant, cotton                    | 30  | 2.50| 1.769| 0.440| 0.385| 0.378| 0.378|
| Dezhou        | 08–DZ| Eggplant                            | 30  | 3.20| 2.007| 0.440| 0.351| 0.345| 0.440|
| Shouguang     | 08–SG| Eggplant                            | 15  | 4.20| 2.319| 0.388| 0.476| 0.459| 0.388|
| Jinan         | 09–JN| Eggplant, tomato                    | 30  | 4.00| 2.221| 0.379| 0.476| 0.468| 0.379|
| Zaozhuang     | 09–ZZ| Eggplant, tomato                    | 30  | 4.40| 2.434| 0.447| 0.486| 0.478| 0.379|
| Liaocheng     | 09–LC| Eggplant, tomato                    | 30  | 3.80| 2.179| 0.552| 0.438| 0.431| 0.552|
| Zibo          | 09–ZB| Eggplant, tomato                    | 30  | 3.80| 2.069| 0.433| 0.433| 0.436| 0.433|
| Linyi         | 09–LY| Eggplant, Japanese hop              | 30  | 3.80| 1.947| 0.447| 0.376| 0.370| 0.447|
| Dezhou        | 09–DZ| Eggplant                            | 30  | 3.20| 2.007| 0.440| 0.351| 0.345| 0.440|
| Zaozhuang     | 10–ZZ| Eggplant                            | 15  | 3.80| 2.507| 0.421| 0.567| 0.548| 0.421|
| Liaocheng     | 10–LC| Eggplant, cotton                    | 30  | 4.00| 2.561| 0.398| 0.586| 0.558| 0.398|
| Zibo          | 10–ZB| Eggplant, cotton                    | 30  | 4.80| 2.059| 0.351| 0.425| 0.418| 0.356|
| Linyi         | 10–LY| Eggplant                            | 30  | 4.80| 2.536| 0.402| 0.587| 0.576| 0.400|
| Dezhou        | 10–DZ| Eggplant, Japanese hop              | 30  | 3.60| 2.267| 0.420| 0.552| 0.543| 0.420|
| Shouguang     | 10–SG| Eggplant, tomato                    | 30  | 4.40| 2.362| 0.482| 0.566| 0.557| 0.479|
| Jinan         | 11–JN| Eggplant                            | 30  | 5.40| 2.759| 0.537| 0.537| 0.527| 0.371|
| Zaozhuang     | 11–ZZ| Eggplant, tomato                    | 29  | 5.40| 2.605| 0.489| 0.579| 0.569| 0.489|
| Liaocheng     | 11–LC| Eggplant                            | 30  | 3.00| 2.572| 0.407| 0.590| 0.580| 0.407|
| Zibo          | 11–ZB| Cotton, Japanese hop                | 30  | 5.00| 2.584| 0.418| 0.504| 0.496| 0.418|
| Linyi         | 11–LY| Eggplant, cotton                    | 30  | 5.40| 2.461| 0.463| 0.510| 0.491| 0.463|
| Dezhou        | 11–DZ| Eggplant, cotton                    | 29  | 4.40| 2.124| 0.414| 0.507| 0.498| 0.412|
| Shouguang     | 11–SG| Eggplant, tomato                    | 30  | 5.20| 2.264| 0.325| 0.444| 0.436| 0.325|
| Jinan         | 12–JN| Eggplant, cotton                    | 28  | 4.60| 2.489| 0.400| 0.546| 0.536| 0.400|
| Zaozhuang     | 12–ZZ| Eggplant                            | 28  | 4.20| 2.402| 0.340| 0.505| 0.495| 0.340|
| Liaocheng     | 12–LC| Eggplant                            | 28  | 4.60| 2.072| 0.313| 0.437| 0.430| 0.350|
| Zibo          | 12–ZB| Eggplant                            | 30  | 4.00| 2.133| 0.333| 0.464| 0.457| 0.361|
| Linyi         | 12–LY| Eggplant                            | 30  | 4.60| 2.059| 0.444| 0.599| 0.589| 0.444|
| Dezhou        | 12–DZ| Eggplant                            | 30  | 5.80| 2.735| 0.444| 0.599| 0.589| 0.444|
| Shouguang     | 12–SG| Eggplant                            | 30  | 5.80| 2.735| 0.444| 0.599| 0.589| 0.444|

For each sample, the following are indicated: sampling location, population code, date of collection, host plant, sample size (*N*), average number of alleles per locus (*Na*), the effective number of alleles (*Ne*), the observed heterozygosity (*Ho*), the expected heterozygosity (*He*), Nei’s expected heterozygosity (*Nei*), and the estimator of the fixation index (*Fis*). Significant values for heterozygote deficiency are in bold.
time rather than location according to hierarchical AMOVA
(Table 2). According to BAPS analysis for the data from 2006 to
2012, specific genetic clusters persisted for only 1–3 years in any
one location (Fig. 2), which suggests that genetic composition cha-

ged rapidly at those locations. This result is consistent with the gen-
etic changes in B. tabaci B over a short time frame in the Lockyer
Valley, Queensland, Australia. In that study, Dinsdale and collea-
gues used eight microsatellite loci and detected a significant temporal
change in the local genetic composition of B. tabaci B during the
growing seasons of 2006–2007.

The mechanism underlying the rapid spatiotemporal changes in
genetic structure of B. tabaci Q in Shandong Province is consistent
with the hypothesis that the populations detected in the field from
2006 to 2012 were derived from multiple, secondary introductions
and from populations that overwintered in local greenhouses. In the
present study, host and climate factors affecting the genetic composi-
tion were similar across the study area because the specimens were
collected from a limited number of host types and within the same
region; thus it seems unlikely that host or climate can explain the
changes and differences in genetic structure of B. tabaci Q. At the
Liaocheng location, for example, the host plants were eggplant and
cotton throughout the sampling period (2007–2012) but the genetic
structure changed. In support of the inference that there were mul-
tiple introductions of the pest, some new genetic clusters suddenly
appeared in different locations in the same year, e.g., three new
genetic clusters appeared in 2010 or 2011. Local whitefly populations
that overwinter in greenhouses may also affect the genetic structure,
e.g., the population in Shouguang in 2012 (12–SG), whose genetic
cluster differed from those of the other six locations in 2012, may be a
mixture of the local population and external whitefly individuals. As
a typical location with greenhouses where whiteflies can survive the
winter, Shouguang may have numerous local whitefly individuals
that survive the winter.

The application of insecticides cannot be ruled out as a factor
influencing genetic change because insecticides are widely used in
the study region. Prior studies suggest that the application of some
insecticides can cause population bottlenecks that enhance genetic
differentiation.

Implications for B. tabaci Q management and for future research.
The finding of rapid genetic changes in field populations of B. tabaci
Q during the initial invasion provides useful information concerning
the insect's invasion mechanism and its control. First, the results
indicate that both secondary introductions from other regions and
overwintering individuals from local greenhouses can contribute to
the substantial genetic variability, which would provide a genetic
basis for the adaptation of B. tabaci Q to new environments.
Second, a change in genetic cluster may be associated with a
change in biological and ecological characteristics. Understanding
the relationship between the genotypes and these characteristics, and
the monitoring of genotypes, can help guide management of B.
tabaci Q. For example, if a new genetic cluster involves a high
level of resistance to specific insecticides, the types of insecticides
can be adjusted accordingly.

Several questions remain regarding the genetic changes of invasive
B. tabaci Q in China. First, we do not know the geographic distri-
bution of the rapid genetic changes of B. tabaci Q populations; addi-
tional research is required to determine whether the changes in the
genetic clusters in Shandong Province reflect the changes at the

| Table 2 | Hierarchical AMOVA table and corresponding values
| for FCT (difference among groups), FSC (differences among collec-
tions within groups), and FST (differences among all collections) |

| Hierarchical structure | FCT  | FSC   | FST   |
|-----------------------|------|-------|-------|
| Location > Time       | -0.00398 | 0.17006** | 0.16676** |
| Time > Location       | 0.08570** | 0.09948** | 0.17666** |

*P ≤ 0.05, **P ≤ 0.001.

Figure 2 | B. tabaci Q sampling locations in Shandong Province during 2006–2012. The color-coded assignment of populations to ten clusters
identified by BAPS is shown in Figure S2 (B). In the graph, each color represents one cluster. The sampling locations are grouped by color to indicate
which groups are likely to represent distinct populations. The map was generated using GeoMapApp (version 2) (http://www.geomapapp.org/).
national scale. Second, the key factors affecting the spatiotemporal genetic changes remain to be determined, although many ecological factors have been explored or discussed for other species \[26,31,38\]. Finally, the potential effects of population genetic changes on the biotic characteristics of the populations should be explored. Two important characteristics concern the harbouring of endosymbionts and the transmission of plant viruses. The presence of the endosymbiont \textit{Rickettsia} in whiteflies enhances whitely fitness and spread \[1,14\], and variation in the percentage of invasive \textit{B. tabaci} Q with secondary endosymbionts was associated with temporal genetic changes in \textit{B. tabaci} Q in China \[1\]. Similarly, the acquisition of certain plant viruses by whitelys can modify whitely feeding behaviour \[23\].

### Methods

#### Sample collection

During July to September of 2006–2012, adult \textit{B. tabaci} were collected from host plants including four field crops (cotton, tomato, eggplant, and cucumber) and one weed (Japanese hop) at seven representative locations (Dezhou, DZ; Liaocheng, LC; Jinan, JN; Shouguang, SG; Zibo, ZB; Zhaohuazhou, ZZ; and Linyi, LY) in Shandong Province, China (Table 1). The geographical locations are shown using GeoMapApp v. 2.2 (Fig. 2). Among the seven locations, Shouguang is considered China’s main vegetable producer, and the abundant greenhouses in Shouguang are used to produce vegetables throughout the winter (http://en.wikipedia.org/wiki/Shouguang). Each \textit{B. tabaci} collection involved sampling whitelys from every second available plant until at least 100 whitely adults had been collected from each host. The living \textit{B. tabaci} individuals were stored in 95% ethyl alcohol at \(-20^\circ\)C until DNA extraction. Four collections were missing (Jinan and Linyi were not sampled in 2006, and Zibo was not sampled in 2006 or 2007), and there were, therefore, 45 rather than 49 collections in total.

#### DNA extraction, PCR amplification, and species determination

Genomic DNA was extracted from individual females (14–30 individuals) from each collection (one collection was defined as the samples from a single location in a single year), as described by Frohlich et al. \[1999\] \[26\]. The extracted genomic DNA was stored at \(-20^\circ\)C and used as template for PCR amplification. Our long-term field survey indicated that only two species from the \textit{B. tabaci} species complex (\textit{B. tabaci} B and Q) were represented in Shandong Province \[20,23\]. \textit{B. tabaci} species was determined using cleaved amplified polymorphic sequences (CAPS) of the mitochondrial cytochrome oxidase I (mtCOI) gene (about 620 bp), which was amplified with primers (C1-J-2195/R-BQ-2819), as reported by Chu et al. \[2011\] \[23\]. The amplified mtCOI fragment was cleaved by the restriction endonuclease \textit{VspI}, mtCOI fragments that can be cut by \textit{VspI} belong to \textit{B. tabaci} Q and those that cannot be cut belong to \textit{B. tabaci} B.

#### Microsatellite genotyping

Microsatellite markers have been widely used to analyse the population structure of \textit{B. tabaci} \[23,26,28\]. In this study, primers for a suite of five microsatellites (BEM6, BEM11, BEM25, BEM1, and BEM37) were used to amplify loci from 1,251 \textit{B. tabaci} Q individuals collected in Shandong Province during 2006–2012, as described by De Barro et al. \[2003\] \[26\]. Amplification products of the microsatellite loci were run on an ABI 3730xl DNA analyser. Allele size was determined by comparing the mobility of the PCR products to that of the \textit{GenScanTM} 400HD size standard (Applied Biosystems).

#### Data analysis

For each of the 45 microsatellite data sets (seven locations) of \textit{B. tabaci} Q, the following genetic diversity indexes were calculated using POPGENE v.1.31 \[26\]: observed number of alleles (\(Q\)), the following genetic diversity indexes were calculated using POPGENE v.1.31 spread31, and variation in the percentage of invasive \textit{B. tabaci} Q with secondary endosymbionts was associated with temporal genetic changes in \textit{B. tabaci} Q in China \[1\]. Similarly, the acquisition of certain plant viruses by whitelys can modify whitely feeding behaviour \[23\].

1. Timm, A. E., Geertsema, H. & Warnich, L. Population genetic structure of \textit{Grapholitha molesta} (Lepidoptera: Tortricidae) in South Africa. \textit{Ann. Entomol. Soc. Amer.} 101, 197–203 (2008).
33. Ryan, W. et al. Global Multi-Resolution Topography Synthesis. Geochem. Geophys. Geosyst. 10, (Q03014) (2009).
34. Frohlich, D. R. et al. A phylogeographical analysis of the Bemisia tabaci species complex based on mitochondrial DNA markers. Mol. Ecol. 8, 1683–1691 (1999).
35. Chu, D., Zhang, Y. J. & Wan, F. H. Cryptic invasion of the exotic Bemisia tabaci biotype Q occurred widespread in Shandong Province of China. Fla. Entomol. 93, 203–207 (2010).
36. Chu, D., Wan, F. H., Zhang, Y. J. & Brown, J. K. Change in the biotype composition of Bemisia tabaci in Shandong Province of China from 2005 to 2008. Env. Entomol. 39, 1028–1036 (2010).
37. Khasdan, V. et al. DNA markers for identifying biotypes B and Q of Bemisia tabaci (Hemiptera: Aleyrodidae) and studying population dynamics. Bull. Entomol. Res. 95, 605–613 (2005).
38. De Barro, P. J. Genetic structure of the whitefly Bemisia tabaci in the Asia–Pacific region revealed using microsatellite markers. Mol. Ecol. 14, 3695–3718 (2005).
39. Delatte, H. et al. Microsatellites reveal extensive geographical, ecological and genetic contacts between invasive and indigenous whitefly biotypes in an insular environment. Genet. Res. 87, 109–124 (2006).
40. De Barro, P. J. et al. Isolation and characterization of microsatellite loci in Bemisia tabaci. Mol. Ecol. Notes 3, 40–43 (2003).
41. Yeh, F. C. et al. POPGENE, the user–friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Edmonton, Canada (1997).
42. Raymond, M. & Rousset, F. GENEPOP (version 1.2); population genetics software for exact tests and ecumenicism. J. Hered. 86, 248–249 (1995).
43. Excoffier, L., Laval, G. & Schneider, S. Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evol. Bioinfor. Online 1, 47–50 (2005).
44. Corander, J., Siren, J. & Arjas, E. Bayesian spatial modeling of genetic population structure. Comput. Stat. 23, 111–129 (2008).

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Author contributions
D.C. and Y.J.Z. designed the experiment. D.C. and D.G. performed the experiment. Y.L.T., J.L. and D.F.J. contributed reagents/materials. D.C. and Y.J.Z. wrote the paper.

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