Distribution of Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) Mitotypes in Commercial Cotton Fields in the Punjab Province of Pakistan

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Source: Florida Entomologist, 103(1) : 41-47

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.103.0407
Distribution of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) mitotypes in commercial cotton fields in the Punjab province of Pakistan

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**Abstract**

The *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) sibling species group is comprised of genetic variants defined by biological differences or a fragment of mitochondrial cytochrome oxidase I gene sequence (mitotype) that allows for phylogeographic affiliation. Some mitotypes may cause damage to crop plants by feeding and transmission of plant viruses. In Pakistan, cotton-vegetable agroecosystems are vulnerable to whitefly-transmitted virus (genus Begomovirus; family Geminiviridae) infection. The identity and distribution of the whitefly *B. tabaci* mitotypes associated with the cotton crop were studied in 8 districts in the Punjab Province from 2014 to 2016. Phylogenetic analysis of the 3′-fragment of the mitochondrial cytochrome oxidase I gene indicated the predominant haplotypes belonged to the Asia II-1 mitotype, with pairwise distances ranging from 0.15 to 3.2%. Pairwise distances showed that *B. tabaci* haplotype diversity varied by district, with the Khewal harboring the highest divergence at 1.37%, compared to the lowest at 0.50% in the Dera Ghazi Khan district. The median-joining network analysis showed genetic expansion, or a ‘recovery’ trend, following the declining genetic diversity that occurred during the late 1990s to the early 2000s. The Asia II-1 mitotype group was the predominant whitefly vector species in Punjab Province. The haplotype network provides documentation of continued genetic expansion among the *B. tabaci* populations in the Punjab, which is consistent with previously reported trends among whiteflies sampled in the same or nearby districts from 2012 to 2014. Genetic expansion varied among districts and could be explained by factors unique to each district, i.e., management practices that influence *B. tabaci* mitotype composition, whitefly susceptibility to cotton leaf curl disease complex, and cotton genotype.

**Key Words:** haplotype; median-joining network analysis; mitochondria cytochrome oxidase I gene; phylogenetic analysis; population genetics

**Resumen**

El complejo de moscas blancas *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), comprende grupos genéticos cuya afiliación filo-geográfica puede ser determinada mediante una serie de rasgos biológicos o el uso de un fragmento del gen mitochondrial citocromo oxidasa I (mitotipo). Algunos mitotipos de *B. tabaci* pueden ocasionar daños económicos importantes en cultivos comerciales debido a su alimentación y a la transmisión efectiva de virus vegetales, por ejemplo, en Paquistán, los agro-ecosistemas de algodón son altamente vulnerables a virus transmitidos por moscas blancas (genero Begomovirus; familia Gemíniviridae). En este artículo, se estudió la identidad y distribución geográfica de los principales mitotipos de *B. tabaci* que se encuentran infestando parcelas comerciales de algodón en 8 distritos de la provincia de Punjab, Paquistán, durante los años 2014-2016. Se determinó mediante un análisis filo-genético del fragmento 3′ del gen citocromo oxidasa I, que el haplótipo más predominante perteneció al mitotipo Asia II-1, con distancias genéticas que variaron entre 0.15-3.2%. Se determinó que las distancias genéticas entre haplotipos varían por distrito geográfico, con la divergencia más alta de 1.37% en Khanewal, comparado con la divergencia más baja de 0.50% en el distrito Dera Ghazi Khan. Se demostró mediante un análisis de red de haplotipos, que la población de moscas blancas continúa en un proceso de expansión genética que varía por distrito, lo que coincide con el escenario de expansión demográfica reportada en poblaciones de mosca blanca colectadas en localidades similares durante el periodo 2012-2014. El patrón geográfico de expansión observado puede ser atribuido a factores únicos de cada distrito geográfico, por ejemplo: prácticas de manejo de plagas que alteran la composición mitotípica de la mosca blanca, eficiencia del vector para transmitir virus y genotipo de algodón sembrado.

**Palabras Claves:** haplotipo; red de haplotipos median-joining; gen mitochondrial citocromo oxidasa I; análisis filogenético; genética de poblaciones

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Supplementary Table S1; supplementary material is online at http://purl.fcla.edu/fcla/entomologist/browse

2020 — Florida Entomologist — Volume 103, No. 1
& Halsey 1978; Cock 1993), with more than 200 plant species recorded as hosts in Pakistan (Attique et al. 2003).

Recently, *B. tabaci* mitotypes and their genetic groups have been differentiated based on phylogenetic analysis of an approximately 580 to 780 base pair fragment of the 3′-end of the mitochondrial cytochrome oxidase I gene (mtCOI-3′) sequence (Frohlich et al. 1999), with several theoretical estimates proposed for species delineation at 3.5 to 4.0% nucleotide (nt) divergence, irrespective of corresponding biological species criteria (Dinsdale et al. 2010; Lee et al. 2013). To date, no consensus has been reached for a globally applicable species demarcation system within the *B. tabaci* taxon. Even so, understanding the mitotype composition and population structure of *B. tabaci* in agro-ecosystems is important, because management practices may cause shifts in the prevalence of the different *B. tabaci* mitotypes, which in turn may govern begomovirus transmission competency, potentially influencing the dynamics of the different mitotypes and cotton leaf curl disease outbreaks, possibly resulting in shifting of 1 or more components of the pathosystem. Different mitotypes of the *B. tabaci* sibling species group have been shown to respond differently to various pesticide regimes, the range of plants suitable as hosts, and environmental factors. These and other factors strongly influence the composition of the *B. tabaci* mitotypes and their dynamics in agro-ecosystems (Bedford et al. 1994; Brown 2007, 2010; Gill & Brown 2010).

Plant viruses transmitted by whiteflies belong to 1 of 5 genera (Jones 2003; Navas-Castillo et al. 2011). Among the economically important plant viruses transmitted by whiteflies, the genus Begomovirus (Geminiviridae) is the most widespread, comprising more than 400 species (https://talk.ictvonline.org/taxonomy/), including many known to cause economically important plant diseases (Brown et al. 2012). In Pakistan and India, over 60 begomoviral species or strains have been described from cultivated and wild plant species (Ho et al. 2017). Cotton leaf curl disease is caused by several monopartite begomoviruses or strains, and associated betasatellites that are endemic to much of southern Asia, including Pakistan (Zhou et al. 1998; Saleem et al. 2016). During the past several decades, the emergence of cotton leaf curl disease has resulted in major economic losses in the cotton producing areas of Pakistan (Briddon et al. 2001; Zubair et al. 2017). Understanding the distribution of the different whitefly mitotypes and their population dynamics, which can be predicted by spatio-temporal patterns of mtCOI diversity, have become essential for devising effective management approaches. Such strategies are further enhanced by knowledge of the host range, pesticide resistance, and other biological characteristics associated with the range of different mitotypes that transmit the equally diverse begomovirus species group have been shown to respond differently to various pesti¬cide regimes, the range of plants suitable as hosts, and environmental factors. These and other factors strongly influence the composition of the *B. tabaci* mitotypes and their dynamics in agro-ecosystems (Bedford et al. 1994; Brown 2007, 2010; Gill & Brown 2010).

The objective of this study was to determine the mitotype composition and spatial patterns of genetic divergence of the *B. tabaci* whitefly vector collected from commercial cotton fields in the southern Punjab districts of Pakistan from 2014 to 2016. Mitotypes of the whitefly *B. tabaci* were identified phylogenetically based on the mtCOI-3′ gene sequence, and the structure of haplotypes was studied using a median-joining network analysis.

## Materials and Methods

### WHITEFLY FIELD COLLECTIONS

Whiteflies were collected from infested cotton plants in 8 districts of the southern Punjab Province, including Bahawalpur, Dera Ghazi Khan, Khanewal, Lodhran, Multan, Muzaffargarh, Rahim Yar Khan, and Vehari, Pakistan, from 2014 to 2016 (Fig. 1). Adult whiteflies were collected using a hand-held aspirator, transferred to a microfuge tube containing 95% ethanol, and stored at −20 °C. Standard management practices and the same cotton varieties were implemented at all of the whitefly collection sites. Twenty-four whiteflies per district (192 total) were sequenced, and haplotypes were assigned to a sister or major clade based on the mtCOI sequence fragment.

### WHITEFLY DNA ISOLATION, POLYMERASE CHAIN REACTION AMPLIFICATION, AND DNA SEQUENCING

Total DNA was purified from individual whiteflies using a previously published protocol (Zhang et al. 1998) with modifications (Paredes-Montero et al. 2019). Briefly, whiteflies were dipped in double-distilled water to dilute the ethanol, followed by blotting against the torn edge of a piece of No. 1 Whatman filter paper (Sigma-Aldrich, Darmstadt, Germany) to remove the distilled water. Each whitefly was ground with a micro-pestle in the bottom of a 1.0 mL microfuge tube, containing 250 μL lysis buffer. The lysis buffer contained 100 mM Tris-HCl, 1.4 M NaCl, 20 mM EDTA, 2% hexadecyltrimethylammonium bromide (CTAB) (Sigma-Aldrich, Darmstadt, Germany), and 20 μg per reaction of protease K. The lysate was incubated at 55 °C for 1 h followed by 10 min at 65 °C. One volume of chloroform:isoamyl-alcohol (24:1) was added, the contents were mixed by inversion, and the emulsion was separated by centrifugation, at 14,000 rpm for 3 min. The DNA was precipitated by the addition of 1 volume isopropanol and 40 μg glycogen, followed by incubation at 4 °C for 10 min. The pellet was collected by micro-centrifugation at 14,000 rpm for 10 min, washed with 70% ethanol, air dried, dissolved in 30 μL ddH$_2$O, and stored at −20 °C.

To detect the possible presence of the exotic B mitotype [North Africa-Mediterranean-Middle East major clade (NAF-MED-ME (III)] among the endemic Asia I and II mitotypes (major clades) (Brown 2010), samples were subjected to polymerase chain reaction amplification using the previously reported B-mitotype-specific primers 5′-BtBF1F-3′ [TATTCTCCTACCCACTATAA] and 5′-WfBr2R-3′ [GCT-TAAATCTTACTAACCCGAC], yielding an expected size fragment of 350 base pairs (Andreason et al. 2017). The negative samples were subjected to polymerase chain reaction amplification using the universal mtCOI primers C1-J-Z195F 5′-TGGATTTTTTGGCTATCCAGAAGT-3′ and L2-N-3014R 5′-TCCAATGCACTAATCTGCACATATAA-3′ that yield an expected size amplicon of about 850 base pairs for *B. tabaci* (Frohlich et al. 2003; Navas-Castillo et al. 2011). Among the economically important plant viruses transmitted by whiteflies, the genus Begomovirus (Geminiviridae) is the most widespread, comprising more than 400 species (https://talk.ictvonline.org/taxonomy/), including many known to cause economically important plant diseases (Brown et al. 2012). In Pakistan and India, over 60 begomoviral species or strains have been described from cultivated and wild plant species (Ho et al. 2017). Cotton leaf curl disease is caused by several monopartite begomoviruses or strains, and associated betasatellites that are endemic to much of southern Asia, including Pakistan (Zhou et al. 1998; Saleem et al. 2016). During the past several decades, the emergence of cotton leaf curl disease has resulted in major economic losses in the cotton producing areas of Pakistan (Briddon et al. 2001; Zubair et al. 2017). Understanding the distribution of the different whitefly mitotypes and their population dynamics, which can be predicted by spatio-temporal patterns of mtCOI diversity, have become essential for devising effective management approaches. Such strategies are further enhanced by knowledge of the host range, pesticide resistance, and other biological characteristics associated with the range of different mitotypes that transmit the equally diverse begomoviral species and strains that cause cotton leaf curl disease, as well as mitotype-virus specific factors that directly or indirectly influence the efficiency of whitefly-mediated transmission.

**Fig. 1.** Map of the Punjab Province in Pakistan showing the locations of whitefly sample collections, and distribution of the Asia II-1 mitotype of *Bemisia tabaci*, represented by the star symbol.
GENETIC DIVERSITY AMONG ASIA II-1 HAPLOTYPES

The corrected pairwise distance among Asia II-1 haplotypes was 0.15 to 3.2%, with extent of divergence varying primarily by district (Fig. 3). Whiteflies collected in the districts of Muzaffargarh and Khanewal exhibited the highest pairwise divergence at 1.06 and 1.37%, respectively, whereas the range of pairwise distances for the other 6 districts was 0.50 to 0.77%, with the Dera Ghazi Khan district showing the lowest divergence at 0.50% (Fig. 3). The differences in genetic diversity are reflective of the dynamic patterns of diversification, which has been shown to influence mitotype distribution. Accordingly, when certain mitotypes show differences in transmission competency over others, the distribution and spread of cotton leaf curl disease-begomoviruses in cotton and vegetable crops can be altered dramatically, or result in the emergence of previously unrecognized species or strains, often due to recombination (Bisaro 1994; Brown 2001; Faquett et al. 2005; Sallem et al. 2016).
Discussion

The results indicated that Asia II-1 group sub-mitotype 1 was the only *B. tabaci* variant among the samples collected from the cotton-growing areas of the Punjab Province. This observation is consistent with recent studies that reported it to be the predominant mitotype in agroecosystems in the Punjab and Sindh provinces (Ashfaq et al. 2014; Masood et al. 2017; Islam et al. 2018; Paredes-Montero et al. 2019). Five other endemic mitotypes or sub-mitotypes have been documented previously in Pakistan agroecosystems, namely Asia I and Asia II-1, Asia II-5, Asia II-7, and Asia II-8 (Firdaus et al. 2013; Masood et al. 2017; Islam et al. 2018; Paredes-Montero et al. 2019). Additionally, there is one report of the B mitotype (Ashfaq et al. 2014) which is phylogeographically affiliated with the NAF-MED-ME (III) major clade, and so is thought to have been introduced only recently (Ashfaq et al. 2014).

The diversity of haplotypes given by the corrected pairwise distances varied by district. The most highly divergent haplotypes at 1.37% were found in Khanewal, whereas in Dera Ghazi Khan, haplotypes differed only by 0.5%. The range of genetic divergence among whiteflies from the different districts studied was found to be similar to divergences reported in several previous studies (Ashfaq et al. 2014; Masood et al. 2017; Paredes-Montero et al. 2019), suggesting that differences in whitefly management practices may have favored or limited establishment or displacement of certain haplotypes compared to others (Chu et al. 2010; Horowitz & Ishaaya 2014). Certain classes of insecticides over others have been shown to favor outbreaks of different *B. tabaci* mitotypes/sub-mitotypes, which appears to be due to differences in their ability to develop insecticide resistance (Bedford et al. 1994; Coats et al. 1994; Anthony et al. 1995; Denholm et al. 1998; Ahmad et al. 2002; Dennehy et al. 2010; Luo et al. 2010; Horowitz & Ishaaya 2014).

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**Fig. 2.** Phylogenetic tree showing the relationship among members of the *Bemisia tabaci* complex found in Asia, based on the 3′-mitochondria cytochrome oxidase I gene (mtCOI-3′). All whiteflies collected for this study during 2014 to 2016, were identified as the Asia II-1 mitotype. The sequence of the *Bemisia afer* species was used as ‘outgroup’ to root the tree. The sequences in the upper-most collapsed clade clustered all of the sequences obtained in this study.
The network of haplotypes exhibited a high frequency of unique mutations that gave rise to several low-frequency haplotypes that diverged from the predominant haplotype in 1 or 2 mutations, forming a star-like structure (Mirol et al. 2008). This pattern is suggestive of genetic expansion or recovery from a recent bottleneck within the Asia II-1 populations. A previous study conducted in these same locations from 2012 to 2014 showed a similar scenario, with haplotype diversity levels starting to recover after a dramatic decline in genetic diversity among Asia II-1 haplotypes starting in the early 2000s (Paredes-Montero et al. 2019). It was hypothesized that this pattern of reduced genetic variation could have been due to the reasonably well-documented overuse of pesticides in cotton agroecosystems over the last 2 decades (Ahmad et al. 2002). Two yr after initial signs of recovery occurred, the rate of genetic expansion was about the same for B. tabaci collected from 2014 to 2016; however, the number of predominant haplotypes increased from 1 to 4 in relation to that reported by Paredes-Montero et al. (2019). In this context, and despite the smaller sample size analyzed in this study, at 107 compared to 538 samples collected in many of the same or additional districts analyzed by Paredes-Montero et al. (2019), the genetic signatures indicated the continued predominance of haplotypes classified in the subclade Asia II-1 in cotton grown in the Punjab Province from 2014 to 2016. Therefore, based on these observations, sustained genetic expansion of the populations is predicted to reach a plateau in subsequent yr in the absence of changes in cotton varieties or management practices.

The exclusive presence of Asia II-1 in cotton-agroecosystems in the southern Punjab Province may explain in part the recent spread of several predominant strains and species of cotton leaf curl disease there (Paredes-Montero et al. 2019). This hypothesis is supported by the high incidence of cotton leaf curl disease observed in cotton plantings where the Asia II-1 mitotype predominates. In contrast, lower disease incidence has been reported in the southern province of Sindh (Simón et al. 2003; Ahmed et al. 2011; Pan et al. 2018), where the B biotype occurs (Ashfaq et al. 2014) together with mitotypes endemic to the Asian continent.
(Ashfaq et al. 2014; Masood et al. 2017; Islam et al. 2018; Paredes-Montero et al. 2019). Based on one study, the (exotic) B mitotype showed low efficiency to no transmission of an isolate of the cotton leaf curl multian virus introduced recently in China (Pan et al. 2018), compared to variable rates of transmission by the introduced Q mitotype, or high rates of transmission by mitotypes endemic to Asia. This evidence for differential transmission specificity, together with a low transmission frequency of cotton leaf curl multian virus-transmission by the B mitotype suggests that, although the introduced B mitotype has established in at least 1 locale in Pakistan, it is unlikely to have a prominent role in the transmission of the majority of begomoviruses occurring in Pakistan. However, the B mitotype has been shown to be a competent vector of other New and Old World begomoviruses, and so it could pose a threat as a begomovirus vector if any of those viruses are introduced into Pakistan in the future.

**Conclusion**

The Asia II-1 was identified as the predominant mitotype among *B. tabaci* samples collected from cotton in southern Punjab, Pakistan. Here, 2 lines of evidence underscore differences in diversification among and between different *B. tabaci* mitotypes associated with the different districts. First, the intra-mitotype diversity was found to vary by district, which most likely is indicative of differential mitotype responsiveness to locally imposed agricultural practices. Second, a continued increase in haplotype diversity, or genetic expansion, was observed following a notable decline in diversity beginning in about 2010 with the initiation of recovery during 2014 to 2016 that could in time lead to restored diversity, perhaps approaching the previously high levels, and the geographic redistribution of the Asia II-1 and other sub-mitotypes in the southern Punjab Province of Pakistan. Several interacting factors are thought to be involved, including altered management practices implemented since 2010, including use of different pesticide regimes for whitefly control, particularly those that cause minimal agroecosystem and natural enemy disturbances. Additionally, with the release of tolerant or resistant cotton leaf curl disease cotton varieties, transmission frequencies may have been reduced, which if so encourages the routinely judicious use of insecticides. Routine monitoring of the population structure and distribution of *B. tabaci* mitotypes and cotton leaf curl disease-begomoviruses in cotton or cotton-vegetable agroecosystems in the Punjab Province is expected to contribute to implementing best practices that contribute to avoiding upsurges in whitefly that lead to cotton leaf curl disease outbreaks. Consequently, the new information presented here as well as that from previous studies of historical fluxes in *B. tabaci* mitotype and begomovirus distribution are extremely valuable for predicting these outbreaks.

**Acknowledgments**

The authors wish to thank Muhammad Naeem Aslam of CAB International-South Asia for his assistance with whitefly sample collections. The first author expresses sincere gratitude to the Higher Education Commission of Pakistan for the International Research Support Initiative Program fellowship that provided funding to cover the cost of travel and lodging, and partial support of the research reported here, carried out at the School of Plant Sciences, University of Arizona, Tucson, Arizona, USA.

**References Cited**

Ahmad MZ, De Barro PJ, Greerf JM, Ren S-X, Naveed M, Qiu B-L. 2011. Genetic identity of the *Bemisia tabaci* species complex and association with high cotton leaf curl disease (CLCuD) incidence in Pakistan. Pest Management Science 67: 307–317.

Andreasen SA, Arif M, Brown JK, Ochoa-Corona F, Fletcher J, Wayadande A. 2017. Single-target and multiplex discrimination of whiteflies (Hemiptera: Aleyrodidae) *Bemisia tabaci* and *Trialeurodes vaporariorum* with modified priming oligonucleotide thermodynamics. Journal of Economic Entomology 110: 1821–1830.

Anthony NM, Brown JK, Markham PG, Frenchenconstant RH. 1995. Molecular analysis of cyclodiene resistance-associated mutations among populations of the sweetpotato whitefly *Bemisia tabaci*. Pesticide Biochemistry and Physiology 51: 220–228.

Ashfaq M, Hebert PDN, Mirza MS, Khan AM, Mansoor S, Shah GS, Zafar Y, Wang X-L. 2014. DNA barcoding of *Bemisia tabaci* complex (Hemiptera: Aleyrodidae) reveals southerly expansion of the dominant whitefly species on cotton in Pakistan. PLoS ONE 9: e104485. doi: 10.1371/journal.pone.0104485

Attique MR, Rafiq M, Ghaffar A, Ahmad Z, Mohyuddin AI. 2003. Hosts of *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodidae) in cotton areas of Punjab, Pakistan. Crop Protection 22: 715–720.

Bandelt Hj, Forster P, Rohly A. 1999. Median-joining networks for inferring intra-specific phylogenies. Molecular Biology and Evolution 16: 37–48.

Bedford ID, Briddon RW, Brown JK, Rosell RC, Markham PG. 1994. Geminivirus transmission and biological characterization of *Bemisia tabaci* (Gennadius) biotypes from different geographic regions. Annals of Applied Biology 125: 311–325.

Bisaro DM. 1994. Recombination in the Geminiviruses: mechanisms for maintaining genome size and generating genomic diversity, pp. 39–60 In Paszkowski J [ed.], Homologous Recombination and Gene Silencing in Plants. Springer, Dordrecht, Netherlands.

Briddon RW, Mansoor S, Bedford ID, Pinner MS, Saunders K, Stanley J, Zafar Y, Malik KA, Markham PG. 2001. Identification of DNA components required for induction of cotton leaf curl disease. Virology 285: 234–243.

Brown JK. 2001. The molecular epidemiology of begomoviruses, pp. 279–316 In Khan JA, Dijkstra J [eds.], Plant Viruses As Molecular Pathogens. CRC Press, New York, USA.

Brown JK. 2007. The *Bemisia tabaci* complex: genetic and phenotypic variability drives begomovirus spread and virus diversification. Plant Disease 1: 25–56.

Brown JK. 2010. Phylogenetic biology of the *Bemisia tabaci* sibbling species group, pp. 31–67 In Stansly PA, Naranjo SE [eds.], *Bemisia*: Bionomics and Management of a Global Pest. Springer, Dordrecht, Netherlands.

Brown JK, Faquet CM, Briddon RW, Zerbini M, Moriones E, Navas-Castillo J. 2012. Geminivirus, pp. 351–373 In King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ [eds.], Virus Taxonomy: Classification and Nomenclature of Viruses: Ninth Report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press, San Diego, California, USA.

Brown JK, Frohlich DR, Rosell RC. 1995. The sweetpotato or silverleaf whiteflies: biotypes of *Bemisia tabaci* or a species complex? Annual Review of Entomology 40: 511–534.

Caballero RDJ. 2007. Systematics of the *Bemisia tabaci* complex and the role of endosymbionts in reproductive compatibility. Ph.D. dissertation, The University of Arizona, Tucson, Arizona, USA.

Chu D, Wan HF, Zhang YJ, Brown JK. 2010. Change in the biotype composition of *Bemisia tabaci* in Shandong province of China from 2005 to 2008. Environmental Entomology 39: 1028–1036.

Coats SA, Brown JK, Hendrix DL. 1994. Biochemical characterization of biotype-specific esterases in the whitefly, *Bemisia tabaci* Genn (Homoptera: Aleyrodidae). Insect Biochemistry and Molecular Biology 24: 723–728.

Cock MJW. 1993. *Bemisia tabaci*: an update 1986–92 on the cotton whitefly with an annotated bibliography. International Institute of Biological Control, Berkshire, United Kingdom.

Darriba D, Taboada GL, Doallo R, Posada D. 2012. *ModelTest* 2: More models, new heuristics and parallel computing. Nature Methods 9: 772–772.

Denholm I, Cahiil M, Dennehy TJ, Horowitz AR. 1998. Challenges with managing insecticide resistance in agricultural pests, exemplified by the whitefly *Bemisia tabaci*. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences 353: 1757–1767.

Dennehy TJ, Degain BA, Harpold VS, Zaborac M, Morin S, Fabrick JA, Nichols RL, Brown JK, Byrne FJ, Li X. 2010. Extraordinary resistance to insecticides reveals exotic Q biotype of *Bemisia tabaci* in the new world. Journal of Economic Entomology 103: 2174–2186.

Dinsdale A, Cook L, Riggins C, Buckley YM, De Barro P. 2010. Refined global analysis of *Bemisia tabaci* (Hemiptera: Sternorrhyncha: Aleyrodidae) mitochondrial cytochrome oxidase 1 to identify species level genetic boundaries. Annals of the Entomological Society of America 103: 196–208.
Gill RJ, Brown JK. 2010. Systematics of Bemisia tabaci species complex: additions from different parts of the world. Insect Science 20: 723–733.

Firdaus S, Vosman B, Hidayati N, Jaya Supena ED, Visser GFR, van Heusden AW. 2019. A phylogeographical analysis of the Bemisia tabaci species complex based on mitochondrial DNA markers. Molecular Ecology 8: 1683–1691.

Gill RJ, Brown JK. 2010. Systematics of Bemisia and Bemisia relatives: can molecular techniques solve the Bemisia tabaci complex conundrum—a taxonomist’s viewpoint, pp. 5–29 in Stanisly PA, Nananj SE [eds.], Bemisia: Bionomics and Management of a Global Pest. Springer, Dordrecht, Netherlands.

Gussow D, Clackson T. 1989. Direct clone characterization from plaques and colonies by the polymerase chain reaction. Nucleic Acids Research 17: 4000. doi:10.1093/nar/17.10.4000

Hadjistylli M, Roderick GK, Brown JK. 2016. Global population structure of a worldwide pest and virus vector: genetic diversity and population history of the Bemisia tabaci sibling species group. PLOS ONE 11: e0165105. doi: 10.1371/journal.pone.0165105

Hasegawa M, Kishino H, Yano T. 1985.Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution 22: 160–174.

Ho ES, Newsom-Stewart CM, Diarra L, McCauley CS. 2017. gb4gv: a genome browser for geminivirus. PeerJ 5: e3165. https://doi.org/10.7717/peerj.3165

Horowitz AR, Ishaya I. 2014. Dynamics of biotypes B and Q of the whitefly Bemisia tabaci and its impact on insecticide resistance. Pest Management Science 70: 1568–1572.

Islam W, Lin W, Qasim M, Islam SU, Ali H, Adnan M, Arif M, Du Z, Wu Z. 2018. A nation-wide genetic survey revealed a complex population structure of Bemisia tabaci in Pakistan. Acta Tropica 183: 119–125.

Jones DR. 2003. Plant viruses transmission by whiteflies. European Journal of Oral Entomology 7: 680. https://doi.org/10.1038/s41598-017-00727-2

Luo C, Jones CM, Devine G, Zhang F, Denholm I, Gorman K. 2010. Insecticide resistance in Bemisia tabaci biotype Q (Hemiptera: Aleyrodidae) from China. Crop Protection 29: 429–434.

Masood M, Amin I, Hassan I, Mansoor S, Brown JK, Briddon RW. 2017. Diversity and distribution of cryptic species of the Bemisia tabaci (Hemiptera: Aleyrodidae) complex in Pakistan. Journal of Economic Entomology 110: 2295–2300.

Mirol PM, Routtu J, Hoikkala A, Butlin RK. 2008. Signals of demographic expansion in Drosophila virilis. BMC Evolutionary Biology 8: 59. doi:10.1186/1471-2148-5-89

Mound LA, Halsey SH. 1978. Whitefly of the world: a systematic catalogue of the Aleyrodidae (Homoptera) with host plant and natural enemy data. British Museum (Natural History) and John Wiley & Sons, Chichester, United Kingdom.

Navas-Castillo J, Fiallo-Olives E, Sanchez-Campos S. 2011. Emerging virus diseases transmitted by whiteflies. Annual Review of Phytopathology 49: 219–248.

Paredes-Montero JR, Hamed U, Zia-Ur-Rehman M, Rasoog G, Haider MS, Hermann H-W, Brown JK. 2019. Demographic expansion of the predominant Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) mitotypes associated with the cotton leaf curl virus epidemic in Pakistan. Annals of the Entomological Society of America 112: 265–280.

R Core Team. 2019. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/ (last accessed 5 Nov 2019)

Saleem H, Nahid N, Shakir S, Ijaz S, Murtaza G, Khan AA, Mubin M, Nawaz-ul-Rehman MS, Melcher U. 2016. Diversity, mutation and recombination analysis of cotton leaf curl geminiviruses. PLOS ONE 11: e0151161. doi: 10.1371/journal.pone.0151161

Simón B, Cenis JL, Betta F, Khalid S, Moreno IM, Fraile A, García-Arenal F. 2003. Genetic structure of field populations of begomoviruses and of their vector Bemisia tabaci in Pakistan. Phytopathology 93: 1422–1429.

Song H, Buhay JE, Whiting MF, Crandall KA. 2008. Many species in one: DNA barcoding overestimates the number of species when nuclear mitochondrial pseudogenes are coamplified. Proceedings of the National Academy of Sciences 105: 13486–13491.

Sseruwagi P, Legg JP, Maruthi MN, Colvin J, Rey MEC, Brown JK. 2005. Genetic diversity of Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) populations and presence of the B biotype and a non-B biotype that can induce silverleaf symptoms in squash, in Uganda. Annals of Applied Biology 147: 253–265.

Sseruwagi P, Maruthi MN, Colvin J, Rey MEC, Brown JK, Legg JP. 2006. Colonization of non-cassava plant species by cassava whiteflies (Bemisia tabaci) in Uganda. Entomologia Experimentalis et Applicata 119: 145–153.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution 30: 2725–2729.

Villesen P. 2007. FaBox: an online toolbox for fast sequences. Molecular Ecology Notes 7: 965–968.

Zhang Y, Uyemoto JK, Kirkpatrick BC. 1998. A small-scale procedure for extracting nucleic acids from woody plants infected with various phytopathogens for PCR assay. Journal of Virological Methods 71: 45–50.

Zhou X, Robinson DJ, Liu Y, Harrison BD. 1998. Four DNA-A variants among Pakistani isolates of cotton leaf curl virus and their affinities to DNA-A of geminivirus isolates from okra. Journal of General Virology 79: 915–923.

Zhang Y, Uyemoto JK, Kirkpatrick BC. 1998. A small-scale procedure for extracting nucleic acids from woody plants infected with various phytopathogens for PCR assay. Journal of Virological Methods 71: 45–50.

Zhou X, Robinson DJ, Liu Y, Harrison BD. 1998. Four DNA-A variants among Pakistani isolates of cotton leaf curl virus and their affinities to DNA-A of geminivirus isolates from okra. Journal of General Virology 79: 915–923.

Zhang Y, Uyemoto JK, Kirkpatrick BC. 1998. A small-scale procedure for extracting nucleic acids from woody plants infected with various phytopathogens for PCR assay. Journal of Virological Methods 71: 45–50.

Zhou X, Robinson DJ, Liu Y, Harrison BD. 1998. Four DNA-A variants among Pakistani isolates of cotton leaf curl virus and their affinities to DNA-A of geminivirus isolates from okra. Journal of General Virology 79: 915–923.

Zhang Y, Uyemoto JK, Kirkpatrick BC. 1998. A small-scale procedure for extracting nucleic acids from woody plants infected with various phytopathogens for PCR assay. Journal of Virological Methods 71: 45–50.

Zhou X, Robinson DJ, Liu Y, Harrison BD. 1998. Four DNA-A variants among Pakistani isolates of cotton leaf curl virus and their affinities to DNA-A of geminivirus isolates from okra. Journal of General Virology 79: 915–923.

Zhou X, Robinson DJ, Liu Y, Harrison BD. 1998. Four DNA-A variants among Pakistani isolates of cotton leaf curl virus and their affinities to DNA-A of geminivirus isolates from okra. Journal of General Virology 79: 915–923.