A THESIS

FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Comparison of the genetic cluster of *Bemisia tabaci* MED (Hemiptera: Aleyrodidae) in Korea with its life history characteristics and insecticide resistance

국내 담배가루이 (노린재목: 가루이과)의 유전적 구조와 생활사 특성 및 살충제 저항성 비교

BY

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ENTOMOLOGY PROGRAM

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ABSTRACT

Comparison of the genetic cluster of *Bemisia tabaci* MED (Hemiptera: Aleyrodidae) in Korea with its life history characteristics and insecticide resistance

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The sweet potato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is a major pest that cause serious economic damage worldwide.

The objective of this study were (1) to examine the population genetic structures and diversities of *B. tabaci* MED from commercial

tomato greenhouses in Korea by using eight microsatellite markers, (2) to compare the difference in life history characteristics of representative different genetic clusters of *B. tabaci* MED through single and cross mating experiments and to verify correlation between genetic clusters and life history characteristics, and (3) to investigate the insecticide resistance status of *B. tabaci* MED populations and to determine correlation between genetic clusters and three insecticide class resistance levels.

In this study, the population genetic structures and diversities of *B. tabaci* MED were conducted among 35 populations of commercial tomato greenhouses in different geographic regions from 2016 to 2018 (17 populations in 2016, 13 populations in 2017, and five populations in 2018) using eight microsatellite markers. The average number of alleles per population (N_A) ranged from 2.000 to 5.875, the expected heterozygosity (H_E) ranged from 0.218 to 0.600, the

observed heterozygosity (H_O) ranged from 0.061 to 0.580, and the fixation index inbreeding coefficient (F_{IS}) ranged from -0.391 to 0.872 over the three years of the study. Some significant correlation ($p < 0.05$) was present between genetic differentiations (F_{ST}) and geographical distance, and a comparatively high proportion of variation was found among the *B. tabaci* MED populations. The *B. tabaci* MED populations were divided into two well-differentiated genetic clusters (cluster 1 and 2) within different geographic regions. Interestingly, its genetic clusters converged rapidly into one genetic cluster.

We compared life history characteristics of these two different genetic clusters of *B. tabaci* MED through single and cross mating experiments on two different host plants, cucumber and tobacco, at 26 °C. Intrinsic rate of increase (r), finite rate of increase (λ), and net reproductive rate (Ro) were significantly higher in the dominating cluster (cluster 2) (0.247, 1.280, and 192.402, respectively on

cucumber; 0.226, 1.253, and 133.792, respectively on tobacco) than in the other cluster (cluster 1) (0.149, 1.161, and 50.539, respectively on cucumber; 0.145, 1.156, and 53.332, respectively on tobacco). Overall performances of cross mating groups, C2fC1m (C2 female × C1 male) and C1fC2m (C1 female × C2 male), were in-between those of C2 and C1, with C2fC1m performing better than C1fC2m.

B. tabaci has been known to rapidly develop insecticide resistance because the use of chemical insecticides is the primary strategy to control *B. tabaci* in many cropping systems worldwide. In this study, to find clues for this phenomenon, we investigated the resistance traits of the two clusters for three insecticide classes (organophosphate, pyrethroid, and neonicotinoid). Since the resistance mutation frequencies in regional samples were either high (i.e., the voltage-sensitive sodium channel L925I/T929V mutations and the F392 acetylcholinesterase 1 mutation) or zero (the nicotinic

acetylcholine receptor R81T mutation), no meaningful correlation was deduced between resistance allele frequency and genetic cluster. However, the actual resistance levels to all three insecticide classes were significantly higher in cluster 2 than those in cluster 1, suggesting that cluster 2 has a higher resistance potential. Furthermore, thiamethoxam treatment to the mixed population of clusters 1 and 2 over three generations exhibited a strong tendency of population displacement from cluster 1 to cluster 2.

This study demonstrated that the *B. tabaci* MED (Q1) populations were divided into two well-differentiated genetic clusters within different geographic regions in Korea. Moreover, this study provided a strong evidence that genetic cluster 2 of *B. tabaci* MED had significantly superior life history characteristics and insecticide resistances than cluster 1 populations. Therefore, this study was provided that the rapidly converged phenomenon of genetic cluster in

B. tabaci MED populations in Korea significant correlated with their life history characteristics and insecticide resistances.

Key words: *Bemisia tabaci,* population genetics, microsatellite, life history characteristics, insecticide resistance

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General introduction

1-1. History and distribution of *B. tabaci*

The sweet potato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is one of the significant global agricultural pest that causes economic damages, predominantly in tropical and subtropical regions (Stansly & Naranjo, 2010). *B. tabaci* was first described in 1889, as *Aleurodes tabaci*, a pest of tobacco in Greece (Gennadius, 1889) and has spread globally with the tread in ornamental plants (Cervera et al., 2000; De Barro et al., 2011). *B. tabaci* originating from subtropical and tropical regions, has recently become distributed nearly around the world (Brown et al., 1995). *B. tabaci* is a complex of 11 well-defined high-level groups consisting of at least 36 putative species morphologically indistinguishable species, identified based on mtCOI (mitochondrial cytochrome oxidase I) (Boykin et al., 2012; De Barro et al., 2011). Two major global putative species of *B. tabaci*, MEAM1 (Middle East-Asia

Minor 1, formerly known as biotype B or *B. argentifolii*) and MED (Mediterranean, formerly known as biotype Q), are highly invasive and colonize large areas worldwide (Hu et al., 2011). Three putative species (MEAM1, MED, and JpL (*Lonicera japonica*)) of *B. tabaci* complex are present in Korea. MEAM1 was first detected in 1998 on poinsettia (*Euphorbia pulcherrima*) and rose (*Rosa hybrida*) (Lee et al., 2000). MED and JpL were recorded in 2004 and 2014 on tomato (*Lycopersicum esculentum* M.) (Lee et al., 2005) and Japanese honeysuckle (*Lonicera japonica* Thunb) (Lee et al., 2014), respectively. Currently, MED is predominant in most regions of the country, MEAM1 and JpL are found in a restricted region (Lee et al., 2016; Lee et al., 2014).

1-2. Life cycle of *B. tabaci*

The *B. tabaci* life cycle comprises an egg, four nymphal instars, and winged adults (Fig 1.) (Inbar & Gerling, 2008). The four nymphal instars are sessile except for the early first instar (Stansly & Naranjo, 2010). The late stage of the fourth instar (red-eyed nymphal or pupal stage), feeding stops until after emergences as an adult that live up to several weeks (Walker et al., 2009). Adults cover their body and wings with wax particles produced by wax plates on their abdomen, giving the wings their white color after initially being transparent (BYRNE & HADLEY, 1988). During oviposition, the female inserts the pedicel on the abaxial side of leaf surface, and secures it with a glue-like secretion that keeps the egg anchored in place (Buckner et al., 2002).

B. tabaci has a haplodiploid (arrhenotokous) sex determination system. Offspring from fertilized eggs develop as

females, and from unfertilized eggs as males. Mated females lay female and male eggs, whereas unmated females lay only male eggs (Byrne & Bellows Jr, 1991; Wang et al., 2009). Depending on the environment, *B. tabaci* produce 11 to 15 generation per year (Brown et al., 1995).

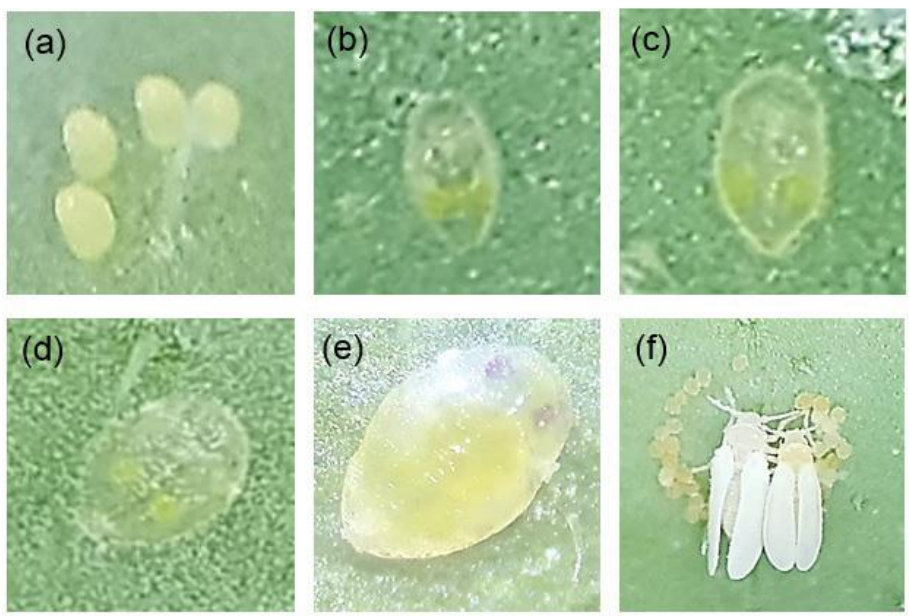

Fig 1. Developmental stages of *B. tabaci* MED (on cucumber). (a) Eggs, (b) first instar, (c) second instar, (d) third instar, (e) fourth instar with red eyes, and (f) female and male adults with fully expanded wings. Photos by Yujeong Park.

1-3. Damage by *B. tabaci*

B. tabaci has and extremely broad host range (Perring, 2001) including edible, ornamental, and fiber crops. Collectively, over 1,000 species host plants have been recorded for *B. tabaci* (Abd-Rabou & Simmons, 2010), causing serious damage directly through feeding and indirectly through the transmission of plant pathogenic viruses. *B. tabaci* is also a vector for more than 100 pathogenic plant viruses (Simon et al., 2003), particularly known vector of begomoviruses (Geminiviridae) (Khan et al., 2012). Begomoviruses are reported as economically the most important in agriculture, as they cause substantial yield losses (Navas-Castillo et al., 2011). Especially, the TYLCV (tomato yellow leaf curl virus) is one of the most devastating viruses of cultivated tomato in the world (Li et al., 2010). Symptoms of the disease consist of prominent upward curling of leaflet margins, reduction of leaflet area and yellowing of young leaves, together with stunting and flower abortion (Moriones

& Navas-Castillo, 2000). In Korea, since the 2008 outbreak of TYLCV has been observed first time and spread rapidly into neighboring areas (Lee et al., 2010).

All stage of *B. tabaci* ingest phloem sap with its mandibular and maxillary stylet and from excretion of honeydew onto the abaxial surfaces of plant leaves and fruits (Hunter et al., 1996; Navas-Castillo et al., 2011). They also enables sooty mold fungi development, and reduce photosynthesis, ultimately resulting in reduced quality of crops and fruits (Chen et al., 2004).

1-4. Chemical and biological control of *B. tabaci*

B. tabaci is found highly polyphagous, high reproductive rate, high capacity for dispersion and resistant to multiple classes of insecticides quickly (Perring, 2001; Pan *et al*., 2011; Kontsedalov *et al*., 2012; Janssen *et al*., 2017). Therefore, *B. tabaci* is the most difficult pest to manage on many greenhouse or field crops. *B. tabaci* infestations have primarily been controlled by insecticides in many cropping systems and chemical control remains an important part of IPM (insect pest management) (Zheng et al., 2017).

While different approaches have been developed to control *B. tabaci*, such as biological control (Liu et al., 2015). The biology control of pest is an important ecosystem service that benefits agricultural production and is influenced by natural ecosystem processes and human management (Calvo et al., 2011; Cock et al., 2010). For this reason, biology control such as insect pathogenic

fungi and natural enemies, and use of plant oils such as spearmint oil or essential oil are being actively studied (Choi & Kim, 2004; Kim et al., 2011). Especially, the natural enemies (e,g., *Amblyseius swirskii*, *Encarsia Formosa*, *Eretmocerus eremicus*, and *Nesidiocoris tenuis*) associated with *B. tabaci* infestation can cause high levels of mortality to populations of this insect pest (Bacci et al., 2007; Basit, 2019; Stansly et al., 2005).

1-5. Objectives of this study

In this study, the population genetic structures and diversities of *B. tabaci* MED (Q1) from commercial tomato greenhouses were identified and their genetic relationships in Korea were examined during 2016 to 2018 (17 populations in 2016, 13 populations in 2017, and five populations in 2018).

Understanding the population and structure and movement of insect pest species is very important for establishing strategies for pest management (Ben Abdelkrim et al., 2017). Using microsatellite and mitochondrial markers, which have consistently proven to be effective tools for population genetic studies (Dalmon et al., 2008). More than a combination of genetic diversity information based on microsatellite markers and environmental approaches therefore has potential to provide a powerful framework for the study *B. tabaci* population dynamics. The objective of this

study were (1) to examine the population genetic structure and diversities of *B. tabaci* MED from commercial tomato greenhouses in Korea by using eight microsatellite markers, (2) to compare the difference in life history characteristics of representative different genetic clusters of *B. tabaci* MED through single and cross mating experiments and to verify correlation between genetic clusters and life history characteristics, and (3) to investigate the insecticide resistance status of *B. tabaci* MED populations and to determine correlation between genetic clusters and three insecticide class resistance levels.

Chapter Ⅱ.

Population genetic structure of *Bemisia tabaci* MED (Hemiptera: Aleyrodidae) in Korea

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2-1. Abstract

The sweet potato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is a major agricultural pest that causes economic damages worldwide. In particular, *B. tabaci* MED (Mediterranean) has resulted in serious economic losses in tomato production of Korea. In this study, 1,145 *B. tabaci* MED females from 35 tomato greenhouses in different geographic regions were collected from 2016 to 2018 (17 populations in 2016, 13 in 2017, and five in 2018) and analyzed to investigate their population genetic structures using eight microsatellite markers. The average number of alleles per population (N_A) ranged from 2.000 to 5.875, the expected heterozygosity (H_E) ranged from 0.218 to 0.600, the observed heterozygosity (H_O) ranged from 0.061 to 0.580, and the fixation index inbreeding coefficient (F_{IS}) ranged from -0.391 to 0.872 over the three years of the study. Some significant correlation ($p < 0.05$) was present

between genetic differentiations (F_{ST}) and geographical distance, and a comparatively high proportion of variation was found among the *B. tabaci* MED populations. The *B. tabaci* MED populations were divided into two well-differentiated genetic clusters within different geographic regions. Interestingly, its genetic structures converged into one genetic cluster during just one year. The reasons for this genetic change were speculated to arise from different fitness, insecticide resistance, and insect movement by human activities.

Key words: *Bemisia tabaci,* Mediterranean, whitefly, population genetics, microsatellite, Korea

2-2. Introduction

The sweet potato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is a major agricultural insect pest that is distributed worldwide. *B. tabaci* has an extremely broad host range (Perring, 2001) and causes serious damage to diverse host plant species. *B. tabaci* is also a vector for more than 100 pathogenic plant viruses (Simon et al., 2003), particularly known to be a vector for begomoviruses (Khan et al., 2012), and a major vector for tomato yellow leaf curl virus (TYLCV), one of the most devastating viruses in cultivated tomatoes in the world (Li et al., 2010). *B. tabaci* is a complex of 11 well-defined high-level groups consisting of at least 36 putative species identified based on mtCOI (mitochondrial cytochrome oxidase I) (Boykin et al., 2012; De Barro et al., 2011). These putative species are morphologically indistinguishable and differ in host range, virus transmission, fecundity, and insecticide resistance (Dinsdale et al.,

2010; Wang et al., 2010a). Two major global putative species of *B. tabaci*, MEAM1 (Middle East-Asia Minor 1, formerly known as biotype B or *B. argentifolii*) and MED (Mediterranean, formerly known as biotype Q), are highly invasive and colonize large areas worldwide (Hu et al., 2011). Three putative species (MEAM1, MED, and JpL (*Lonicera japonica*)) of the *B. tabaci* species complex are present in Korea. MEAM1 and MED were first detected in 1998 and 2004 (Lee et al., 2005; Lee et al., 2000), respectively. JpL was first recorded in 2014 (Lee et al., 2014). Currently, MED is predominant in most regions of the country, and MEAM1 and JpL are found only in a restricted region (Lee et al., 2016; Lee et al., 2014). Understanding the population genetic structure of a pest species is important for establishing pest management strategies (Kim et al., 2017). Pest population structure assessments are helpful to reveal the origins and spread patterns of a target species (Kim et al., 2006), to delineate potential boundaries for

their control (Streito et al., 2017), and to provide the statistical ability to differentiate between genetic groups (Hedrick, 2001), as well as to check whether they have mixed with other populations or not. When all population genetics information based on microsatellite markers is combined with environmental approaches, the construction of a powerful framework for managing *B. tabaci* is facilitated (Ben Abdelkrim et al., 2017).

Over the past decades, various molecular genetics tools have considerably extended the boundary of population genetics (Sunnucks, 2000). Diverse DNA markers for insect genetics research (i.e., the amplified fragment length polymorphism (AFLP) marker, expressed sequence tags (EST), mitochondrial DNA (mtDNA), microsatellites, and random amplified polymorphic DNA (RAPD) (Behura, 2006)) have been identified and developed to determine the population genetic structure of a species. Among them, microsatellites are especially

popular genetic markers because of their co-dominance, high abundant variation and polymorphism rates, multiple alleles, and quick allele detection by a wide variety of methods (Ellegren, 2004). Microsatellite markers are also very effective tools in population genetic studies for insect species (Boopathi et al., 2014; Dalmon et al., 2008). Through molecular genetic diagnosis using population genetic analyses, effective control can be achieved in a short time at a low cost (Oliveira et al., 2006). Different microsatellite markers were employed in several recent studies (Chu et al., 2011; Díaz et al., 2015; Dickey et al., 2013; Mouton et al., 2015; Tahiri et al., 2013) to investigate the population genetic structure, genetic differentiation, genetic evolution, gene flow, and dispersal pattern of *B*. *tabaci* over relatively large geographic scales. In this study, the population genetic structures and diversities of *B*. *tabaci* MED from tomato greenhouses were identified and their genetic relationships in Korea were examined.

2-3. Materials and methods

2-3-1. *B. tabaci* sampling

In total, 1,145 *B*. *tabaci* female adults were collected from 35 commercial tomato greenhouses in Korea using an aspirator during 2016 – 2018 (17 populations in 2016, 13 populations in 2017, and five populations in 2018) (Fig 1 and Table 1). The *B*. *tabaci* samples were collected from tomatoes plants at least 1 m apart to avoid the collection of full siblings in the greenhouses. All individual samples were preserved in 99.8% ethanol before DNA extraction.

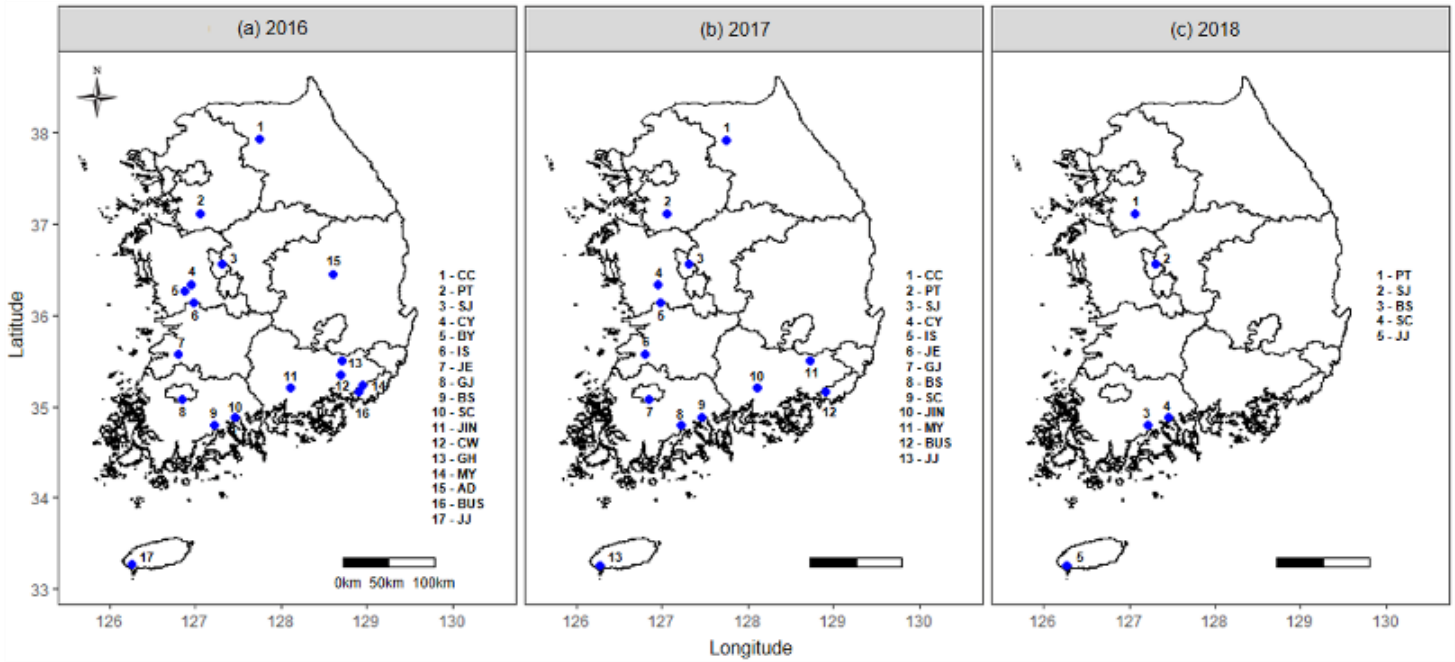


Fig. 1. *B*. *tabaci* MED sampling sites (see Table 1 for details) in Korea from (a) 2016, (b) 2017, and (c) 2018.

Table 1. Details of sampling information of *B. tabaci* MED in Korea

Sample site	Population	Collection date	GPS coordinates	Host plant	Sample size
Seogwipo-si	16'JJ	2016-04-25	37°27′39.0″N,126°57′28.0″E	Tomato	40
	17'JJ	2017-04-19	33°15′15.0″N,126°16′09.0″E		20
	18'JJ	2018-10-10	33°15′15.0″N,126°16′09.0″E		20
JinJu-si *	16'JIN	2016-05-25	35°12′40.0″N,128°06′56.0″E	Tomato	40
	17'JIN	2017-06-07			30
Changwon-si	16'CW	2016-05-25	35°20′37.0″N,128°42′04.0″E	Tomato	40
Busan *	16'BUS	2016-05-25	35°10′18.0″N,128°54′56.0″E	Tomato	40
	17'BUS	2017-06-09			30
Gimhae-si	16'GH	2016-05-26	35°14′06.0″N,128°57′42.0″E	Tomato	40
Miryang-si *	16'MY	2016-05-26	35°30′08.0″N,128°43′18.0″E	Tomato	40
	17'MY	2017-06-08			30
Jeongeup-si *	16'JE	2016-06-01	35°34′28.0″N,126°48′07.0″E	Tomato	40
	17'JE	2017-06-20			30
Suncheon-si *	16'SC	2016-06-01	37°27′39.0″N,126°57′28.0″E	Tomato	40
	17'SC	2017-06-19			30
	18'SC	2018-07-11			20
Gwangju *	16'GJ	2016-06-02	35°04′31.0″N,126°51′11.0″E	Tomato	40
	17'GJ	2017-06-20			30
Boseong-gun *	16'BS	2016-06-02	34°47′33.0″N,127°13′15.0″E	Tomato	40
	17'BS	2017-06-19			30
	18'BS	2018-07-11			20
Iksan-si	16'IS	2016-06-09	36°08'21.0''N,126°58'59.0''E	Tomato	40
	17'IS	2017-06-20	36°08′20.0″N,126°58′55.0″E		15
Andong-si	16'AD	2016-06-09	36°27′23.0″N,128°36′11.0″E	Tomato	40
Buyeo-gun	16'BY	2016-06-30	36°15′60.0″N,126°52′49.0″E	Tomato	40

Table 1. *Continued*.

Cheongyang-gun *	16'CY	2016-06-30	36°20′21.0″N,126°57′18.0″E	Tomato	40
	17'CY	2017-06-21			30
Sejong-si	16'SJ	2016-06-30	36°34′11.6″N,127°19′02.8″E	Tomato	40
	17'SJ	2017-06-19	36°34′19.0″N,127°18′40.0″E		30
	18'SJ	2018-07-12	36°34′19.0″N,127°18′40.0″E		20
Chuncheon-si	16'CC	2016-07-29	37°56′02.9″N 127°44′57.7″E	Tomato	40
	17'CC	2017-06-29	37°55′38.0″N 127°45′15.0″E		30
Pyeongtaek-si	16'PT	2016-08-05	37°07′20.0″N,127°03′29.0″E	Tomato	40
	17'PT	2017-06-26	37°07′25.0″N,127°03′14.0″E		30
	18'PT	2018-08-10	37°07′20.0″N,127°03′29.0″E		20

*Same tomato greenhouse during two or three years

2-3-2. Molecular methods

2-3-2-1. DNA extraction

Genomic DNA (gDNA) extraction was performed using a Qiagen Gentra Puregene Tissue Kit (Qiagen, Gaithersburg, MD, USA) according to the manufacturer's instructions. Since *B*. *tabaci* is a haplo-diploid species, producing male progeny from unfertilized eggs and female progeny from fertilized eggs (LIU et al., 2012), only adult females were used for the genetic analysis of each individuals. The extracted gDNA samples were finally stored at -20 ˚C until use. DNA quantification was performed with ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA).

2-3-2-2. Identification of *B. tabaci* putative species

Two individuals per population were randomly selected in order to identify the *B*. *tabaci* putative species. A fragment of the mtCOI gene was PCR-amplified using the primer pair C1-J-2195 (5'-TTGATTTTTTGGTCATCCAGAAGT-3') and L2-N-3014 (5'-TCCAATGCACTAATCTGCCATATTA-3') (Frohlich et al., 1999). All PCR reactions were conducted using 1 μl forward primer (10 pmol/μl), 1 μl reverse primer (10 pmol/μl), and 2 μl template DNA in 20 μl reaction volumes consisting of 25 mM dNTPs, 10 mM Tris-HCl (pH 9), 30 mM KCl, 1.5 mM MgCl2, and 1 unit of Taq DNA polymerase using Accupower PCR PreMix (Bioneer, Seoul, Korea). The reaction conditions included an initial denaturation for 5 min at 94 ˚C, followed by 34 cycles of 1 min each at 94 ˚C, 1 min at 52 ˚C, and 1 min at 72 ˚C, with a final extension for 5 min at 72 ˚C (Lee et al., 2014). PCR products were sent for sequencing to NICEM (Seoul, Korea). Putative

species identification was based on direct sequence comparisons using NCBI BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

2-3-2-3. PCR amplification of eight microsatellites

PCR primers were used to amplify microsatellite DNA loci 11, 53 (Delatte et al., 2005), 68, 145, 177 (Dalmon et al., 2008), BT4, BT159 (Tsagkarakou & Roditakis, 2003), and Bem23 (De Barro et al., 2003) using the individual gDNA of *B*. *tabaci* MED as templates. PCR amplifications for the microsatellite primers and PCR reactions were performed as previously described (Dalmon et al., 2008). A total of 1,145 individuals were genotyped using eight microsatellite loci distributed in two PCR multiplex sets. Two multiplex PCRs were performed for each individual at 10 pmol/μl (multiplex 1 loci: 11, 145, 177, BT4, and BT159; multiplex 2 loci: 53, 68, and Bem23). In order to analyze the length of the PCR products using a laser detection system,

some of the forward and reverse primers were labeled with a fluorescent dye. The rTaq PCR kit (Takara Bio Inc., Kyoto, Japan) was used for these reactions. The total reaction volume was 10 µl, which contained 2.9 µl or 4.1 µl (multiplex 1: 2.9 µl, multiplex 2: 4.1 µl) distilled water, 1.0 µl 10X PCR buffer, 1.0 µl 2.5mM dNTP mixture, 0.2 µl of each primer, 0.1 µl of Taq polymerase, and 2.0 µl template DNA. The multiplex PCR products were analyzed using an ABI 3730xl (Applied Biosystems Inc., Foster, CA, USA). Allele size was detected using GENEMAPPER v.3.7 (Applied Biosystems Inc.). Multiplex 1 was amplified in PTC100 Thermocyclers (MJ Research, Waltham, MA, USA) as follows: 15 min at 94 ˚C, followed by 40 cycles for 30 s at 94 ˚C, 1 min 30 s at 57 ˚C, 1 min at 72 ˚C, ending with 30 min at 60 ˚C. Multiplex 2 was amplified as above except that the annealing temperature was increased from 57 to 60 ˚C. PCR was carried out as described by Dalmon et al. (Dalmon et al., 2008). The 1 µl PCR

product was diluted with 8.5 μl of Hi-Di formamide (Applied Biosystems Inc.) and 0.5 μl Genescan ROX-500 size standard (Applied Biosystems Inc.).

2-3-3. Analyses of genetic diversity

GENEPOP v.4.0 (Raymond & Rousset, 1995) and Micro-Checker v.2.2.3 (Brookfield, 1996; Van Oosterhout et al., 2004) were used to determine the microsatellite data for scoring errors, allelic dropouts, and null alleles. The estimated frequency of null alleles per loci for each population was calculated in FreeNa (Chapuis & Estoup, 2007) using the expectation maximization (EM) algorithm (Dempster et al., 1977). Each of the 1,145 collected samples were used to test deviations from Hardy-Weinberg equilibrium (HWE) conditions, the number of alleles (N_A), allele size range, and the observed (H_O) and expected heterozygosities (H_E), and the inbreeding coefficient (F_{IS})

were computed using GenAlEx v.6.5 (Peakall & Smouse, 2012) and Microsatellite Toolkit (Park, 2001).

2-3-3-1. Analysis of molecular variance (AMOVA)

AMOVA was performed using GenAlEx v.6.5. AMOVA was used to characterize genetic variation patterns and to estimate variance components. A two-part AMOVA analysis was conducted to check genetic divergence (F_{ST}) as a factor of variation among and within the populations. AMOVA computations were performed with 999 permutations to test for significance

2-3-4. Analyses of genetic structure

The number of genetic clusters (*K*) was estimated in STRUCTURE v.2.3.2 with 60,000 Markov Chain Monte Carlo (MCMC) steps and a burn-in period of 600,000. The log-likelihood estimate was

run for *K* = ranges from 1 to 10 with ten replicates each. They were used to determine the number of clusters based on a combination of the mean estimated Ln probability of the data (Pritchard et al., 2000b) and the second-order rate of change in the log-probability of the data (*ΔK*) (Evanno et al., 2005). The Evanno method was then implemented in STRUCTURE HARVESTER Web v.0.6.93 (Earl, 2012).

2-3-5. Principal coordinate analysis (PCoA)

PCoA was conducted between multi-locus genotypes in all individuals. The codominant-genotypic option of GeneAlex v.6.5 was used to calculate the similarity genetic distance matrix (Peakall & Smouse, 2012). The PCoA plot was based on factor scores along the two principal axes (axis 1 and 2) and enabled the visualization of population differences.

2-3-5-1. Discriminant analysis of principal components (DAPC)

DAPC was performed in the '*adegenet*' package (Jombart & Ahmed, 2011) of R software v.3.5.1 (R Development Core Team, 2018) to identify an optimal number of genetic clusters to describe the data. DAPC is a multivariate algorithm, similar to principal component analysis (PCA) that identifies genetic clusters and can be used as an efficient genetic clustering tool (Jombart et al., 2010). The number of clusters was identified based on Bayesian information criterion (BIC). If the value of BIC is positive and low, it is a suitable model. When the BIC value is negative, a high number is a suitable model.

2-3-5-2. Isolation by distance (IBD)

The Mantel test (Mantel, 1967) was performed to assess isolation by distance. The relationship between pairwise geographic

distance (Ln km) and pairwise genetic distance in terms of $F_{ST}/(1-F_{ST})$ with 1,000 random permutations was conducted using the GenAlEx v.6.5, GENEPOP v.4.0, and '*ade4'* package (Chessel et al., 2004) of R software v.3.5.1. The IBD graph was generated by using the R software v.3.5.1 with '*ggpolt2*' package.

2-3-5-3. Bottleneck test

The BOTTLENECK v.1.2.02 (Piry et al., 1999) was used to detect the effect of a recent reduction in all population sizes. The possibility of bottleneck events in the 35 populations was examined using a one-tailed Wilcoxon signed-rank test under three mutation models, the infinite allele model (IAM), the two-phase model (TPM), and the stepwise mutation model (SMM) (parameters for TPM: variance = 30.0%, probability = 70.0%, 1,000 replications). The Wilcoxon signed-rank test has been shown to be effective and reliable

when eight microsatellite loci are analyzed (Piry et al., 1999).

2-3-5-4. Pairwise comparisons of fixation index (F_{ST})

To assess the level of genetic differentiation between the samples, pair-wise fixation index (F_{ST}) value estimates were computed using GENEPOP v.4.0. To correct for null alleles, pairwise estimators of F_{ST} values were calculated from each microsatellite dataset that potentially harbored null alleles using the excluding null alleles (ENA) correction method ($F_{ST\text{-}ENA}$) following 1,000 bootstrapping permutations over the loci. The ENA correction method was used to obtain unbiased pairwise F_{ST} values using FreeNA. To investigate the relationship between the genetic distance revealed by the F_{ST} values and geographic distance, an isolation-by-distance analysis was performed using a regression of $F_{ST}/(1-F_{ST})$ values against the logarithm of the geographical distance (km) between the populations.

Significance of the correlation between the two data matrices was assessed using a Mantel test with 1,000 permutations. This was performed with the ISOLDE program implemented in GENEPOP v.4.0.

2-4. Results

2-4-1. Identification of the *B. tabaci* populations

All *B*. *tabaci* individuals collected were successfully sequenced and analyzed. Approximately 810 bp of the mtCOI gene was amplified from *B*. *tabaci* individuals by PCR. All populations identified belonged to the MED (Q1) species based on representative samples. The information of GenBank accession numbers are presented in Appendix 2.

2-4-2. Genetic diversity

The values of the genetic diversity indexes for the Korea populations of *B*. *tabaci* MED are shown in Table 2. There were one to eight alleles per loci in the eight microsatellites and the estimated average frequency of null alleles ranged from 0.031 to 0.407 among the 35 populations. The average number of alleles per population (N_A)

ranged from 2.000 (17'JIN) to 5.875 (16'SJ). The expected heterozygosity (H_E) ranged from 0.218 (16'JJ) to 0.600 (16'PT), whereas the observed heterozygosity (H_O) ranged from 0.061 (16'CW) to 0.580 (16'IS). The value of H_E in each population was higher than the value of H_O, except for 12 populations that showed negative values for F_{IS}. The estimator of the fixation index inbreeding coefficient (F_{IS}) ranged from -0.391 (17'CC) to 0.872 (16'CW). A positive value for F_{IS} indicates the presence of heterozygotic deficiencies, whereas a negative value indicates the presence of homozygotic deficiencies. The analysis of genetic diversity for all different eight microsatellite loci of *B*. *tabaci* MED screened is given in Appendix 1.

Table 2. Genetic diversity of the *B. tabaci* MED populations

Population	N	N_A	H_E	H_O	F_{IS}	F_{null}
16'JJ	40	2.625	0.218	0.160	0.266	0.241
16'JIN	40	5.500	0.423	0.274	0.353	0.217
16'CW	40	3.500	0.480	0.061	0.872	0.393
16'BUS	40	2.625	0.407	0.118	0.710	0.407
16'GH	40	3.250	0.414	0.159	0.614	0.327
16'MY	40	4.625	0.459	0.107	0.768	0.307
16'JE	40	4.250	0.478	0.337	0.295	0.296
16'SC	40	4.625	0.458	0.282	0.383	0.184
16'GJ	40	2.875	0.462	0.231	0.499	0.284
16'BS	40	4.750	0.521	0.187	0.642	0.292
16'IS	40	5.750	0.549	0.580	-0.057**	0.174
16'AD	40	5.125	0.486	0.272	0.440	0.284
16'BY	40	3.000	0.256	0.136	0.466	0.231
16'CY	40	2.875	0.391	0.180	0.540	0.031
16'SJ	40	5.875	0.594	0.148	0.751	0.333
16'CC	40	5.625	0.445	0.237	0.468	0.255
16'PT	40	5.500	0.600	0.264	0.560	0.300
17'JJ	20	3.000	0.369	0.391	-0.058**	0.268
17'JIN	30	2.000	0.246	0.209	0.150	0.238
17'MY	30	3.375	0.378	0.388	-0.026**	0.331
17'BUS	30	3.250	0.406	0.304	0.251	0.329
17'SJ	30	3.750	0.409	0.417	-0.020**	0.211
17'SC	30	2.875	0.376	0.373	0.010*	0.323
17'BS	30	3.375	0.325	0.339	-0.041**	0.259
17'GJ	30	3.000	0.443	0.425	0.041*	0.382
17'JE	30	3.375	0.394	0.499	-0.265**	0.308
17'IS	15	3.000	0.379	0.426	-0.123**	0.272
17'CY	30	3.000	0.356	0.299	0.160	0.406

Table 2. *Continued*.

17'PT	30	3.250	0.424	0.513	-0.211**	0.240
17'CC	30	2.625	0.387	0.539	-0.391**	0.343
18'SC	20	2.500	0.368	0.413	-0.122**	0.303
18'BS	20	2.875	0.302	0.319	-0.054**	0.273
18'SJ	20	3.375	0.420	0.413	0.019*	0.175
18'PT	20	4.000	0.546	0.250	0.542	0.304
18'JJ	20	2.375	0.286	0.350	-0.225**	0.292

N, number of individuals sampled; N_A, Mean number of alleles per population; H_E, Mean expected heterozygosity; H_O, Mean observed heterozygosity; F_{IS}, Mean fixation index inbreeding coefficient; and F_{null}, average proportion of Homozygous for null allele. Significance F_{IS} value is obtained after 1,000 permutation tests (*$p < 0.05$; **$p < 0.01$).

2-4-2-1. AMOVA

AMOVA among the 35 *B*. *tabaci* MED populations showed that 48.0% of the total genetic variation was accounted for by variation among the populations and 52.0% of the variation was accounted for by individual variation within the populations (Table 3). The AMOVA results revealed a relatively high proportion of variation among the populations.

Table 3. Analysis of molecular variance (AMOVA) for the 35 *B. tabaci* MED populations collected from different regions in Korea using eight microsatellite markers

Source of variation	**Degrees of freedom**	**Sums of squares**	**Mean sums of squares**	**Estimated variance**	**% of variation**	***p*-value**
Among population	34	5557.909	163.468	4.845	48.0%	0.01
Within population	1110	5820.817	5.244	5.244	52.0%	
Total	1144	11378.726		10.089	100%	

Significant at $p < 0.01$ (based on 999 permutations)

2-4-2-2. Genetic relationships and population structure analysis

The genetic structure analysis of 35 *B*. *tabaci* MED populations using eight microsatellite marker genotypes revealed two dominant genetic clusters. The highest likelihood value was obtained for $K = 2$ (Fig 2a). The 16 populations (16'CC, 16'PT, 16'SJ, 16'BY, 16'CY, 16'IS, 16'JE, 16'BS, 16'SC, 16'CW, 16'GH, 16'MY, 16'AD, 17'IS, 17'JE, and 18'PT) formed one cluster, and 19 populations (16'JIN, 16'GJ, 16'BUS, 16'JJ, 17'CC, 17'PT, 17'SJ, 17'CY, 17'GJ, 17'BS, 17'SC, 17'JIN, 17'MY, 17'BUS, 17'JJ, 18'JJ, 18'SJ, 18'BS, and 18'SC) formed the other cluster (Fig 2b and 2c). The populations of *B*. *tabaci* MED converged rapidly into one cluster (orange color) over time (Fig 3).

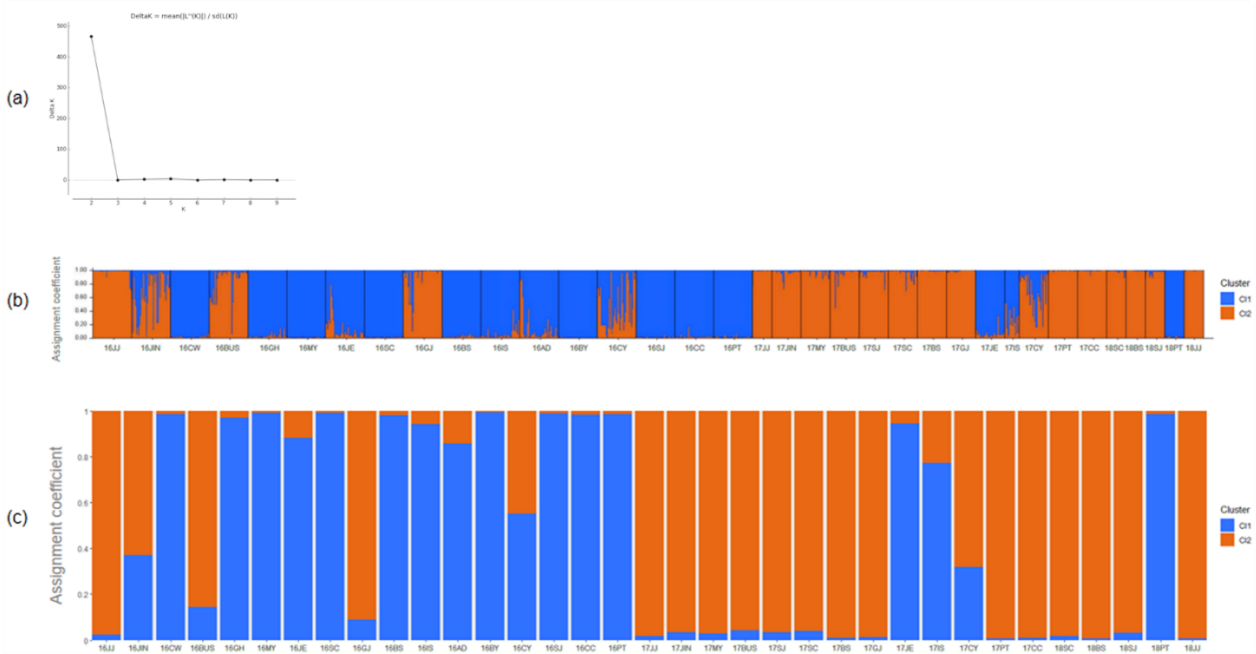


Fig 2. Scatter plots of ΔK = 2. (a) The maximum value among the genotypes was 466.35 at ΔK = 2, using ΔK = m(|L“K|) / s[L(K)]. Bar plot of the population structure for *B. tabaci* from 35 populations in Korea (b) using STRUCTURE v.2.3.2 and (c) R software v.3.5.1. Each population is represented by a vertical line with different colors representing the probabilities assigned to each of the genetic clusters.

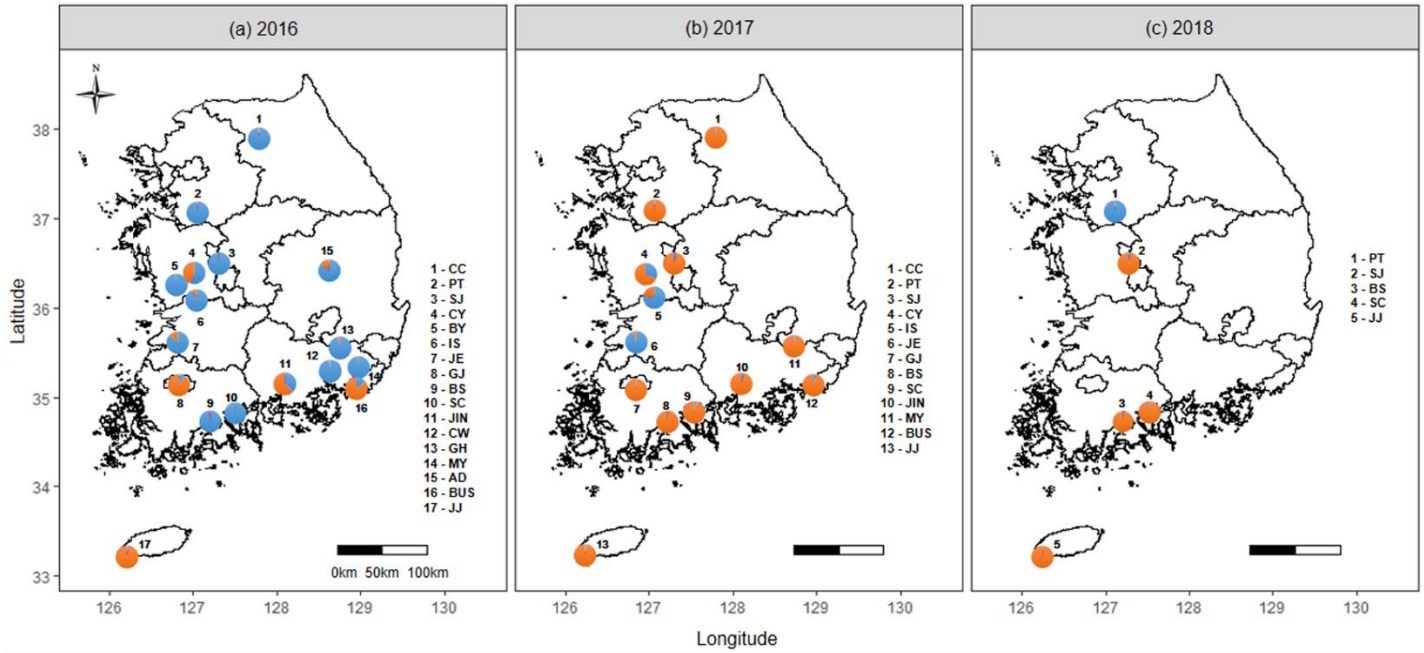


Fig 3. Bayesian clustering results from the structure for all samples (*K* = 2). The geographical distribution of the population and the genetic structure of the *B. tabaci* MED in Korea revealed by STRUCTURE analysis in samples from (a) 2016, (b) 2017, and (c) 2018. Genetic changes were observed in six of the populations from 2016 to 2017. The maps were created by using the R software v.3.5.1.

2-4-2-3. PCoA of *B. tabaci* MED

Principal component analysis of the 35 *B*. *tabaci* MED populations showed that the first principal components accounted for 27.6% of the total variation, followed by the second component, which accounted for 43.3% of the variation (Fig 4a). The first and second components of PCoA for each year are as follows: 32.3%, 52.6% for 2016 (Fig 4b), 30.7%, 53.1% for 2017 (Fig 4c), and 39.8%, 69.1% for 2018 (Fig 4d), respectively.

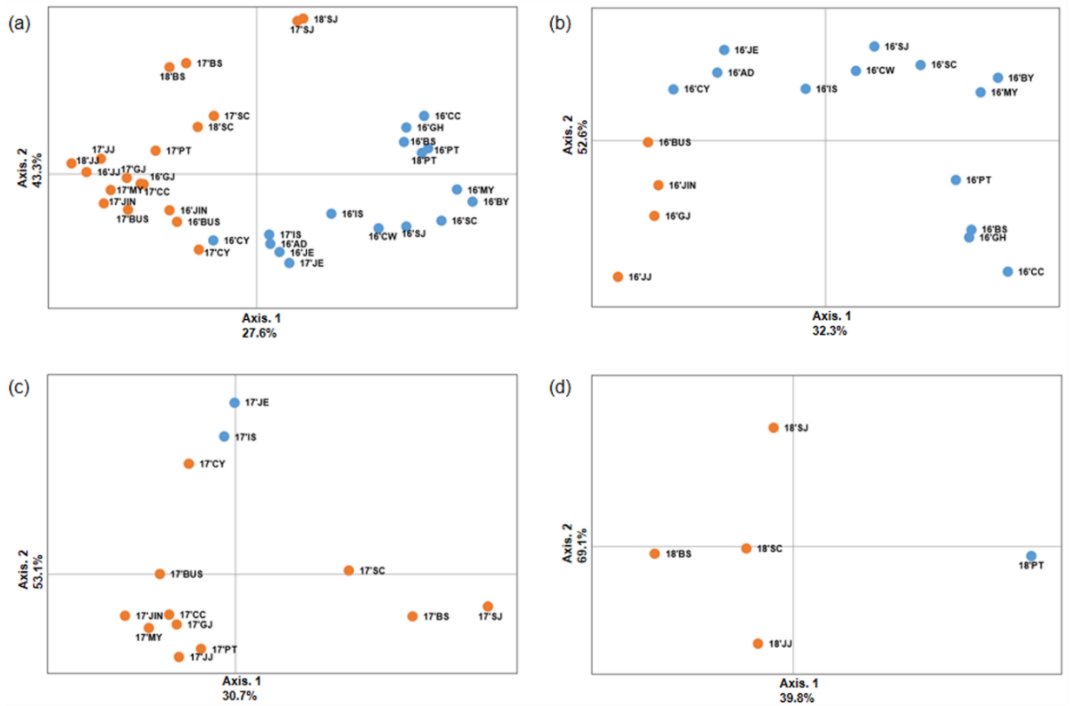


Fig 4. Principal component analysis (PCoA) plotting the relationships of 35 *B. tabaci* MED population samples. Each axis indicates the percentage of the total variations. STRUCTURE with marked color is the same as one of Bayesian clustering (blue and orange colors) from (a) 2016 - 2018, (b) 2016, (c) 2017, and (d) 2018.

2-4-2-4. DAPC

In DAPC, the elbow in the curve of BIC was at $K = 2$ using the *find*. *cluster* function of R software v.3.5.1 (Deperi et al., 2018). In this study, the value of BIC was found to be 166.05, which was positive and the smallest value (Fig 5a). The DAPC results showed that the populations of *B*. *tabaci* MED were split into two well-differentiated genetic clusters with low overlap between them. The first cluster contained populations from 2016 and the second cluster contained populations from 2017 and 2018 (Fig 5b). The DAPC results agreed with the STRUCTURE results.

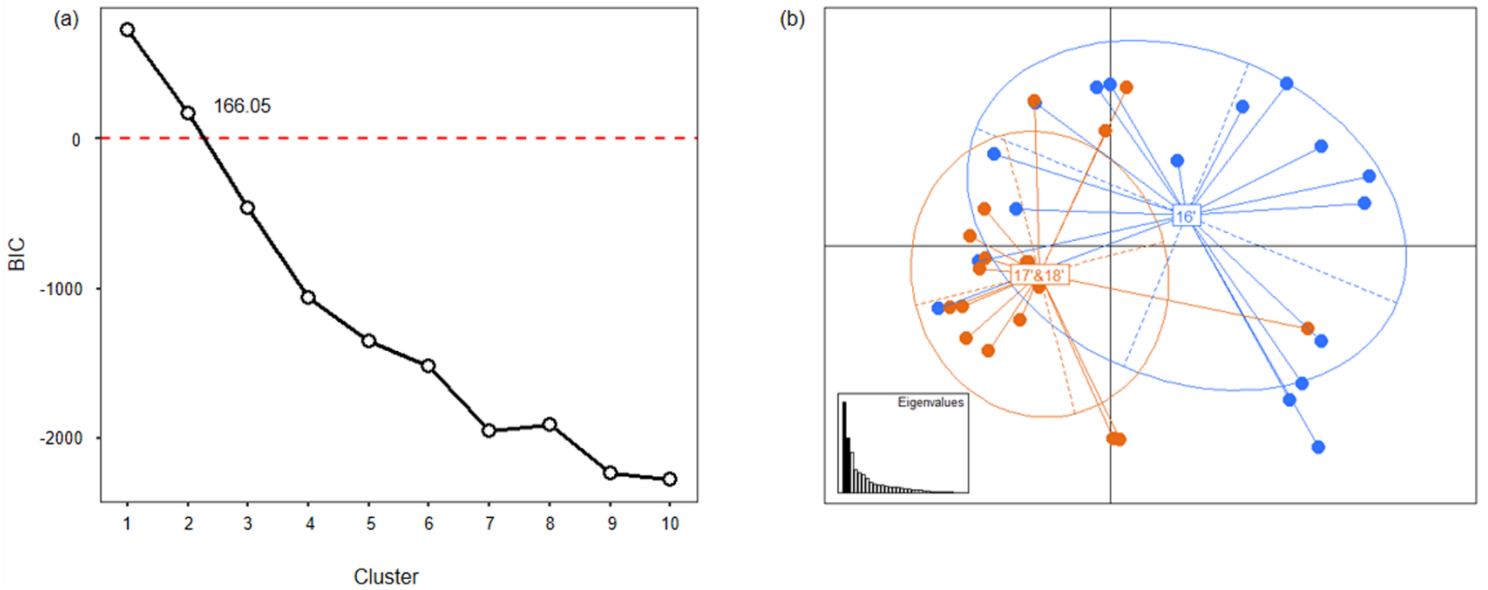


Fig 5. Discriminant analysis of principal components (DAPC) analysis of 35 *B. tabaci* MED populations in Korea. (a) The Bayesian information criteria (BIC) supported two distinct genetic clusters. (b) The eigenvalues of the analysis suggest that the first two components explained the maximum genetic structure of the dataset. Scatter-plot of the distribution of *B. tabaci* MED formed two genetic clusters (blue and orange colors).

2-4-2-5. IBD

A significant correlation was detected between genetic and geographic distances in the *B*. *tabaci* MED populations based on the Mantel tests of IBD (r^2 = 0.557; p = 0.01), indicating a pattern of isolation by distance (Fig 6). Multiple points in the scatterplot fit to the linear regression along the geographic distance range. This result indicates that gene flow between population increases with geographic distance. IBD analysis revealed that geographic distance had an effect on the population structure of the *B*. *tabaci*.

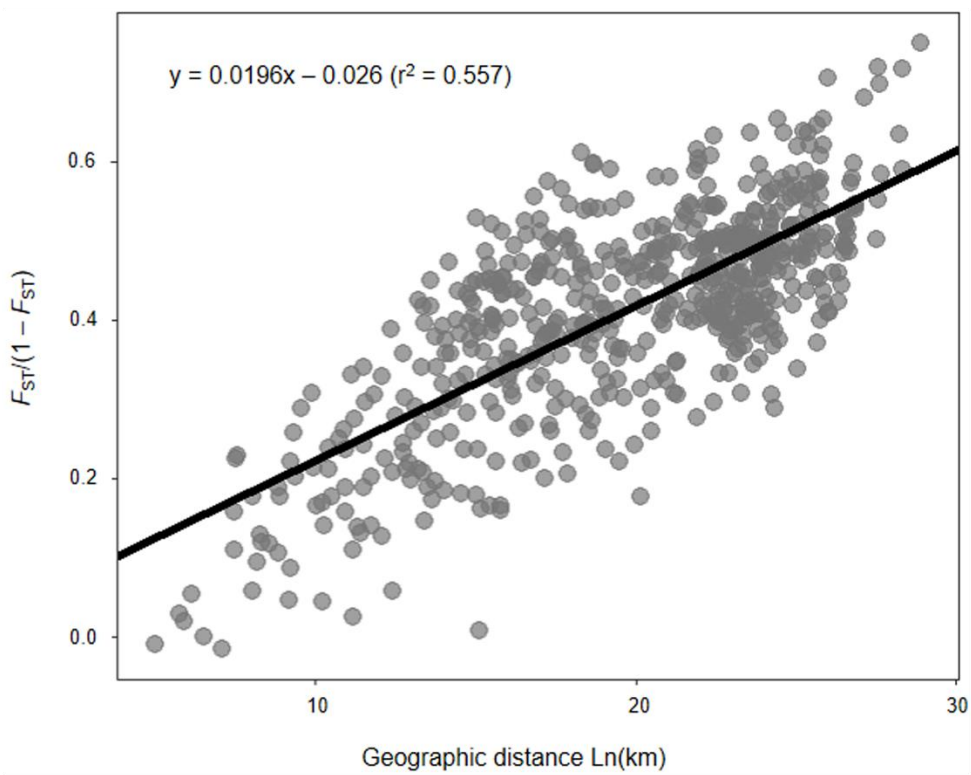


Fig 6. Relationship between genetic distance and the log of the geographical distance for *B. tabaci* MED. The line represents the regression line and circles represent the logarithm transformation of distance (p = 0.01, 1,000 permutations).

2-4-2-6. Bottleneck test

The mode-shift analysis of bottleneck test, a signature of recent population reduction was found only for the 16'GJ and 18'PT populations (Table 4). Departure from mutation-drift equilibrium was observed in two populations, indicating that they remained relatively unstable in recent evolutionary history. Significant heterozygosity excess (Wilcoxon test *p*-values) was detected in eight populations under the IAM (16'CW, 16'BUS, 16'GJ, 16'CY, 17'BUS, 17'GJ, 17'CY, and 18'PT) and two populations under the TPM (16'GJ, 17GJ), which accounted for 22.8% and 5.7% of the Korea populations (Table 4 bolded numbers), respectively. Under the SMM, however, significant heterozygosity excess was not detected in any population.

Table 4. Wilcoxon signed-rank tests for heterozygosity excess for the 35 *B. tabaci* MED populations

Population	WILCOXON Tests * (Heterozygosity Excess *p*-values)			Mode-Shift
	IAM	TPM	SMM	
16'JJ	0.94531	0.97266	0.98047	Normal
16'JIN	0.84375	0.99609	1.00000	Normal
16'CW	**0.03711**	0.52734	0.72656	Normal
16'BUS	**0.01953**	0.19147	0.52734	Normal
16'GH	0.12500	0.37109	0.96289	Normal
16'MY	0.67969	0.97266	0.99023	Normal
16'JE	0.14844	0.59375	0.07813	Normal
16'SC	0.52734	0.76953	0.99609	Normal
16'GJ	**0.00781**	**0.01172**	0.05469	Shifted mode
16'BS	0.32031	0.97266	0.99414	Normal
16'IS	0.27344	0.76953	0.99023	Normal
16'AD	0.62891	0.99414	1.00000	Normal
16'BY	0.37109	0.97266	1.00000	Normal
16'CY	**0.01953**	0.15625	0.52734	Normal
16'SJ	0.32031	0.80859	0.98633	Normal
16'CC	0.72656	0.97266	0.99609	Normal
16'PT	0.12500	0.37109	0.84375	Normal
17'JJ	0.42188	0.76953	0.84375	Normal
17'JIN	0.28906	0.46875	0.65625	Normal

Table 4. *Continued*.

17'MY	0.46875	0.76563	0.96094	Normal
17'BUS	**0.03906**	0.65625	0.94531	Normal
17'SJ	0.52734	0.67969	0.98047	Normal
17'SC	0.32031	0.52734	0.76953	Normal
17'BS	0.65625	0.96094	0.97266	Normal
17'GJ	**0.00781**	**0.01563**	0.07813	Normal
17'JE	0.40625	0.81250	0.94531	Normal
17'IS	0.40625	0.65625	0.81250	Normal
17'CY	**0.03906**	0.28906	0.94531	Normal
17'PT	0.34375	0.46875	0.46875	Normal
17'CC	0.15625	0.47266	0.76953	Normal
18'SC	0.05469	0.23438	0.28906	Normal
18'BS	0.57813	0.78125	0.96094	Normal
18'SJ	0.23438	0.34375	0.65625	Normal
18'PT	**0.01953**	0.18750	0.40625	Shifted mode

Infinite allele model (IAM), two-phase model (TPM), and stepwise mutation model (SMM) for detection of a recent population bottleneck event within each *B. tabaci* MED population.

*One-tailed Wilcoxon signed-rank test; Bolded numbers indicate they are significant at $p < 0.05$.

2-4-2-7. Pairwise comparisons of fixation index (F_{ST})

The fixation index (F_{ST}) reflects the degree of genetic differentiation among the populations. F_{ST} is close to 0 when the genetic variation shows no difference in fixation among the populations. It is close to 1 when genetic differentiation is high. In this study, the F_{ST} values ranged from -0.0155 to 0.7501 and the ENA-corrected F_{ST} values ranged from -0.0139 to 0.7327 among the populations (Table 5). The highest F_{ST} value was detected between the 16'JJ and 16'BY populations (0.7327). The lowest F_{ST} value was found between the 17'SJ and 18'SJ populations (-0.0139). In practice, an F_{ST} value of 0.00 - 0.05 indicates low differentiation and F_{ST} values > 0.15 indicate a high level of differentiation. Negative F_{ST} values are allowed because correlations vary from -1 to +1 (Wright, 1949). As a result, most *B*. *tabaci* in Korea showed high levels of differentiation.

Table 5. Pairwise F_{ST} values based on variation at eight microsatellite loci between the *B. tabaci* MED populations

	16'JJ	16'JIN	16'CW	16'BUS	16'GH	16'MY	16'JE	16'SC	16'GJ	16'BS	16'IS	16'AD	16'BY	16'CY	16'SJ	16'CC	16'PT	17'JJ	17'JIN	17'MY	17'BUS	17'SJ	17'SC	17'BS	17'GJ	17'JE	17'IS	17'CY	17'PT	17'CC	18'SC	18'BS	18'SJ	18'PT	18'JJ
16'JJ		0.2894	0.5640	0.3427	0.5955	0.6336	0.5115	0.6201	0.2432	0.5249	0.5102	0.4856	0.7501	0.4542	0.5407	0.5687	0.5179	0.0949	0.2578	0.1192	0.2356	0.6150	0.5451	0.5986	0.2960	0.5896	0.5962	0.4948	0.3874	0.3084	0.5548	0.6104	0.6324	0.5848	**0.0294**
16'JIN	0.2487		0.3983	0.1616	0.4852	0.4822	0.2634	0.4194	0.1808	0.4207	0.3661	0.2193	0.6080	0.2214	0.3667	0.4630	0.3748	0.2120	0.1569	0.1375	0.1259	0.5103	0.3496	0.4617	0.2111	0.3315	0.3913	0.2578	0.2599	0.1876	0.3223	0.4546	0.5083	0.4060	0.2415
16'CW	0.5356	0.3746		0.3854	0.4038	0.3810	0.3126	0.3222	0.3871	0.3753	0.3226	0.2892	0.4910	0.3956	0.1769	0.4414	0.3712	0.4565	0.5095	0.4458	0.4164	0.5157	0.4877	0.5196	0.4245	0.3181	0.4082	0.4004	0.4775	0.4553	0.4810	0.5184	0.5052	0.4088	0.5084
16'BUS	0.3232	0.1343	0.3635		0.4928	0.4916	0.2589	0.4466	0.2229	0.4599	0.3217	0.2005	0.5890	0.1597	0.3524	0.5230	0.4210	0.2364	0.2492	0.1951	0.0575	0.4963	0.3764	0.4729	0.2371	0.3245	0.3243	0.1835	0.3469	0.2198	0.3456	0.4646	0.4976	0.4529	0.2892
16'GH	0.5750	0.4727	0.3767	0.4713		0.4588	0.4982	0.4018	0.4518	0.3322	0.4360	0.4740	0.5783	0.5415	0.4095	0.2679	0.3062	0.5041	0.5784	0.5009	0.4969	0.5081	0.5537	0.5572	0.4917	0.5378	0.5366	0.5588	0.4988	0.4980	0.5573	0.5690	0.5042	0.3471	0.5467
16'MY	0.5957	0.4491	0.3105	0.4568	0.3863		0.4042	0.3066	0.5012	0.3076	0.4082	0.4239	0.4962	0.4622	0.3077	0.4060	0.3447	0.5371	0.5740	0.5212	0.4983	0.5172	0.4973	0.5836	0.5208	0.4280	0.4962	0.4795	0.5148	0.5017	0.5021	0.5896	0.5059	0.3903	0.5791
16'JE	0.4903	0.2438	0.3042	0.2360	0.4778	0.3796		0.2899	0.3520	0.4515	0.3373	0.1653	0.5605	0.1794	0.2595	0.5084	0.3638	0.3963	0.4148	0.3445	0.2818	0.5044	0.4140	0.4878	0.3754	**0.0460**	0.3915	0.2079	0.4202	0.3661	0.3815	0.4831	0.4956	0.3938	0.4450
16'SC	0.6078	0.4208	0.3201	0.4449	0.3631	0.2871	0.3052		0.4662	0.4038	0.3726	0.3251	0.5146	0.3628	0.2420	0.3949	0.2365	0.5267	0.5424	0.4941	0.4604	0.5176	0.4588	0.5711	0.5021	0.2988	0.4496	0.3931	0.5011	0.4727	0.4527	0.5724	0.5089	0.2592	0.5659
16'GJ	0.2337	0.1458	0.3484	0.1597	0.4267	0.4396	0.3243	0.4479		0.4079	0.3568	0.2797	0.5975	0.3004	0.3999	0.4662	0.3857	0.1408	0.2699	0.1313	0.1874	0.4769	0.3933	0.4359	**0.0443**	0.4008	0.3797	0.2958	0.2083	0.2246	0.3845	0.4260	0.4724	0.4118	0.1761
16'BS	0.5050	0.4093	0.3140	0.4370	0.2907	0.2504	0.4293	0.3864	0.3734		0.3923	0.4359	0.4639	0.4870	0.3374	0.2334	0.2769	0.4252	0.4931	0.4296	0.4379	0.4645	0.4848	0.5463	0.4239	0.4909	0.4754	0.5045	0.4108	0.4171	0.4911	0.5509	0.4519	0.3319	0.4716
16'IS	0.4959	0.3545	0.2974	0.3289	0.4141	0.3624	0.3324	0.3668	0.3350	0.3629		0.3021	0.4615	0.3630	0.2219	0.4727	0.3570	0.3991	0.4670	0.3922	0.3559	0.4677	0.4132	0.5022	0.3855	0.3707	0.1102	0.3558	0.4128	0.3770	0.3983	0.4954	0.4569	0.3778	0.4458
16'AD	0.4574	0.1882	0.2726	0.1810	0.4452	0.3766	0.1237	0.3191	0.2588	0.4001	0.2916		0.4904	0.1654	0.2954	0.4993	0.3674	0.3683	0.3857	0.3293	0.2689	0.4945	0.3818	0.4609	0.3139	0.1988	0.3046	0.1459	0.3932	0.3530	0.3525	0.4561	0.4854	0.3877	0.4200
16'BY	0.7327	0.6041	0.4835	0.6019	0.5491	0.4690	0.5580	0.5133	0.5848	0.4452	0.4461	0.4874		0.5714	0.4263	0.5271	0.4362	0.6802	0.7056	0.6539	0.6181	0.6354	0.6359	0.6983	0.6199	0.6044	0.5386	0.5878	0.6455	0.6364	0.6529	0.7170	0.6386	0.5056	0.7192
16'CY	0.4280	0.1899	0.3687	0.1489	0.5053	0.4182	0.1626	0.3460	0.2527	0.4528	0.3429	0.1610	0.5633		0.3328	0.5438	0.4008	0.3323	0.3006	0.2823	0.1726	0.5237	0.3575	0.4804	0.3114	0.2333	0.3489	**0.0255**	0.3965	0.2837	0.3307	0.4749	0.5234	0.4378	0.3850
16'SJ	0.5188	0.3442	0.1298	0.3333	0.3651	0.2527	0.2440	0.2380	0.3489	0.2789	0.2008	0.2587	0.4266	0.2997		0.4219	0.2885	0.4372	0.4821	0.4169	0.3793	0.4592	0.4129	0.4940	0.4191	0.2921	0.3021	0.3452	0.4286	0.4015	0.3907	0.4845	0.4445	0.3060	0.4779
16'CC	0.5446	0.4558	0.3950	0.5085	0.2155	0.3435	0.4867	0.3608	0.4410	0.2069	0.4456	0.4658	0.4969	0.5128	0.3771		0.2052	0.4913	0.5450	0.4867	0.4964	0.5087	0.5366	0.5689	0.4859	0.5531	0.5510	0.5727	0.4741	0.4871	0.5480	0.5791	0.4997	0.2733	0.5232
16'PT	0.5045	0.3676	0.3051	0.3968	0.2395	0.2828	0.3490	0.2172	0.3495	0.2447	0.3328	0.3362	0.4351	0.3743	0.2344	0.1729		0.4150	0.4813	0.4142	0.4084	0.4249	0.4122	0.4900	0.4037	0.4164	0.4146	0.4235	0.3970	0.4128	0.4073	0.4887	0.4096	**0.0075**	0.4548
17'JJ	0.1137	0.1934	0.4268	0.2116	0.4815	0.4971	0.3769	0.5198	0.1342	0.4088	0.3835	0.3419	0.6658	0.3078	0.4135	0.4781	0.4049		0.2023	0.0581	0.1409	0.4861	0.4044	0.4507	0.1688	0.4722	0.4612	0.3579	0.2493	0.1886	0.3991	0.4493	0.4911	0.4589	**0.0199**
17'JIN	0.1919	0.1463	0.4985	0.2682	0.5684	0.5526	0.4082	0.5464	0.2399	0.4907	0.4580	0.3705	0.7034	0.2922	0.4741	0.5344	0.4787	0.1724		0.1285	0.1053	0.5953	0.4455	0.5641	0.2622	0.4860	0.5104	0.3576	0.3406	0.1569	0.4490	0.5750	0.6073	0.5363	0.2287
17'MY	0.1117	0.1209	0.4169	0.1620	0.4798	0.4859	0.3333	0.4927	0.1089	0.4120	0.3816	0.3040	0.6444	0.2539	0.3960	0.4731	0.4040	**0.0466**	0.1135		0.0856	0.5171	0.3906	0.4737	0.1658	0.4203	0.4321	0.3025	0.2136	0.1086	0.3784	0.4707	0.5184	0.4519	0.0543
17'BUS	0.2123	0.1147	0.3807	**0.0491**	0.4710	0.4581	0.2590	0.4534	0.1304	0.4159	0.3458	0.2388	0.6129	0.1434	0.3510	0.4811	0.3874	0.1098	0.1301	0.0657		0.4967	0.3591	0.4613	0.1887	0.3568	0.3633	0.2021	0.2783	0.1176	0.3405	0.4542	0.4969	0.4413	0.1766
17'SJ	0.5808	0.4871	0.4695	0.4679	0.4718	0.4656	0.4873	0.4982	0.4380	0.4285	0.4474	0.4720	0.6113	0.4870	0.4215	0.4767	0.3945	0.4584	0.5704	0.4831	0.4599		0.3850	0.4446	0.4899	0.5469	0.5373	0.5447	0.4717	0.4862	0.4037	0.4541	**-0.0155**	0.4676	0.5422
17'SC	0.5147	0.3346	0.4464	0.3637	0.5174	0.4587	0.3982	0.4443	0.3429	0.4546	0.3993	0.3630	0.6230	0.3265	0.3838	0.5090	0.3893	0.3870	0.4435	0.3727	0.3484	0.3222		0.4272	0.4082	0.4687	0.4540	0.3943	0.3823	0.3277	**-0.0004**	0.4328	0.3748	0.4549	0.4694
17'BS	0.5535	0.4179	0.4811	0.4274	0.5289	0.5364	0.4585	0.5515	0.3894	0.5132	0.4804	0.4277	0.6750	0.4395	0.4627	0.5422	0.4618	0.4104	0.5205	0.4253	0.4079	0.4077	0.3754		0.4386	0.5402	0.5797	0.5008	0.5057	0.5104	0.4168	**-0.0090**	0.4445	0.5363	0.5206
17'GJ	0.2570	0.1775	0.3932	0.1874	0.4730	0.4779	0.3466	0.4923	**0.0413**	0.4109	0.3746	0.2914	0.6128	0.2695	0.3881	0.4743	0.3875	0.1429	0.2284	0.1256	0.1320	0.4630	0.3733	0.3953		0.4286	0.4022	0.3191	0.2120	0.2379	0.3997	0.4318	0.4860	0.4331	0.2208
17'JE	0.5528	0.3073	0.3220	0.3023	0.5127	0.4031	**0.0499**	0.3069	0.3693	0.4648	0.3589	0.1696	0.5906	0.2140	0.2874	0.5226	0.3958	0.4405	0.4679	0.3964	0.3250	0.5156	0.4386	0.5007	0.3933		0.4438	0.2747	0.4782	0.4358	0.4469	0.5415	0.5435	0.4555	0.5270
17'IS	0.5543	0.3683	0.3920	0.3368	0.5073	0.4588	0.3790	0.4408	0.3433	0.4486	0.1003	0.3096	0.5353	0.3189	0.2951	0.5181	0.3958	0.4252	0.4880	0.4007	0.3426	0.4970	0.4279	0.5361	0.3752	0.4175		0.3405	0.4378	0.3959	0.4502	0.5809	0.5320	0.4469	0.5243
17'CY	0.4606	0.2164	0.3974	0.1638	0.5385	0.4563	0.1890	0.3979	0.2645	0.4869	0.3560	0.1587	0.5969	**0.0245**	0.3400	0.5521	0.4166	0.3233	0.3242	0.2654	0.1569	0.5207	0.3746	0.4596	0.2798	0.2546	0.3391		0.4062	0.3054	0.3754	0.4983	0.5447	0.4608	0.4163
17'PT	0.3562	0.2511	0.4435	0.3172	0.4814	0.4811	0.4080	0.4963	0.1767	0.4039	0.4019	0.3760	0.6363	0.3570	0.4040	0.4634	0.3905	0.2466	0.3117	0.1993	0.2509	0.4397	0.3531	0.4709	0.1919	0.4545	0.4074	0.3764		0.1761	0.3923	0.5011	0.4647	0.4248	0.2876
17'CC	0.2495	0.1793	0.4270	0.2110	0.4729	0.4679	0.3580	0.4688	0.1806	0.4003	0.3679	0.3349	0.6273	0.2505	0.3824	0.4672	0.3999	0.1486	0.1477	0.0869	0.1141	0.4469	0.3146	0.4659	0.1934	0.4144	0.3672	0.2725	0.1557		0.3303	0.5083	0.4860	0.4469	0.2246
18'SC	0.5227	0.3110	0.4460	0.3442	0.5241	0.4650	0.3734	0.4413	0.3369	0.4622	0.3888	0.3383	0.6383	0.3066	0.3724	0.5184	0.3861	0.3809	0.4470	0.3613	0.3346	0.3418	**-0.0044**	0.3607	0.3653	0.4206	0.4263	0.3586	0.3649	0.3164		0.4250	0.3953	0.4470	0.4735
18'BS	0.5666	0.4143	0.4869	0.4249	0.5462	0.5491	0.4596	0.5573	0.3838	0.5249	0.4759	0.4287	0.6976	0.4369	0.4611	0.5563	0.4671	0.4145	0.5342	0.4254	0.4045	0.4194	0.3840	**-0.0064**	0.3921	0.5037	0.5384	0.4608	0.4690	0.4648	0.3718		0.4541	0.5368	0.5280
18'SJ	0.5997	0.4883	0.4632	0.4744	0.4720	0.4572	0.4824	0.4931	0.4375	0.4195	0.4376	0.4663	0.6166	0.4884	0.4114	0.4694	0.3840	0.4664	0.5843	0.4875	0.4637	**-0.0139**	0.3120	0.4080	0.4627	0.5133	0.4931	0.5239	0.4348	0.4476	0.3333	0.4205		0.4512	0.5512
18'PT	0.5779	0.4134	0.3525	0.4479	0.2800	0.3409	0.3892	0.2336	0.3949	0.3150	0.3621	0.3687	0.5085	0.4221	0.2693	0.2308	**0.0098**	0.4621	0.5455	0.4558	0.4368	0.4415	0.4380	0.5129	0.4331	0.4386	0.4391	0.4657	0.4344	0.4461	0.4349	0.5215	0.4316		0.5093
18'JJ	**0.0441**	0.2128	0.4835	0.2585	0.5312	0.5446	0.4286	0.5592	0.1658	0.4604	0.4280	0.3947	0.7042	0.3508	0.4610	0.5147	0.4497	**0.0181**	0.1705	**0.0431**	0.1401	0.5144	0.4395	0.4731	0.1804	0.4917	0.4764	0.3713	0.2707	0.1681	0.4407	0.4821	0.5255	0.5145	

Significant values ($p < 0.05$) for pairwise F_{ST} are in bold.

2-5. Discussion

This study is the first comprehensive genetic structure analysis of *B*. *tabaci* MED (Q1) populations in Korea using eight microsatellite loci. The Korean populations of tomato *B*. *tabaci* MED appeared to be classified into two genetic clusters based on STRUCTURE and DAPC analyses, and their genetic structure converged rapidly into one genetic cluster. This phenomenon was reported previously by Dinsdale et al. (Dinsdale et al., 2012) in Australia. They reported that the genetic cluster of *B*. *tabaci* rapidly changed even in a period of just four months. The results of this study and those by Dinsdale et al. (Dinsdale et al., 2012), suggested that one out of the two *B*. *tabaci* MED genetic clusters in Korea might become the dominant species in the future.

This phenomenon could be caused by different fitness between the two *B*. *tabaci* MED genetic clusters in Korea. Although the two *B*. *tabaci* MED genetic clusters might have been mixed when they were first introduced in new areas, one genetic cluster would become dominant if there is fitness difference between them. Fitness difference between two genetic clusters could result from different susceptibilities to insecticides. The use of various insecticides, such

as neonicotinoids, organophosphates, and carbamates, has been the main control method for *B*. *tabaci* MED in Korea. Extensive use of these insecticides has rapidly resulted in high levels of insecticide resistance in *B*. *tabaci* MED populations (Lee et al., 2002). The two genetic clusters of *B*. *tabaci* MED might have different potentials for developing resistance to different insecticides. This differentiation was partially supported by changing the frequencies and diversity caused by chemical control (Chu et al., 2014; Gauthier et al., 2014). Results of the current study also showed low genotype frequencies and diversities, and limited founder or bottleneck effects. However, the speed of this genetic cluster change in Korea could differ by areas. For example, the Jeju populations showed one genetic cluster of *B*. *tabaci* MED and this trend was maintained during the past three years.

However, in the Pyeongtaek area, the genetic cluster of *B*. *tabaci* MED changed every year. The differences in the speed of genetic cluster change could be caused by human-related factors because *B*. *tabaci* has a low dispersal ability over long distances (Byrne, 1999). In the case of Jeju, the *B*. *tabaci* MED populations should not have been affected by other populations because almost all growers produce tomato seedlings themselves and Jeju is isolated because it is an island. On the other hand, the Pyeongtaek tomato

growers have purchased tomato seedlings from different nurseries every year. Moreover, the city of Pyeongtaek has one of the most active agricultural trades of all Korean cities. Whitefly populations are generally affected by human activities, such as the movement of infested plants from nurseries, material shipments, and commercial trading, rather than by active flight (Chu et al., 2014; Hadjistylli et al., 2016). Thus, the populations in areas with high human activities and diverse nursery routes (i.e., the Pyeongtaek populations) might show accelerated genetic cluster changes compared to populations in isolated areas with limited nursery routes (i.e., the Jeju populations).

The information on the genetic characteristics of *B*. *tabaci* in areas where it usually occurs should be useful for efficient management of *B*. *tabaci* (Cullingham et al., 2012; Karsten et al., 2013; Rollins et al., 2006).The genetic structure information gathered from the long-term and large-scale field analysis in this study facilitates a better understanding of the population dynamics of *B*. *tabaci* MED as an invasive pest in Korea. Thus, the results of this study could be a valuable foundation to develop efficient management strategies for *B*. *tabaci* MED in Korea. However, further studies are needed to clearly find the fitness differences between the two *B*. *tabaci* MED genetic clusters in Korea.

Appendix 1. Genetic diversity for all different eight microsatellite loci screened for *B*. *tabaci* MED in Korea

Population	Statistic / Loci	11	145	177	BT4	BT159	53	68	Bem23
16'JJ	N_A[a]	36	36	40	40	40	36	38	38
	H_o[b]	0.000	0.000	0.050	0.000	0.175	0.000	0.763	0.289
	H_E[c]	0.000	0.105	0.384	0.000	0.160	0.000	0.609	0.483
	F_{IS}[d]		1.000	0.870		-0.096		-0.252	0.401
	Null[e]	0.001	0.156	0.194	0.001	0.000	0.001	0.037	0.132
16'JIN	N_A	38	39	39	40	40	35	37	38
	H_o	0.000	0.667	0.359	0.125	0.450	0.000	0.351	0.237
	H_E	0.000	0.478	0.630	0.327	0.713	0.000	0.469	0.768
	F_{IS}		-0.396	0.430	0.617	0.368		0.250	0.691
	Null	0.001	0.000	0.377	0.190	0.014	0.001	0.057	0.294
16'CW	N_A	38	40	40	38	40	40	40	39
	H_o	0.211	0.050	0.025	0.079	0.100	0.000	0.000	0.026
	H_E	0.194	0.509	0.646	0.417	0.560	0.469	0.521	0.527
	F_{IS}	-0.088	0.902	0.961	0.810	0.821	1.000	1.000	0.951
	Null	0.000	0.302	0.340	0.254	0.303	0.323	0.344	0.329
16'BUS	N_A	37	38	39	36	38	35	37	37
	H_o	0.000	0.079	0.000	0.000	0.000	0.000	0.378	0.486
	H_E	0.000	0.491	0.480	0.742	0.555	0.000	0.389	0.602
	F_{IS}		0.839	1.000	1.000	1.000		0.026	0.192
	Null	0.001	0.277	0.213	0.426	0.634	0.001	0.009	0.119
16'GH	N_A	39	40	36	37	38	35	38	38
	H_o	0.179	0.175	0.000	0.000	0.605	0.000	0.053	0.263
	H_E	0.168	0.654	0.198	0.542	0.703	0.000	0.419	0.625
	F_{IS}	-0.068	0.733	1.000	1.000	0.139		0.874	0.579
	Null	0.000	0.289	0.285	0.354	0.085	0.001	0.270	0.232

Appendix 1. *Continued*.

16'MY	N_A	40	40	38	40	40	38	35	36
	H_o	0.075	0.175	0.026	0.050	0.075	0.000	0.286	0.167
	H_E	0.206	0.498	0.380	0.228	0.731	0.373	0.507	0.752
	F_{IS}	0.636	0.648	0.931	0.780	0.897	1.000	0.437	0.778
	Null	0.156	0.220	0.666	0.174	0.382	0.293	0.138	0.332
16'JE	N_A	36	38	38	38	39	40	40	36
	H_o	0.694	0.211	0.184	0.026	0.231	0.000	0.875	0.472
	H_E	0.706	0.229	0.214	0.652	0.542	0.000	0.677	0.801
	F_{IS}	0.017	0.079	0.138	0.960	0.574		-0.292	0.411
	Null	0.111	0.024	0.000	0.380	0.194	0.001	0.006	0.195
16'SC	N_A	38	39	39	39	40	36	38	36
	H_o	0.474	0.410	0.128	0.128	0.200	0.028	0.500	0.389
	H_E	0.637	0.707	0.121	0.423	0.310	0.027	0.836	0.598
	F_{IS}	0.256	0.420	-0.057	0.697	0.355	-0.014	0.402	0.350
	Null	0.082	0.185	0.384	0.231	0.099	0.000	0.190	0.162
16'GJ	N_A	40	40	40	39	40	40	40	40
	H_o	0.000	0.900	0.000	0.000	0.450	0.000	0.250	0.250
	H_E	0.000	0.585	0.621	0.544	0.749	0.139	0.399	0.658
	F_{IS}		-0.538	1.000	1.000	0.399	1.000	0.373	0.620
	Null	0.001	0.000	0.400	0.360	0.192	0.179	0.117	0.263
16'BS	N_A	40	40	38	37	40	39	40	38
	H_o	0.075	0.250	0.000	0.081	0.350	0.000	0.500	0.237
	H_E	0.205	0.360	0.665	0.493	0.645	0.586	0.544	0.671
	F_{IS}	0.635	0.305	1.000	0.835	0.457	1.000	0.082	0.647
	Null	0.156	0.010	0.156	0.295	0.203	0.371	0.000	0.263

Appendix 1. *Continued*.

	N_A	39	39	34	34	35	39	40	24
	H_o	0.692	0.897	0.441	0.912	0.229	0.231	0.825	0.417
16'IS	H_E	0.739	0.512	0.695	0.805	0.252	0.255	0.618	0.517
	F_{IS}	0.063	-0.751	0.365	-0.133	0.092	0.094	-0.336	0.195
	Null	0.054	0.000	0.034	0.002	0.000	0.057	0.012	0.112
	N_A	37	38	40	36	36	35	38	39
	H_o	0.216	0.658	0.450	0.111	0.167	0.029	0.263	0.282
16'AD	H_E	0.591	0.623	0.432	0.459	0.578	0.028	0.414	0.762
	F_{IS}	0.634	-0.056	-0.043	0.758	0.712	-0.014	0.365	0.630
	Null	0.234	0.000	0.210	0.264	0.257	0.000	0.059	0.281
	N_A	37	37	37	36	35	36	37	36
	H_o	0.000	0.270	0.000	0.000	0.000	0.000	0.432	0.389
16'BY	H_E	0.000	0.472	0.193	0.000	0.000	0.000	0.749	0.632
	F_{IS}		0.427	1.000				0.423	0.385
	Null	0.001	0.091	0.001	0.001	0.001	0.001	0.157	0.157
	N_A	35	36	36	40	35	37	39	37
	H_o	0.000	0.278	0.000	0.000	0.000	0.000	0.513	0.649
16'CY	H_E	0.000	0.517	0.000	0.646	0.490	0.102	0.591	0.782
	F_{IS}		0.463		1.000	1.000	1.000	0.133	0.170
	Null	0.001	0.151	0.279	0.393	0.330	0.000	0.016	0.075
	N_A	40	40	37	39	40	40	38	38
	H_o	0.175	0.225	0.054	0.051	0.075	0.000	0.316	0.289
16'SJ	H_E	0.522	0.443	0.422	0.640	0.511	0.718	0.694	0.803
	F_{IS}	0.665	0.492	0.872	0.920	0.853	1.000	0.545	0.639
	Null	0.218	0.172	0.321	0.364	0.298	0.179	0.209	0.285

Appendix 1. *Continued*.

	N_A	40	40	32	38	40	40	40	33
	H_o	0.050	0.025	0.125	0.053	0.075	0.250	0.800	0.515
16'CC	H_E	0.073	0.206	0.642	0.126	0.487	0.383	0.837	0.804
	F_{IS}	0.316	0.879	0.805	0.581	0.846	0.346	0.044	0.359
	Null	0.000	0.201	0.351	0.101	0.278	0.371	0.029	0.164
	N_A	40	40	37	40	40	39	40	40
	H_o	0.125	0.075	0.135	0.125	0.375	0.026	0.900	0.350
16'PT	H_E	0.445	0.723	0.748	0.605	0.762	0.122	0.785	0.612
	F_{IS}	0.719	0.896	0.819	0.793	0.508	0.790	-0.146	0.428
	Null	0.223	0.376	0.351	0.299	0.216	0.057	0.000	0.169
	N_A	19	17	20	20	20	19	20	20
	H_o	0.000	0.176	0.100	0.250	0.800	0.000	1.000	0.800
17'JJ	H_E	0.100	0.164	0.666	0.501	0.499	0.000	0.545	0.480
	F_{IS}	1.000	-0.074	0.850	0.501	-0.604		-0.835	-0.667
	Null	0.152	0.000	0.344	0.185	0.000	0.000	0.000	0.000
	N_A	27	30	28	28	30	29	30	30
	H_o	0.000	0.000	0.036	0.000	0.100	0.000	1.000	0.533
17'JIN	H_E	0.000	0.000	0.357	0.000	0.359	0.000	0.500	0.748
	F_{IS}			0.900		0.722		-1.000	0.287
	Null	0.001	0.001	0.257	0.001	0.222	0.001	0.000	0.158
	N_A	27	29	30	30	29	30	30	30
	H_o	0.000	0.310	0.033	0.033	0.828	0.000	1.000	0.900
17'MY	H_E	0.000	0.402	0.613	0.214	0.592	0.000	0.605	0.601
	F_{IS}		0.228	0.946	0.844	-0.398		-0.653	-0.499
	Null	0.001	0.088	0.362	0.199	0.041	0.154	0.000	0.016

Appendix 1. *Continued*.

	N_A	28	30	30	30	30	30	30	30
	H_o	0.000	0.200	0.000	0.033	0.267	0.000	0.967	0.967
17'BUS	H_E	0.000	0.474	0.482	0.585	0.603	0.000	0.499	0.605
	F_{IS}		0.578	1.000	0.943	0.558		-0.935	-0.598
	Null	0.001	0.200	0.336	0.354	0.210	0.418	0.000	0.000
	N_A	30	30	29	30	30	28	30	30
	H_o	0.867	0.100	0.034	0.167	0.667	0.036	0.600	0.867
17'SJ	H_E	0.491	0.206	0.216	0.215	0.709	0.035	0.673	0.727
	F_{IS}	-0.765	0.515	0.840	0.225	0.060	-0.018	0.109	-0.193
	Null	0.000	0.125	0.195	0.000	0.084	0.000	0.000	0.000
	N_A	27	29	29	29	30	30	30	30
	H_o	0.000	0.000	0.000	0.414	0.967	0.500	0.933	0.167
17'SC	H_E	0.000	0.340	0.238	0.509	0.745	0.375	0.616	0.187
	F_{IS}		1.000	1.000	0.187	-0.298	-0.333	-0.515	0.110
	Null	0.001	0.281	0.233	0.109	0.000	0.154	0.000	0.000
	N_A	29	30	30	30	30	30	30	30
	H_o	0.276	1.000	0.100	0.267	0.100	0.000	0.267	0.700
17'BS	H_E	0.238	0.545	0.206	0.235	0.299	0.000	0.518	0.562
	F_{IS}	-0.160	-0.835	0.515	-0.135	0.666		0.485	-0.246
	Null	0.000	0.000	0.125	0.000	0.165	0.418	0.014	0.000
	N_A	30	30	30	30	30	30	30	30
	H_o	0.000	0.967	0.000	0.133	0.733	0.000	1.000	0.567
17'GJ	H_E	0.000	0.529	0.571	0.569	0.681	0.000	0.500	0.696
	F_{IS}		-0.826	1.000	0.766	-0.078		-1.000	0.186
	Null	0.001	0.000	0.366	0.283	0.049	0.000	0.000	0.087

Appendix 1. *Continued*.

	N_A	30	28	30	30	30	30	30	30
	H_o	0.900	0.357	0.133	0.100	0.800	0.000	0.933	0.767
17'JE	H_E	0.499	0.363	0.184	0.402	0.596	0.000	0.603	0.505
	F_{IS}	-0.802	0.016	0.277	0.751	-0.342		-0.547	-0.518
	Null	0.000	0.516	0.088	0.227	0.000	0.145	0.000	0.000
	N_A	15	15	15	15	14	15	15	15
	H_o	0.267	0.867	0.000	0.267	0.071	0.000	1.000	0.933
17'IS	H_E	0.231	0.491	0.338	0.436	0.196	0.000	0.580	0.760
	F_{IS}	-0.154	-0.765	1.000	0.388	0.636		-0.724	-0.228
	Null	0.000	0.000	0.283	0.118	0.154	0.001	0.000	0.000
	N_A	27	29	27	30	30	30	30	30
	H_o	0.000	0.759	0.000	0.000	0.000	0.000	0.933	0.700
17'CY	H_E	0.000	0.504	0.000	0.560	0.464	0.000	0.553	0.768
	F_{IS}		-0.505		1.000	1.000		-0.688	0.088
	Null	0.001	0.000	0.001	0.365	0.333	0.001	0.000	0.069
	N_A	30	30	27	30	30	30	30	30
	H_o	0.000	1.000	0.074	0.133	0.233	1.000	0.967	0.700
17'PT	H_E	0.000	0.531	0.742	0.215	0.349	0.500	0.499	0.556
	F_{IS}		-0.883	0.900	0.380	0.331	-1.000	-0.935	-0.259
	Null	0.001	0.000	0.084	0.094	0.115	0.000	0.000	0.000
	N_A	28	29	30	30	30	29	30	30
	H_o	0.000	0.586	0.000	0.200	0.800	0.759	1.000	0.967
17'CC	H_E	0.000	0.492	0.331	0.283	0.491	0.471	0.516	0.515
	F_{IS}		-0.191	1.000	0.293	-0.629	-0.611	-0.938	-0.877
	Null	0.001	0.015	0.278	0.086	0.000	0.000	0.000	0.000

Appendix 1. *Continued*.

	N_A	20	20	20	20	20	20	20	20
	H_o	0.000	0.000	0.000	0.450	0.950	0.750	0.950	0.200
18'SC	H_E	0.000	0.320	0.180	0.484	0.739	0.469	0.566	0.184
	F_{IS}		1.000	1.000	0.070	-0.286	-0.600	-0.678	-0.088
	Null	0.001	0.268	0.023	0.083	0.000	0.000	0.000	0.000
	N_A	20	20	20	20	20	20	20	20
	H_o	0.000	1.000	0.150	0.250	0.050	0.000	0.250	0.850
18'BS	H_E	0.000	0.564	0.219	0.224	0.271	0.000	0.524	0.618
	F_{IS}		-0.774	0.314	-0.117	0.816		0.523	-0.377
	Null	0.001	0.000	0.084	0.000	0.205	0.001	0.158	0.000
	N_A	20	20	20	20	20	20	20	20
	H_o	0.900	0.150	0.050	0.200	0.550	0.000	0.650	0.800
18'SJ	H_E	0.495	0.289	0.224	0.270	0.738	0.000	0.654	0.695
	F_{IS}	-0.818	0.481	0.777	0.259	0.254		0.006	-0.151
	Null	0.000	0.134	0.187	0.000	0.125	0.001	0.000	0.003
	N_A	20	20	20	20	20	20	20	20
	H_o	0.000	0.050	0.200	0.150	0.350	0.000	0.850	0.400
18'PT	H_E	0.375	0.541	0.734	0.686	0.756	0.000	0.613	0.666
	F_{IS}	1.000	0.908	0.727	0.781	0.537		-0.388	0.400
	Null	0.290	0.328	0.311	0.317	0.238	0.001	0.000	0.172
	N_A	20	20	20	20	20	20	20	20
	H_o	0.000	0.150	0.050	0.050	0.650	0.000	1.000	0.900
18'JJ	H_E	0.000	0.141	0.446	0.219	0.439	0.000	0.545	0.495
	F_{IS}		-0.062	0.888	0.771	-0.481		-0.835	-0.818
	Null	0.001	0.000	0.296	0.180	0.000	0.001	0.000	0.000

[a]Number of alleles. [b]Expected heterozygosity. [c]Observed heterozygosity. [d]Mean fixation index inbreeding coefficient. [e]Average proportion of homozygous for null allele.

Appendix 2. Information of GenBank accession number

Sample site	Population	Collection date	GenBank accession No.
Seogwipo-si	16'JJ 17'JJ 18'JJ	2016-04-25 2017-04-19 2018-10-10	HM802268 KY249477 KY249414
JinJu-si *	16'JIN 17'JIN	2016-05-25 2017-06-07	EU386987 EF694108
Changwon-si	16'CW	2016-05-25	KY468417
Busan *	16'BUS 17'BUS	2016-05-25 2017-06-09	FJ375358 HM597869
Gimhae-si	16'GH	2016-05-26	EU263626
Miryang-si *	16'MY 17'MY	2016-05-26 2017-06-08	EU760729 HM597849
Jeongeup-si *	16'JE 17'JE	2016-06-01 2017-06-20	EF667474 EU263630
Suncheon-si *	16'SC 17'SC 18'SC	2016-06-01 2017-06-19 2018-07-11	MH357338 KY468420 HM597847
Gwangju *	16'GJ 17'GJ	2016-06-02 2017-06-20	KY468410 KY468415
Boseong-gun *	16'BS 17'BS 18'BS	2016-06-02 2017-06-19 2018-07-11	EU263629 HM597859 KY249401
Iksan-si	16'IS 17'IS	2016-06-09 2017-06-20	HM597859 EU427722
Andong-si	16'AD	2016-06-09	KP137475
Buyeo-gun	16'BY	2016-06-30	MH357340

Appendix 2. *Continued.*

Cheongyang-gun *	16'CY 17'CY	2016-06-30 2017-06-21	EU760736 KY249451
Sejong-si	16'SJ 17'SJ 18'SJ	2016-06-30 2017-06-19 2018-07-12	KY249434 MG565975 EU376987
Chuncheon-si	16'CC 17'CC	2016-07-29 2017-06-29	MH357339 KY468408
Pyeongtaek-si	16'PT 17'PT 18'PT	2016-08-05 2017-06-26 2018-08-10	MH357340 MH205752 KY249438

Chapter Ⅲ.

Comparison of life history characteristics of two different genetic clusters of *Bemisia tabaci* MED (Hemiptera: Aleyrodidae)

3-1. Abstract

Bemisia tabaci Mediterranean (Gennadius) (Hemiptera: Aleyrodidae) is one of serious insect pests with economic importance worldwide. Previously, we have reported that most *B. tabaci* Mediterranean (MED) populations on greenhouse tomatoes in Korea converge from well-differentiated two genetic clusters (C1 and C2) to one (C2) during one year period. To elucidate factors responsible for this phenomenon, we compared life history characteristics of these two different genetic clusters of *B. tabaci* MED through single and cross mating experiments on two different host plants, cucumber and tobacco, at 26 °C. Intrinsic rate of increase (r), finite rate of increase (λ), and net reproductive rate (R_O) were significantly higher in the dominating cluster (C2) (0.247, 1.280, and 192.402, respectively on cucumber; 0.226, 1.253, and 133.792, respectively on tobacco) than in the other cluster (C1) (0.149, 1.161, and 50.539, respectively on cucumber; 0.145, 1.156, and 53.332, respectively on tobacco). Overall performances of cross mating groups, C2fC1m (C2 female $\times$ C1 male) and C1fC2m (C1 female $\times$ C2 male), were in-between those of C2 and C1, with C2fC1m performing better than C1fC2m. Thus, maternal inheritance appeared to be significantly associated with their life

history characteristics, with partial involvement of paternal inheritance. Our results demonstrated that the rapid convergence of genetic clusters of *B. tabaci* MED populations was clearly associated with differences in their life history characteristics.

Key words: *Bemisia tabaci,* Mediterranean, whitefly, life history characteristics, life table, Korea

3-2. Introduction

The sweet potato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) causes significant economic damage to major vegetables, fruits, and ornamental crops worldwide (Chen et al., 2004; Navas-Castillo et al., 2011; Tsagkarakou et al., 2012). In Korea, *B. tabaci* MED (Mediterranean or biotype Q) is currently predominant in most regions whereas *B. tabaci* MEAM1 (Middle East-Asia Mininor 1) and *B. tabaci* JpL are only present in a few regions (Lee et al., 2016; Lee et al., 2014).

Previously, we have reported that there are two clusters of *B. tabaci* MED populations in greenhouse tomatoes and that their genetic clusters converge into one genetic cluster in most regions (Park et al., 2019). We hypothesized that the dominating genetic cluster (cluster 2) population could efficiently compete out the other cluster (cluster 1) which was prevalent at the beginning. Potentially different insecticide resistance of these genetic clusters, if any, might be partly involved in genetic cluster change. Similar phenomenon has been reported previously in Australia (Dinsdale et al., 2012) and China (Chu et al., 2014). However, there have been no follow-up studies that delve into causes. Life table analysis is considered as one of the most effective

analytical tools to evaluate life history characteristics of insects because life table parameters provide comprehensive understanding of fitness of insect species (Chi, 1990; Fang et al., 2014; Mitchell, 1981; MUSA & REN, 2005). Especially, intrinsic rate of increase (*r*) is a basic parameter for describing population traits (Birch, 1948).

The objective of this study was to find evidence for the change in compositions of genetic cluster that resulted in dominance of one genetic cluster of *B. tabaci* MED in Korea. To test our hypothesis that differences in fitness between two genetic clusters contributed significantly to this change, we compared life history characteristics of two different genetic clusters of *B. tabaci* MED on two different host plants, cucumber and tobacco, through single and cross mating.

3-3. Materials and Methods

3-3-1. *B. tabaci* MED cultures and plants

We used two different representative genetic cluster populations of *B. tabaci* MED cluster 1 and cluster 2, collected from tomato greenhouses in Pyeongtaek and Sejong, respectively, in Korea in 2018 (Park et al., 2019). We confirmed genetic structures of these populations according to the following procedures. PCR primers were used to amplify microsatellite DNA loci 11, 53 (Delatte et al., 2005), 68, 145, 177 (Dalmon et al., 2008), BT4, BT159 (Tsagkarakou & Roditakis, 2003), and Bem23 (De Barro et al., 2003) using individual gDNAs of *B. tabaci* MED as templates. PCR reaction conditions followed the protocol by Dalmon et al. (Dalmon et al., 2008). PCR products were analyzed using an ABI 3730xl (Applied Biosystems Inc., Foster, CA, USA) at NICEM (Seoul, Korea). Then 1 μl PCR product was diluted with 8.5 μl of Hi-Di formamide (Applied Biosystems Inc.) and 0.5 μl Genescan ROX-500 size standard (Applied Biosystems Inc.). These genetic data were analyzed using GENEMAPPER v.3.7 (Applied Biosystems Inc.), GenAlEx v.6.5 (Peakall & Smouse, 2012), STRUCTURE v.2.3.2 (Pritchard et al., 2000a), and STRUCTURE HARVESTER Web v.0.6.93 (Earl, 2012).

Host plants used in this study were cucumber (*Cucumis sativus* L.) and tobacco (*Nicotiana tabacum* L.). This is because *B. tabaci* prefers plants with pubescent leaves for oviposition and feeding (Shah & Liu, 2013; Zhao et al., 2014). These two plants belong to the most preferred host plants of *B. tabaci* (Al-Zyoud et al., 2005). Both *B. tabaci* MED populations were separately maintained on both cucumber and tobacco plants under the same experimental conditions. *B. tabaci* colonies were reared in cages (40 × 40 × 40 cm^3) at 26 ± 1 °C with relative humidity (RH) of 50 ± 10% and a photoperiod of 14:10 (L:D) h. These colonies served as stock colonies for experiments. The purity of each culture was monitored for every generation by microsatellite analysis. After ten generations of rearing, *B. tabaci* colonies were used for experiments.

3-3-2. Life table experiments

Life table experiments and analyses were conducted following Maia et al. (De Holanda Nunes Maia et al., 2014; Maia et al., 2000). Data collection was made from the onset of oviposition of adults until completion of development of their progeny. Followings are our experimental procedures.

To obtain newly emerged virgin adults of *B. tabaci* (< 12 h old) (De Barro & Hart, 2000; Li et al., 1989; Luan et al., 2008; Perring & Symmes, 2006), plant leaves with pupae (late 4th instar nymphs with red eyes) were excised from stock colonies of two genetic clusters. The cut of leaf petioles was maintained on a moistened pad until adult emergence. The sex of newly emerged adults was determined under a stereomicroscope (× 200). These adults were separated by sex and placed into insect breeding dishes (10 cm in diameter, 4.2 cm in height) (SPL Life sciences, Pocheon, Korea) before experiments.

Life table experiments were conducted for single and cross mating groups between two different genetic clusters of *B. tabaci* MED on two different host plants, cucumber and tobacco (Table 1). All experiments were conducted at 26 ± 1 °C, 50 ± 10% RH, and a photoperiod of L:D (14:10) h in an incubator. Preparation of single and cross mating groups was made using the 'single-pair mating' method (Sun et al., 2011; Xu et al., 2010). For single-pair mating, we used one female and two male adults of *B. tabaci* in each replicate to assure successful copulation. Each treatment had 30 pairs of *B. tabaci* MED adults. All pairs of *B. tabaci* adults were placed separately on a leaf disc (5 cm in diameter) which was placed on a moistened pad on the

bottom of an insect breeding dish (5 cm in diameter, 1.5 cm in height) (SPL Life sciences, Pocheon, Korea). Adults were transferred onto fresh leaf discs in new insect breeding dishes using brushes (Brush 320 Series No. 1, Hwahong, Hwaseong, Korea) every two days. Dead male adults were replaced from colonies. Oviposition and post-oviposition periods, fecundity, and longevity of female adults were observed and counted daily until they died. The survival of offspring for each treatment group was checked for all progeny of individual female adults every two days until they died or became adults. Emerged *B. tabaci* adults were counted and their sex was identified under a stereomicroscope ($\times$ 200). Since examination for progeny was made for each female adult with 30 adults for each treatment group, survival rate and sex ratio of all offspring were calculated for each treatment group with 30 replications. To observe developmental period from egg to adult for offspring in each treatment group, a total of 60 eggs were randomly selected among the above described progeny of each group. To ascertain representation of proper progeny of each group, three to five eggs were selected over various randomly allocated dates. Marking was made on lids of insect breeding dishes to identify selected eggs with a permanent marker pen (Name pen X,

Monami Co. Ltd, Yong-in, Korea). Their development period was observed daily until they died or became adults. The pad on the bottom of an insect breeding dish was wetted with distilled water using pipette tips every day to maintain healthy leaves.

Table 1. Single and cross mating groups between cluster 1 (C1) and cluster 2 (C2) of *B. tabaci* MED

Host plant	Treatment	Culture type	Mating method	Crosses
Cucumber/ Tobacco	C1	Single cluster	Single	C1 (1♀ × 2♂)
	C2			C2 (1♀ × 2♂)
	C1fC2m	Mixed cluster	Single crossing	C1 (1♀) × C2 (2♂)
	C2fC1m			C2 (1♀) × C1 (2♂)

3-3-3. Proportion of genetic cluster

To characterize the genetic cluster of each treatment group (i.e., single and cross mating), a total of 20 female individuals from each treatment group were examined using previously described microsatellite analysis procedure. We used a burn-in of 60,000 Markov Chain Monte Carlo (MCMC) steps and a burn-in period of 600,000. Log-likelihood estimates were calculated for K = 1 to 10 with ten replicates of each. Structure Harvester analysis was performed to detect the likelihood of the number of occurring clusters among individuals of *B. tabaci* MED.

3-3-4. Body weight and length of adult *B. tabaci*

Body weight and length were measured for 100 female and 100 male adults of *B. tabaci* selected randomly from each treatment group. Adults were frozen. Their body weights and lengths were measured. The body length was measured from the top of the head to the end of the abdomen using a Leica Application Suite X program (Leica Microsystems, Inc., Buffalo Grove, IL, USA). The body weight was measured using a BM-22 microbalance (A&D Co. Ltd., Tokyo, Japan) with 10 individuals as a group.

3-3-5. Statistical analysis

Two-way analysis of variance (ANOVA) was conducted to determine effects of clusters and host plants on female adult longevity, fecundity, oviposition period, adult body weight, adult body length, offspring's sex ratio, and offspring's survival rate using PROC ANOVA in SAS (SAS institute, 2013) (Sas & Guide, 2013). PROC GLM in SAS (SAS Institute, 2013) was used for development period of offspring because of different sample sizes among treatments. Mean separation was conducted by Tukey's studentized range test at $p < 0.05$.

3-3-6. Life table analysis

Fertility life table analysis and jackknife estimation were conducted using the R program (R Development Core Team, 2019) of Maia et al. (De Holanda Nunes Maia et al., 2014). Required data for the analysis were the number, longevity, and daily fecundity of female adults from the parent, and the development period, survivorship, and sex ratio from the offspring. Age-specific survival rate (l_x) and fecundity (m_x) were calculated as follows:

$$l_x = SURV \times \frac{NSF_x}{NF}$$

$$m_x = NEGG_x \times SR$$

Cumulative survival estimation comprises survival of the offspring multiplied by the survival during adult stage which is the number of survived females up to time x (NSF_x) and the initial number of females for each treatment group (NF). It is necessary to calculate the number of eggs laid at each pivotal age ($NEGG_x$) by the sex ratio of offspring (SR) (Maia et al., 2000). To calculate the pivotal age (female adult age plus 0.5), average developmental period of the offspring was used (De Holanda Nunes Maia et al., 2014; Maia et al., 2000). Jackknife estimation and Tukey's studentized range test for population parameters were conducted for all treatment groups for both host plants.

Population parameters were as follows:

The intrinsic rate of increase (*r*)

$$\sum_{x=0}^{\infty} e^{-rx} l_x m_x = 1$$

The finite rate of increase (λ)

$$\lambda = e^{r}$$

The net reproductive rate (R_O)

$$R_0 = \sum_{x=0}^{\infty} l_x m_x$$

The mean generation time (T)

$$T = (\ln R_0 / r)$$

3-4. Results

3-4-1. Proportion of genetic cluster in experimental *B. tabaci* MED groups

In C1 and C1fC2m groups, cluster 1 was dominant. By contrast, cluster 2 was dominant in C2 and C2fC1m groups (Table 2). In single mating, the ratio of the cluster 1 and 2 was over 90 and 70% in C1 and C2, respectively. In cross mating, the cluster 1 and 2 ratio was over 70% in C1fC2m and C2fC1m, respectively. The genetic cluster proportion of each treatment groups showed similar pattern on cucumber and tobacco (Fig 1).

Table 2. The proportion of membership according to Bayesian clustering method for two clusters in each treatment groups of *B. tabaci* (n = 20)

Host plant	Treatment	Inferred clusters	
		Cluster 1	Cluster 2
Cucumber	C1	0.968	0.032
	C2	0.258	0.742
	C1fC2m	0.756	0.244
	C2fC1m	0.157	0.843
Tobacco	C1	0.968	0.032
	C2	0.202	0.798
	C1fC2m	0.749	0.251
	C2fC1m	0.166	0.834

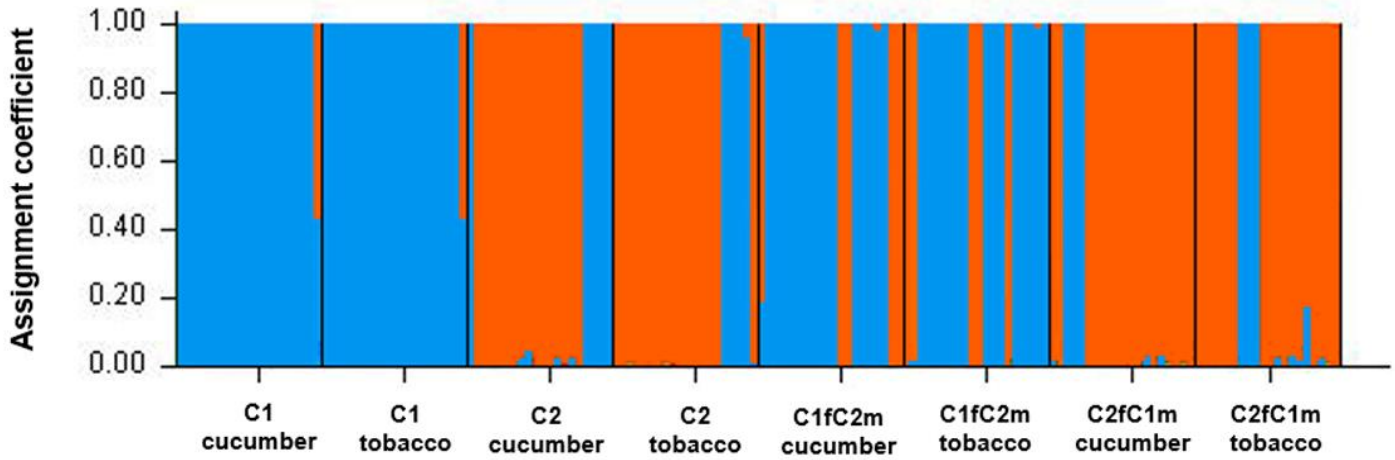


Fig 1. Scatter plot of STRUCTURE results reporting proportional each treatment of *B. tabaci*. Each treatment is represented by a vertical line with different colors representing probabilities assigned to each of the genetic clusters. Cluster 1 and cluster 2 are shown in blue and orange, respectively.

3-4-2. Life history characteristics

Fecundity, longevity, ovipostion period, survival rate, sex ratio, development period, body weight, and body length of *B. tabaci* MED were significantly different among genetic clusters and between host plants. An interaction effect was also found between genetic cluster and host plants for some characteristics such as fecundity, survival rate, and sex ratio of offspring (Appendix 3).

Overall, biological characteristics of *B. tabaci* MED were significantly superior in C2, the lowest in C1, and those of mixed mating groups were in-between. Maternal inheritance was significantly associated with their life history characteristics, with partial involvement of paternal inheritance. Total fecundity was the highest for C2 (292.8 ± 2.31 and 244.9 ± 2.29 eggs on cucumber and tobacco, respectively) (mean ± SE), followed by that for C2fC1m, C1fC2m, and C1 on both host plants (Table 3). Female longevity was significantly longest for C2fC1m followed by that for C2 and C1fC2m. The survival rate of offspring (egg to adult) was rather similar among genetic cluster groups (Table 4). Sex ratio (female %) was distinctively higher in C2. It was the lowest in C1. Those of mixed mating groups were in-between. The developmental period (female + male, female, and male)

on both host plants from short to long was in the following order: C2, C2fC1m, C1fC2m, and C1 (Table 5). Adult body weight and body length were in the following order: C2 > C2fC1m > C1fC2m > C1 (Table 6).

Overall, C2 outperformed other groups regarding life history characteristics on both host plants (Table 7). Intrinsic rate of increase, finite rate of increase, and net reproductive rate (0.247, 1.280, and 192.402, respectively, on cucumber; 0.226, 1.253, and 133.792, respectively, on tobacco) of C2 were distinctively higher than those of C1 (0.149, 1.161, and 50.539, respectively, on cucumber; 0.145, 1.156, and 53.332, respectively, on tobacco). In cross mating, C2fC1m (0.210, 1.234, and 129.912, respectively, on cucumber; 0.196, 1.216, and 96.196, respectively, on tobacco) outperformed C1fC2m (0.172, 1.188, and 64.292, respectively, on cucumber; 0.168, 1.183, and 57.392, respectively, on tobacco). Intrinsic rate of increase value, finite rate of increase, and net reproductive rate were the highest in C2, followed by those in C2fC1m, C1fC2m, and C1 groups for both host plants.

Table 3. Total fecundity, daily fecundity, longevity, oviposition period, and post-oviposition period (mean ± S.E.) of female *B*. *tabaci* (n = 30)

Host plant	Treatment	Total fecundity	Daily fecundity	Longevity	Oviposition period	Post-oviposition period
Cucumber	C1	167.7 ± 3.79dF*	6.9 ± 0.16bC	24.6 ± 0.88cC	23.3 ± 0.75cC	2.3 ± 0.23bC
	C2	292.8 ± 2.31aA	9.6 ± 0.12aA	30.5 ± 0.35bB	29.1 ± 0.33bB	2.4 ± 0.16bC
	C1fC2m	187.3 ± 5.61cE	6.1 ± 0.13cD	30.8 ± 0.92bB	28.7 ± 0.95bB	3.2 ± 0.47bBC
	C2fC1m	271.8 ± 1.9bB	7.1 ± 0.10bC	38.6 ± 0.43aA	34.1 ± 0.47aA	5.6 ± 0.46aA
Tobacco	C1	152.5 ± 1.96dG	6.2 ± 0.12bD	24.8 ± 0.58cC	23.2 ± 0.52cC	2.6 ± 0.18bC
	C2	244.9 ± 2.29aC	7.8 ± 0.08aB	31.5 ± 0.29bB	30.0 ± 0.24bB	2.5 ± 0.18bC
	C1fC2m	174.4 ± 2.53cEF	5.9 ± 0.14bD	29.8 ± 0.70bB	27.9 ± 0.63bB	3.0 ± 0.21aBC
	C2fC1m	201.5 ± 2.01bD	5.3 ± 0.07cE	38.2 ± 0.31aA	35.4 ± 0.30aA	3.9 ± 0.20aB

*Mean followed by the same letter (lower case letter, comparison among genetic clusters within a host plant; capital case letter, comparison among genetic clusters throughout both host plants) within a column are not significantly different at α=0.05, Tukey's studentized range test.

Table 4. Survival rate and sex ratio (mean ± S.E.) in offspring of *B. tabaci*

Host plant	Treatment	Survival rate of offspring (%)	Sex ratio (%)
Cucumber	C1	81.4 ± 1.16aAB[*] (4063/5032)[**]	38.8 ± 0.90dF (1575/4063)[***]
	C2	82.2 ± 0.53aAB (7220/8785)	80.2 ± 0.38aA (5791/7220)
	C1fC2m	83.3 ± 1.01aAB (4653/5637)	42.2 ± 0.61cE (1959/4653)
	C2fC1m	80.2 ± 0.76aB (6529/8154)	59.7 ± 0.47bC (3895/6529)
Tobacco	C1	83.1 ± 1.00abAB (3794/4574)	41.7 ± 1.27cEF (1576/3794)
	C2	81.3 ± 0.66bB (5964/7348)	67.4 ± 0.61aB (4023/5964)
	C1fC2m	75.8 ± 1.10cC (3947/5249)	42.4 ± 0.61cE (1670/3947)
	C2fC1m	85.2 ± 0.84aA (5143/6048)	56.3 ± 0.45bD (2892/5143)

[*]Mean followed by the same letter (lower case letter, comparison among genetic clusters within a host plant; capital case letter, comparison among genetic clusters throughout both host plants) within a column are not significantly different at α=0.05, Tukey's studentized range test following arcsine transformation for proportions.
[**](survived number / initial number)
[***](female number / total adult number)

Table 5. Developmental period (mean ± S.E.) of *B. tabaci*

Host plant	Treatment	Developmental period		
		Female + Male (n)	Female (n)	Male (n)
Cucumber	C1	21.0 ± 0.17aA* (44)	19.7 ± 0.12aB (15)	21.6 ± 0.14aA (29)
	C2	15.1 ± 0.07dD (50)	14.9 ± 0.05dF (40)	15.9 ± 0.10dD (10)
	C1fC2m	17.9 ± 0.16bB (46)	16.8 ± 0.14bC (19)	18.6 ± 0.13bB (27)
	C2fC1m	16.5 ± 0.09cC (47)	16.1 ± 0.07cE (29)	17.2 ± 0.09cC (18)
Tobacco	C1	21.3 ± 0.14aA (42)	20.5 ± 0.14aA (14)	21.7 ± 0.15aA (28)
	C2	15.3 ± 0.07dD (49)	15.0 ± 0.04cF (35)	16.0 ± 0.00dD (14)
	C1fC2m	17.9 ± 0.18bB (45)	16.7 ± 0.11bCD (18)	18.7 ± 0.15bB (27)
	C2fC1m	16.8 ± 0.12cC (47)	16.3 ± 0.09bDE (29)	17.6 ± 0.14cC (18)

*Mean followed by the same letter (lower case letter, comparison among genetic clusters within a host plant; capital case letter, comparison among genetic clusters throughout both host plants) within a column are not significantly different at α=0.05, Tukey's studentized range test.

Table 6. Comparison of body weight and body length (mean ± S.E.) of *B. tabaci*

Host plant	Treatment	Body weight (mg)		Body length (mm)	
		Female	Male	Female	Male
Cucumber	C1	0.255 ± 0.0061cD*	0.217 ± 0.0025bBC	0.666 ± 0.0037dD	0.514 ± 0.0040dD
	C2	0.319 ± 0.0010aA	0.290 ± 0.0017aA	0.802 ± 0.002aA	0.682 ± 0.0034aA
	C1fC2m	0.297 ± 0.0052bBC	0.222 ± 0.0049bB	0.761 ± 0.0045cC	0.594 ± 0.0074cC
	C2fC1m	0.312 ± 0.0022abAB	0.282 ± 0.0032aA	0.786 ± 0.0030bB	0.648 ± 0.0045bB
Tobacco	C1	0.241 ± 0.0054cD	0.208 ± 0.0023bC	0.661 ± 0.0033dD	0.506 ± 0.0038dD
	C2	0.318 ± 0.0012aA	0.287 ± 0.0016aA	0.801 ± 0.0028aA	0.681 ± 0.0032aA
	C1fC2m	0.290 ± 0.0043bC	0.220 ± 0.0039bBC	0.761 ± 0.0038cC	0.594 ± 0.0052cC
	C2fC1m	0.313 ± 0.0029aAB	0.279 ± 0.0025aA	0.782 ± 0.0031bB	0.634 ± 0.0049bB

*Mean followed by the same letter (lower case letter, comparison among genetic clusters within a host plant; capital case letter, comparison among genetic clusters throughout both host plants) within a column are not significantly different at α=0.05, Tukey's studentized range test.

Table 7. Estimates (mean ± S.E.) of life table parameters of *B. tabaci*

Host plant	Treatment	*r*	λ	R_0	*T*
Cucumber	C1	0.149 ± 0.0006dG*	1.161 ± 0.0007dG	50.539 ± 1.3619dE	26.253 ± 0.2220aB
	C2	0.247 ± 0.0007aA	1.280 ± 0.0010aA	192.402 ± 1.3592aA	21.300 ± 0.0605dE
	C1fC2m	0.172 ± 0.0008cE	1.188 ± 0.0010cE	64.292 ± 2.5132cD	24.226 ± 0.2735bC
	C2fC1m	0.210 ± 0.0005bC	1.234 ± 0.0006bC	129.912 ± 0.9356bB	23.136 ± 0.0617cD
Tobacco	C1	0.145 ± 0.0006dG	1.156 ± 0.0007dG	53.332 ± 0.7421cE	27.365 ± 0.1071aA
	C2	0.226 ± 0.0005aB	1.253 ± 0.0006aB	133.792 ± 1.1781aB	21.680 ± 0.0476dE
	C1fC2m	0.168 ± 0.0012cF	1.183 ± 0.0014cF	57.392 ± 0.7397cE	24.145 ± 0.1895bC
	C2fC1m	0.196 ± 0.0007bD	1.216 ± 0.0008bD	96.196 ± 0.9831bC	23.329 ± 0.0681cD

*Mean followed by the same letter (lower case letter, comparison among genetic clusters within a host plant; capital case letter, comparison among genetic clusters throughout both host plants) within a column are not significantly different at α=0.05, Tukey's studentized range test after jackknife estimates.

r, intrinsic rate of increase; λ, finite rate of increase; R_0, net reproductive rate; and *T*, mean generation time

3-5. Discussion

This study compared life history characteristics between two genetically different populations of *B. tabaci* MED based on fertility life table analysis for the first time. Life table parameters of *B. tabaci* have been previously reported regarding different putative species, host plants, or temperatures (Delatte et al., 2009a; Fang et al., 2014; Guo et al., 2012; Liu, 2007; Mansaray & Sundufu, 2009; MUSA & REN, 2005; Tsai & Wang, 1996). Different from these previous studies, our life table study was focused on genetically different populations of *B. tabaci* MED to elucidate if difference in life history characteristics of different genetic clustered populations might be responsible for rapid convergence of one genetic cluster of *B. tabaci* MED in Korea.

Overall, genetic cluster 2 (C2) of *B. tabaci* MED outperformed genetic cluster 1 (C1) for various aspects of life history characteristics through both single mating and cross mating (C2 and C2fC1m vs. C1 and C1fC2m) experiments on both host plants, cucumber and tobacco. These results confirmed that the competitive ability of cluster 2 population was significantly higher than that of cluster 1 regardless of host plant species, indicating that the rapid convergence of genetic clusters of *B. tabaci* MED in Korea populations might be highly related

to their different life history characteristics.

Fecundity was the highest in C2, followed by that in C2fC1m, C1fC2m, and C1. The same trend was observed for sex ratio, body weight, and body length. The development period was the shortest in C2, followed by that in C2fC1m, C1fC2m, and C1. Since these biological characteristics were apparently associated with life history characteristics, life table parameters also showed the same pattern. Biological and life history characteristics of *B. tabaci* MED appeared to be mainly associated with maternal inheritance. To some extent, paternal inheritance was also associated with these characteristics. This trend was supported by proportions of genetic clusters in four single and cross mating genetic cluster groups determined by individual-based STRUCTURE analysis (Fig 1 and Table 2). Such genetic inheritance characteristics could accelerate the prevalence of cluster 2 populations. In this study, we did not examine the potential difference in insecticide resistance of two genetic clusters of *B. tabaci* MED. Insecticide resistance might also play a role in the prevalence of genetic cluster 2. Further study is needed to clarify this.

In conclusion, this study provided a strong evidence that genetic cluster 2 of *B. tabaci* MED had significantly superior life history characteristics than cluster 1. Thus, the rapid convergence of genetic

clusters in *B. tabaci* MED populations is strongly related to their different life history characteristics. Further study is needed to determine potential difference in insecticide resistance between these two genetic clusters of *B. tabaci* MED.

Appendix 3. Results of two-way ANOVA for testing effects of cluster and host plant on biological characteristics, body weight, and body length of *B. tabaci*

Parameter	Source	df	MS	*F*	*p*
Total fecundity	Cluster	3	150130.049	538.85	< 0.0001
	Host	1	803337.004	288.35	< 0.0001
	Cluster × Host	3	11381.782	40.85	< 0.0001
	Error	232	278.610		
Daily fecundity	Cluster	3	94.110	227.15	< 0.0001
	Host	1	78.296	188.98	< 0.0001
	Cluster × Host	3	9.848	23.77	< 0.0001
	Error	232	0.414		
Female longevity	Cluster	3	1887.989	171.18	< 0.0001
	Host	1	0.067	0.01	0.9381
	Cluster × Host	3	10.922	0.99	0.3981
	Error	232	11.029		
Oviposition period	Cluster	3	1327.304	135.45	< 0.0001
	Host	1	5.704	0.58	0.4463
	Cluster × Host	3	13.126	1.34	0.2623
	Error	232	9.800		
Post-oviposition period	Cluster	3	70.315	28.40	< 0.0001
	Host	1	7.004	2.83	0.0940
	Cluster × Host	3	12.515	5.05	0.0021
	Error	232	2.476		
Survival rate of immature stage	Cluster	3	64.121	4.56	0.0040
	Host	1	4.637	0.33	0.5663
	Cluster × Host	3	234.644	16.70	< 0.0001
	Error	232	14.053		
Sex ratio	Cluster	3	5234.002	1028.43	< 0.0001
	Host	1	282.528	55.51	< 0.0001
	Cluster × Host	3	287.260	56.44	< 0.0001
	Error	232	5.089		

Appendix 3. *Continued*.

Developmental period (Female + Male)	Cluster	3	577.018	744.40	< 0.0001
	Host	1	4.210	5.43	0.0203
	Cluster × Host	3	0.440	0.57	0.6368
	Error	362	0.775		
Developmental period (Female)	Cluster	3	188.633	1080.82	< 0.0001
	Host	1	2.311	13.24	0.0004
	Cluster × Host	3	1.242	7.11	0.0001
	Error	191	0.175		
Developmental period (Male)	Cluster	3	236.876	549.22	< 0.0001
	Host	1	1.311	3.04	0.0832
	Cluster × Host	3	0.280	0.65	0.5852
	Error	163	0.431		
Body weight (Female)	Cluster	3	0.020	128.13	< 0.0001
	Host	1	0.001	3.16	0.0795
	Cluster × Host	3	0.000	1.46	0.2326
	Error	72	0.000		
Body weight (Male)	Cluster	3	0.031	343.75	< 0.0001
	Host	1	0.000	4.18	0.0444
	Cluster × Host	3	0.000	0.65	0.5862
	Error	72	0.000		
Body length (Female)	Cluster	3	0.759	654.17	< 0.0001
	Host	1	0.001	0.87	0.3504
	Cluster × Host	3	0.000	0.18	0.9118
	Error	792	0.001		
Body length (Male)	Cluster	3	1.088	485.70	< 0.0001
	Host	1	0.006	2.65	0.1038
	Cluster × Host	3	0.002	0.88	0.4525
	Error	792	0.002		

Chapter Ⅳ.

Comparison of the insecticide resistance trait as a potential driving force for genetic cluster change in *Bemisia tabaci* MED (Hemiptera: Aleyrodidae)

4-1. Abstract

Previously, we reported that most of *Bemisia tabaci* Mediterranean (MED) populations converged from two dominant genetic clusters (cluster 1 and 2) to one (cluster 2) during one year in greenhouse tomatoes in Korea. To find clues for this phenomenon, we investigated the resistance traits of the two clusters for three insecticide classes (organophosphate, pyrethroid, and neonicotinoid).

Since the resistance mutation frequencies in regional samples were either high (i.e., the voltage-sensitive sodium channel L925I/T929V mutations and the F392 acetylcholinesterase 1 mutation) or zero (the nicotinic acetylcholine receptor R81T mutation), no meaningful correlation was deduced between resistance allele frequency and genetic cluster. However, actual resistance levels to all three insecticide classes were significantly higher in cluster 2 than cluster 1, suggesting that cluster 2 has a higher resistance potential. Furthermore, thiamethoxam treatment to the mixed population of cluster 1 and 2 over three generations exhibited a strong tendency of population displacement from cluster 1 to cluster 2.

Our results demonstrated that insecticide resistance trait is one of the driving forces for rapid genetic cluster change in *B*. *tabaci* MED

populations.

Key words: *Bemisia tabaci*, whitefly, Mediterranean, insecticide resistance, Korea

4-2. Introduction

The sweet potato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is an economically important global pest that attacks a broad range of agricultural crops through direct phloem sap sucking, honeydew excretion, and transmission of many plant viruses (Byrne & Bellows Jr, 1991; De Barro et al., 2003; Oliveira et al., 2001), thereby deteriorating plant quality. *B. tabaci* is comprised of more than 36 putative species complexes and morphologically indistinguishable lineages (Boykin et al., 2012; De Barro et al., 2011). The MEAM1 (Middle East-Asia Minor 1, biotype B) and MED (Mediterranean, biotype Q) are the top two complexes predominant worldwide (Zheng et al., 2017). These putative species complexes have developed resistance to multiple insecticide classes (Kontsedalov et al., 2012; Pan et al., 2011; Perring, 2001).

Chemical insecticide use is the primary strategy to control *B. tabaci* in many cropping systems (Byrne et al., 2010; Palumbo et al., 2001; Wang et al., 2010b). Various insecticide classes, such as organophosphates, pyrethroids, and neonicotinoids have been widely used to control *B. tabaci* in fields and greenhouses (Chung et al., 2011; Naveen et al., 2017; Wang et al., 2010b). Organophosphates and

pyrethroids insecticides act on the insect nervous system by altering the normal gating kinetics of the *para*-type voltage-gated sodium channel and by inhibiting the enzyme acetylcholinesterase, respectively (Alon et al., 2008; Gauthier et al., 2014; Tsagkarakou et al., 2009). Neonicotinoids are selective agonists of the insect nicotinic acetylcholine receptor (nAChR), a pentameric cys-loop ligand-gated ion channel located in the central nervous system (Tomizawa & Casida, 2003). As organophosphates and pyrethroids alternatives, systemic neonicotinoid insecticides have been used as a primary options for whiteflies control in fields and greenhouses, resulting in rapid development of neonicotinoid resistance (Chen et al., 2018).

In Korea, the *B. tabaci* MED was first discovered on the tomato (*Lycopersicum esculentum* M.) in 2004, and it has now spread to most areas (Lee et al., 2014; Lee et al., 2012). High levels of pyrethroids (e.g., bifenthrin) and neonicotinoids (e.g., thiamethoxam and imidacloprid) resistance have been reported for *B. tabaci* in Korea (Lee et al., 2012). Our previous study demonstrated that *B. tabaci* MED was classified into two genetic clusters (clusters 1 and 2) in Korea based on eight microsatellite markers. Cluster 1 was the dominant in 2016 but was rapidly displaced with cluster 2 in 2017 all over the country (Park et al., 2019). This sudden genetic displacement is likely due to

various factors including the differences in thermotolerance (Mahadav et al., 2009), host plant preference (Malka et al., 2018), bacterial symbionts (Chiel et al., 2007), pathogen-vector interaction (Liu et al., 2013), life history characteristics (Delatte et al., 2009b), and insecticide resistance (Horowitz et al., 2005; Kontsedalov et al., 2012) between genetically different groups. Comparing the life history characteristics revealed that cluster 2 was significantly superior to cluster 1 in total fecundity, female sex ratio, body weight, body length, developmental period, and intrinsic rate of increase.

In this study, we focused on the insecticide resistance traits in the *B. tabaci* MED populations in Korea as a potential driving force for this displacement of population. To test the hypothesis, the correlation between the insecticide resistance traits and the genetic cluster change in *B. tabaci* MED was investigated. The frequencies of resistance mutations on insecticide target genes were examined for regional samples with known cluster types. Further, the actual resistance levels to acephate, bifenthrin, and thiamethoxam were measured by leaf-dip bioassays in two groups, each representing cluster 1 and 2. To confirm the insecticide resistance trait as a driving force for the cluster change, mixed groups of cluster 1 and 2 were maintained over three generations in the presence or absence of

thiamethoxam exposure, and their cluster ratios in each generation were examined.

4-3. Materials and Methods

4-3-1. *Bemisia tabaci* strains

In our previous study, *B. tabaci* MED adults were collected from 35 commercial tomato greenhouses in Korea from 2016 to 2018 (17 population in 2016, 13 populations in 2017, and five populations in 2018). This study included two different *B. tabaci* clusters MED (Pyeongtaek, cluster 1; Sejong, cluster 2) collected from tomato (*Lycopersicon esculentum* M.) greenhouses in 2018. All collected samples were directly put into 99.8% ethanol and stored at – 20 °C until use. Two strains representing Pyeongtaek (PT, cluster 1) and Sejong (SJ, cluster 2) each were maintained on cucumber plant (*Cucumis sativus* L.) in the rearing cages (40 × 40 × 40 cm^3) under the 26 ± 1 °C, 50 ± 10% relative humidity (RH) with a photoperiod of 16:8 (L:D) h.

4-3-2. Detection of single nucleotide polymorphisms (SNPs) related with insecticides resistance

Mutations, well known to be associated with target site insensitivity resistance, were examined. The frequencies of F392W mutation in acetylcholinesterase 1 gene (*ace1*) and the L925I/T929V

in voltage-sensitive sodium channel gene (*vssc*), conferring organophosphate and pyrethroid resistance in *B. tabaci*, respectively, were investigated (Alon et al., 2008; Morin et al., 2002; Roditakis et al., 2006). However, since no target site insensitivity mutation in the nicotinic acetylcholine receptor (nAChR) gene has been reported in *B. tabaci*, any mutation at R79 corresponding to the location of the R81T mutation in the nAChR β1 subunit gene (*nAChRβ1*), previously reported to be associated with neonicotinoid resistance in *Myzus persicae*, was checked for neonicotinoid resistance (Bass et al., 2011). In addition, since deleting an exon containing the R81T mutation site is known to result in resistance in *M. persicae* (Wang et al., 2017), any mutation in the AG/GT of exon boundaries was also checked.

The gDNA of each sample was extracted from 30 *B. tabaci* MED to calculate the abundance of resistant alleles through quantitative sequencing (Amos et al., 2000). For the amplification of all three genes, two primer sets were designed, and if the first PCR result was faint, another primer set was used for nested PCR. The PCR products were purified and sequenced. The resistance ratio was calculated from the sequencing results, as done in quantitative sequencing. Primer sequences and PCR conditions for each gene are summarized in Appendix 5.

4-3-3. Genetic cluster determination

To confirm the genetic structure, eight microsatellite markers were amplified using the individual gDNA of *B. tabaci* MED as templates under the PCR conditions (Dalmon et al., 2008). PCR products were analyzed using an ABI 3730xl (Applied Biosystems Inc., Foster, CA, USA) at the NICEM (Seoul, Korea). Then, 1 μl PCR product was diluted with 8.5 μl of Hi-Di formamide (Applied Biosystems Inc.) and 0.5 μl of Genescan ROX-500 size standard (Applied Biosystems Inc.). All the genetic data were analyzed using GENEMAPPER v.3.7 (Biosystems, 2004), GenAlEx v.6.5 (Peakall & Smouse, 2012), STRUCTURE v.2.3.2 (Pritchard et al., 2000b), and STRUCTURE HARVESTER Web v.0.6.93 (Earl, 2012).

4-3-4. Insecticides

Technical-grade acephate, bifenthrin, and thiamethoxam (Sigma-Aldrich, St Louis, MO, USA) were purchased. These insecticides were selected as representative of organophosphates, pyrethroids, and neonicotinoids, respectively, as they were commonly used to control *B. tabaci* MED in the tomato greenhouses from which

the *B. tabaci* MED populations were collected.

4-3-5. Bioassays

For insecticide resistance bioassays, insecticides stocks in acetone were 10-fold diluted with deionized water containing 0.1% Triton X-100 (Merck, Darmstadt, German), making final concentrations as follows: acephate 5000, 1000, 100, 20, and 4 mg kg^{-1}; bifenthrin 50, 10, 2, and 0.4 mg kg^{-1}; and thiamethoxam 500, 100, 20, 4, and 0.8 mg kg^{-1}, respectively.

Bioassay was performed based on a leaf-dip bioassay method following IRAC (Insecticide Resistance Action Committee) and Naveen et al. (Naveen et al., 2012). The cucumber leaves trimmed into 3 cm diameter discs, dipped in the sonicated test compound solutions for 20 s, and then air-dried in a fume hood at room temperature for 30 min. Leaves dipped only in diluents served as the untreated controls. In total, 15 to 20 female adults were used for each replicate, anesthetized with CO_2, and placed on a leaf disc located in a plastic tube (3 cm in diameter, 5 cm in height) with mesh-covered holes on top for ventilation and moistened pad beneath the leaf disc. Bioassay were conducted at 26 ± 1 °C, 50 ± 10% RH, and 16:8 (L:D)

h, consisting of three replicates per each treatment including controls. Final mortality was scored after 48 h. *B. tabaci* showing no sign of movement were scored as dead under a stereomicroscope (× 200).

4-3-6. Toxicity test with synergist

PBO (piperonyl butoxide, Sigma-Aldrich, St Louis, MO, USA) was treated with thiamethoxam to prove that cytochrome P450 (CYP450) is a factor for the resistance level difference between clusters. The final solutions for the bioassay included 10 mM PBO, 10% acetone as a solvent, 0.1% triton X-100 as an emulsifier, and 500, 100, 20, 4, and 0.8 mg kg^{-1} thiamethoxam. The toxicity test was performed using the leaf-dip bioassay described in section 2.5. Mortality was checked after 48 h.

4-3-7. Chronic thiamethoxam treatment to a mixed population of clusters 1 and 2

To prove insecticides resistance to be a major factor for the cluster displacement, the effects of insecticide treatment to cluster composition of *B. tabaci* were tested. Thiamethoxam was chosen as a representative insecticide, considering that it showed the highest

resistance difference between clusters 1 and 2 and has been most extensively used for whitefly control in Korea (Lee et al., 2002; Seo et al., 2007). Two hundreds of 1:1-mixed female adults (100 females from each cluster) were separately introduced into two different rearing cages (40 × 40 × 40 cm^3) and maintained under the condition of 26 ± 1 °C, 50 ± 10% RH, and a photoperiod of 14:10 (L:D) h. One cage was maintained without thiamethoxam exposure and the other cage was constantly exposed to thiamethoxam by providing thiamethoxam-treated cucumber plants. The cucumber plants (28-day old) were treated by dipping intact leaves into the test compound (60 mg kg^{-1}) for 20 s, and then allowing the plant to air-dry in a fume hood under laboratory conditions for 30 min. Each *B. tabaci* culture was monitored for three generations. The host plants were replaced every week.

The clusters of 20 female individuals from each generation were screened using eight previously described microsatellite markers. A burn-in of 60,000 Markov Chain Monte Carlo (MCMC) steps and a burn-in period of 600,000 were used. The log-likelihood estimates were calculated for K = 1 to 10 with ten replicates of each. The Structure Harvester analysis was used to detect the likelihood of the number of occurring clusters among individuals of *B. tabaci*.

4-3-8. Data analysis

A standard probit analysis was conducted to determine the LC_{50} values of each treatment, using POLO program PC PoloPlus (Leora Software, Berkeley, CA, USA). The LC_{50} values of a specific insecticide against *B. tabaci* MED were calculated to be significantly different ($p < 0.05$) if their 95% fiducial limits (FL) did not overlap.

4-4. Results

4-4-1. Detection of resistance mutations

Resistance mutation frequencies in regional and seasonal samples are presented in Table 1 and Appendix 6. All samples showed 100% F392W mutation in *ace1*, indicating a saturated state of OP resistance. The L925I and T929V mutation in *vssc* do not coexist in a single haplotype, thus, the actual level of pyrethroid resistance can be represented by the sum of the two mutation frequencies. The combined frequencies of L9251 and T929V mutations ranged from 0.68 (CC in 2016) to 1 (JE, SJ, SC in 2016 and etc.). The average frequency was 0.96, suggesting that pyrethroid resistance prevails in most regional and seasonal populations. The composition of L925I and T929V mutations was different in each sample. The R81T mutation in *nAChRβ1* was not found in any of the regional samples. In summary, since the resistance mutation frequencies in regional samples were either too high (i.e., the F392 *ace1* muation and *vssc* L925I/T929V muations) or zero (the *nAChRβ1* R81T mutation) and there was no apparent difference in the frequencies between clusters 1 and 2, no meaningful correlation was deduced between the resistance allele frequency and genetic cluster.

Table 1. Point mutation alleles ratio of *B. tabaci* MED in Korea from 2016 to 2018

No.	Population	2016			2017			2018		
		OPs/CXs	Neonics	Pyrs	OPs/CXs	Neonics	Pyrs	OPs/CXs	Neonics	Pyrs
1	CC	1.00	n.d	0.68	1.00	n.d	0.93	-	-	-
2	PT	1.00	n.d	0.95	1.00	n.d	0.98	1.00	n.d	0.94
3	SJ	1.00	n.d	1.04	1.00	n.d	1.03	1.00	n.d	0.94
4	CY	1.00	n.d	1.00	1.00	n.d	0.99	-	-	-
5	BY	1.00	n.d	0.94	-	-	-	-	-	-
6	IS	1.00	n.d	0.91	1.00	n.d	0.98	-	-	-
7	JE	1.00	n.d	1.03	1.00	n.d	0.98			
8	GJ	1.00	n.d	1.00	1.00	n.d	0.98	-	-	-
9	BS	1.00	n.d	0.97	1.00	n.d	0.98	1.00	n.d	0.92
10	SC	1.00	n.d	1.03	1.00	n.d	0.98	1.00	n.d	0.95
11	JIN	1.00	n.d	1.00	1.00	n.d	0.99	-	-	-
12	CW	-	-	-	-	-	-	-	-	-
13	GH	1.00	n.d	0.93	-	-	-	-	-	-
14	MY	-	-	-	1.00	n.d	1.00	-	-	-
15	AD	1.00	n.d	0.91	-	-	-	-	-	-
16	BUS	1.00	n.d	1.00	1.00	n.d	0.95	-	-	-
17	JJ	1.00	n.d	-	1.00	n.d	0.99	1.00	n.d	0.95

OPs/CXs, organophosphates/carbamates; Neonics, neonicotinoids; Pyrs, pyrethroid

4-4-2. Insecticide resistance status

The LC_{50} values of the cluster 2 population were significantly higher than those of the cluster 1 population for all three insecticides (Table 2 and Appendix 4). Thiamethoxam showed the largest difference (23.6-fold), followed by acephate (14.3-fold) and bifenthrin (12.3-fold).

4-4-3. Synergistic effects of PBO with insecticides

LC_{50} value of cluster 2 significantly decreased following PBO treatment (from 462 to 20.5 mg kg^{-1}), while that of cluster 1 remained constant (from 19.6 to 19.6 mg kg^{-1}) (Table 2 and Appendix 4). The 23.6-fold resistance difference between cluster 1 and 2 changed to 1.05 following PBO treatment mainly due to the reduction of resistance in cluster 2. The sharp decrease (22.5 fold) in cluster 2 following PBO treatment to the level of cluster 1 suggests that thiamethoxam resistance in cluster 2 is primarily due to the enhanced CYP450 activity.

Table 2. Probit mortality data for the two different populations of *B. tabaci* MED, tested with three classes of insecticides using leaf-dip bioassays

Insecticide	Genetic cluster	N	LC_{50}	RR_{50}	CL 95%	Slope (± SE)	Synergism ratio
Acephate	cluster 1	370	184	14.3	81.3 - 440	0.71 ± 0.06	
	cluster 2	366	2628		1,071 - 10,613	0.37 ± 0.06	
Bifenthrin	cluster 1	344	16.4	12.3	9.37 - 36.0	0.56 ± 0.09	
	cluster 2	371	201		78.4 - 1180	0.72 ± 0.11	
Thiamethoxam	cluster 1	203	19.6	23.6	12.6 - 29.5	0.80 ± 0.08	
	cluster 2	206	462		71.6 - 207.8	0.80 ± 0.08	
Thiamethoxam with PBO	cluster 1	197	19.6	1.05	12.6 - 29.6	0.48 ± 0.07	1.0
	cluster 2	193	20.5		13.2 - 31.0	0.80 ± 0.09	22.5

N, total number of *B. tabaci* individuals used in bioassays; LC, lethal concentration; RR, resistance ratio; CL, confidence limits; SE, standard error

4-4-4. Changes in genetic cluster in a mixed population over three generations following constant thiamethoxam exposure

Genetic cluster analysis based on microsatellite markers over three generations revealed two dominant clusters. The proportions of each treatment are shown in Fig 1 and Table 3. The probability of assignment of each individuals of *B. tabaci* is indicated from 0 to 1. The proportions of cluster 1 and 2 in the parent populations (F_0 generation) were 0.991 and 0.872, respectively. While the composition of control group maintained an almost 1:1 ratio consistently over three generations (0.515 - 0.567 - 0.591 of cluster 1 ratio), the proportion of cluster 1 gradually decreased over time in the thiamethoxam-treated group (0.466 - 0.353 - 0.227).

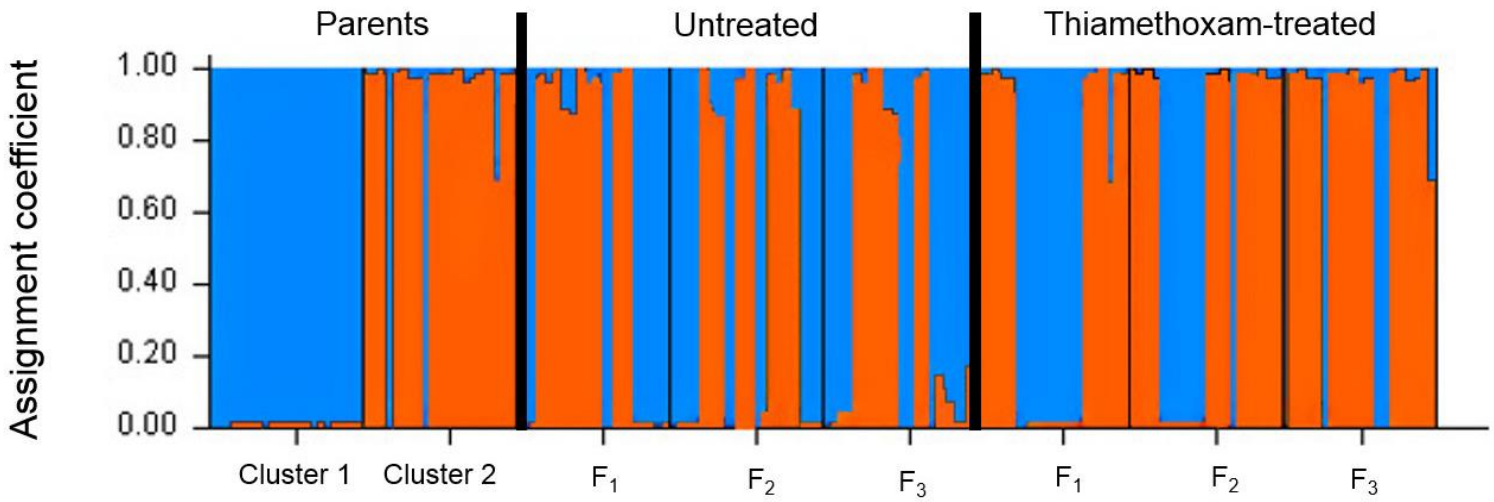


Fig 1. The scatter plot of STRUCTURE analysis results reporting the proportional each treatment of *B. tabaci* MED. Each treatment is represented by a vertical line with different colors representing the probabilities assigned to each genetic cluster. Clusters 1 and cluster 2 are shown in blue and orange, respectively.

Table 3. The proportion of membership of each pre-defined treatment of *B. tabaci* MED in each cluster (n = 20)

No.	Treatment		Inferred clusters		
			Cluster 1	Cluster 2	Pie chart
1	Parents	Cluster 1	0.991	0.009	
2		Cluster 2	0.128	0.872	
3	Untreated	F_1	0.515	0.485	
4		F_2	0.567	0.433	
5		F_3	0.591	0.409	
6	Thiamethoxam-treated	F_1	0.466	0.534	
7		F_2	0.353	0.647	
8		F_3	0.227	0.773	

4-5. Discussion

In this study, we investigated the possible involvement of the insecticide resistance traits in the sudden genetic displacement of cluster 1 of *B. tabaci* MED from tomato greenhouses in Korea (Park et al., 2019). First, the possibility of cluster classification based on the frequencies of resistance-related mutations in insecticide target genes was checked. Although the link between clusters and resistance mutation frequencies was unclear, the prevalence of resistance mutations in Korea was confirmed. The completely saturated F392W mutation in *ace1* in all 33 regional samples collected for three years (Table 1) demonstrated that OP and carbamate resistance are widespread in Korea. A similar surveillance result has also been reported in China (Yuan et al., 2012). Such a high frequency of *ace1* mutation, despite the significant reduction in the use of OPs and carbamates, indicates that the *ace1* mutation little causes fitness cost. The pyrethroid resistance mutation allele was also almost saturated across the country, with an average frequency of 0.96 (Table 1). No orthologous mutation or exon deletion, which is identified in any of the samples examined (Table 1). No orthologous mutation or exon deletion, which is responsible for neonicotinoid resistance in *M.*

persicae (Bass et al., 2011) and *Aphis gossypii* (Koo et al., 2014), was identified in any of the samples examine (Table 1). Considering that many reports, including transcriptome comparison analysis between susceptible and resistant strains, failed to find resistance-related mutations in nAChRs of *B. tabaci*, target gene insensitivity may not be a main factor for neonicotinoid resistance in *B. tabaci* (Ilias et al., 2015). In this study, the absolute levels of neonicotinoid resistance in the regional samples could not be determined owing to the lack of a laboratory-susceptible strain. However, considering the long use history of neonicotinoid insecticides for *B. tabaci* control in Korea, resistance development mediated by mechanisms other than the *nAChRβ1* R81T mutation is still possible in the field population.

To determine and compare the actual resistance level between cluster 1 and 2, a leaf-dip bioassay was conducted for three insecticides with two representative populations, PT and SJ. Although it was not distinguishable from resistance mutation data, the bioassay results showed that cluster 2 had higher resistance levels than cluster 1 (Table 2). The difference was the highest with thiamethoxam, reflecting the high selection pressure by thiamethoxam in Korea. However, the significant reduction in resistance following PBO treatment in cluster 2 strongly indicated that the high thiamethoxam

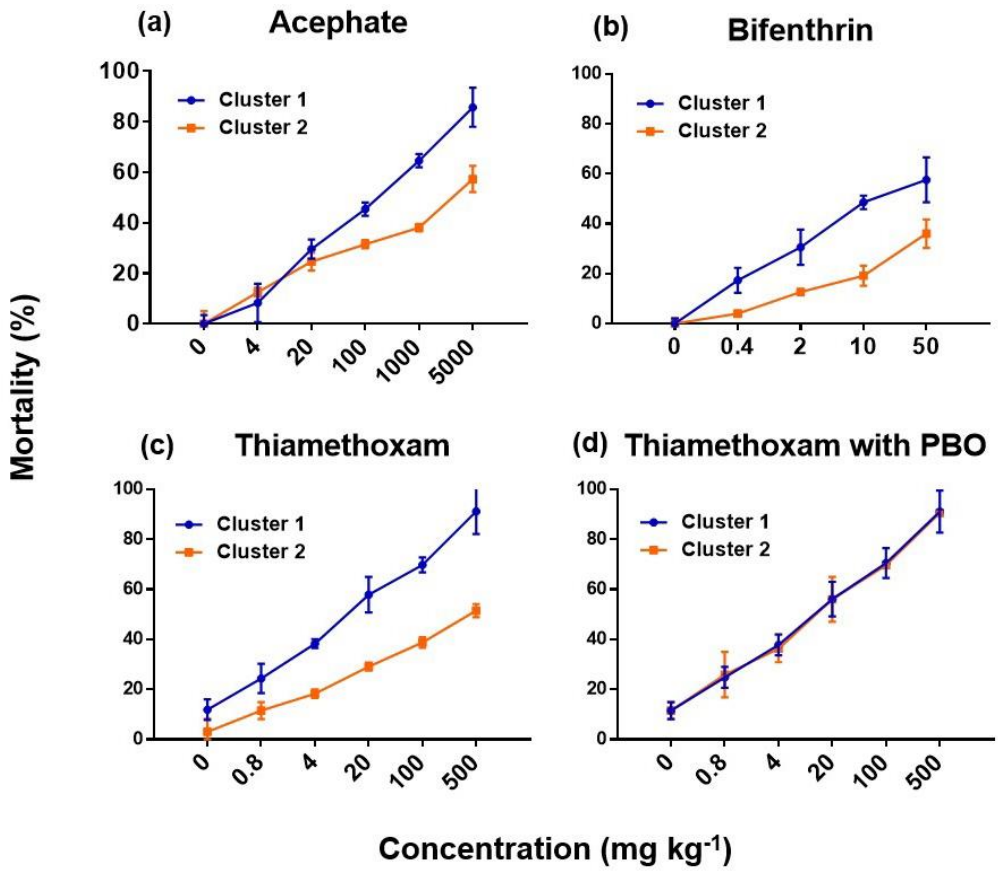
resistance level in cluster 2 resulted from the elevated CYP450 activity. Thus, it can be speculated that cluster 2 is generally more resistant to thiamethoxam than cluster 1 owing to the metabolic factor. Although there was no apparent difference in the frequencies of the *ace1* F392W and *vssc* L925I/T929V mutations between clusters 1 and 2, cluster 2 was more resistant to acephate and bifenthrin than cluster 1, suggesting that the differences in acephate and bifenthrin resistance between the clusters are likely derived from metabolic factors, including CYP450.

To determine whether the higher resistance level of cluster 2 acts as a driving force for the displacement, a 1:1 mixed population was maintained in the presence or absence of thiamethoxam, and the changes in the population genetic structure were monitored. As the fecundity of cluster 2 is higher than that of cluster 1, it was expected that the cluster 1 ratio of the thiamethoxam-untreated control group would decrease or remain constant and that the decrease would be accelerated in thiamethxoam-treated group because of the difference in resistance. As expected, thiamethoxam treatment resulted in a gradual increase in the cluster 2 ratio, demonstrating that insecticide resistance is one of the factors for rapid cluster change in *B. tabaci* MED in Korea (Table 3). Interestingly, the cluster 2 ratio of control

group remained constant or slightly decreased rather than increasing (0.485 to 0.409) despite its fecundity. This suggests that cluster 1 can be competitive over cluster 2 in a coexisting population without insecticide selection pressure. The displacement of cluster 1 by cluster 2 seems similar to the case of MEAM1 and MED. MED shows higher neonicotinoids resistance level than MEAM1, but its fecundity is reduced when they coexist (Pascual & Callejas, 2004). Thus, MEAM1 is dominant in open fields, whereas MED is dominant in protected conditions (Kontsedalov et al., 2012). Sudden displacements in just a few years have been reported in MEAM1-preoccupying area through MED introduction (Kontsedalov et al., 2012; Lee et al., 2005; Tang et al., 2020; Wang et al., 2010c), and this has been expected to result from insecticides resistance (Horowitz et al., 2005). Since MEAM1 has been displaced by MED globally, the change from cluster 1 to cluster 2 might already be prevalent worldwide.

This study revealed that genetic cluster 2 of *B. tabaci* MED, as judged by a representative population, has significantly higher resistance to acephate, bifenthrin, and thiamethoxam than cluster 1 and this thiamethoxam resistance difference comes from the elevated CYP activity. A mixed population test proved that cluster 2 can displace cluster 1 under thiamethoxam exposure owing to its high

resistance. In conclusion, this study provides an evidence that the rapid convergence of genetic clusters in the *B. tabaci* MED populations correlates with their resistance to thiamethoxam. However, larger scale experiments with other insecticides would be required to confirm the involvement of the insecticide resistance trait as a generalized factor in the sudden change in the genetic cluster of the *B. tabaci* MED populations.



Appendix 4. Dose-response curves for acephate, bifenthrin, and thiamethoxam against female *B. tabaci* MED adults using leaf-dip bioassays (after 48 h). (a) acephate, (b) bifenthrin, (c) thiamethoxam, and (d) thiamethoxam with PBO.

Appendix 5. Detailed of primer information and PCR conditions for each gene

Target gene	Gene symbol	Primer name	Sequence	Tm (℃)	Product size	PCR condition
Acetylcholinesterase 1	LOC109029639	Btace1_F1	GTGCTCGTGGAGAACGAGAG	62.5	316	95 ºC 3 min 95 ºC 30 sec, 55 ºC 30 sec, 52.4 ºC 30 sec 34 cycles 72 ºC 5 min
		Btace1_R1	GGTTCAGCCAGTCCGTGTAC	62.5		
		Btace1_F2	TGGCGACGAAGAACTTCAAG	58.4	171	95 ºC 3 min 95 ºC 30 sec, 55 ºC 30 sec, 52.4 ºC 30 sec 34 cycles 72 ºC 5 min
		Btace1_R2	TGAAGTTGTACGGGTTGAGC	58.4		
para sodium channel	LOC109039786	Btpsc_F1	CCCCAGTTCCGATGTATGTC	60.5	386	95 ºC 3 min 95 ºC 30 sec, 52.3 ºC 30 sec, 52.4 ºC 30 sec 34 cycles 72 ºC 5 min
		Btpsc_R1	AAGTCCTGTAGCTAGGGGAC	60.5		
		Btpsc_F2	TTAGCGAAATCCTGGCCAAC	58.4	165	95 ºC 3 min 95 ºC 30 sec, 52.3 ºC 30 sec, 52.4 ºC 30 sec 34 cycles 72 ºC 5 min
		Btpsc_R2	GGGACTGAACATCATACCTG	60.5		
Nicotinic acetylcholine receptor *β*1 subunit	LOC109043254	BtnAChR_F1	ATTGCCAAGCTACTTGCAAACC	60.1	509	95 ºC 3 min 95 ºC 30 sec, 52.3 ºC 30 sec, 72 ºC 30 sec 34 cycles 72 ºC 5 min
		BtnAChR_R1	ATGGCTGCTGCTACTAAGGG	60.5		
		BtnAChR_F2	TCTACTTTTGTGCACCACTC	56.4	178	95 ºC 3 min 95 ºC 30 sec, 52.3 ºC 30 sec, 72 ºC 30 sec 34 cycles 72 ºC 5 min
		BtnAChR_R2	ACGTCATACAAACGTCAACG	56.4		

Appendix 6. Detailed point mutation alleles ratio information of *B. tabaci* MED in Korea from 2016 to 2018.

No.	Population	2016			2017			2018		
		L925I	T929V	Total	L925I	T929V	Total	L925I	T929V	Total
1	CC	0.18	0.50	0.68	0.23	0.70	0.93	-	-	-
2	PT	0.36	0.58	0.95	0.43	0.54	0.98	0.46	0.48	0.94
3	SJ	0.24	0.80	1.04	0.44	0.59	1.03	0.38	0.57	0.94
4	CY	0.49	0.51	1.00	0.36	0.63	0.99	-	-	-
5	BY	0.35	0.59	0.94	-	-	-	-	-	-
6	IS	0.40	0.51	0.91	0.25	0.73	0.98	-	-	-
7	JE	0.38	0.64	1.03	0.44	0.54	0.98	-	-	-
8	GJ	0.00	1.00	1.00	0.24	0.74	0.98	-	-	-
9	BS	0.38	0.59	0.97	0.29	0.69	0.98	0.20	0.72	0.92
10	SC	0.41	0.62	1.03	0.29	0.69	0.98	0.37	0.58	0.95
11	JIN	0.00	1.00	1.00	0.24	0.75	0.99	-	-	-
12	CW	-	-	-	-	-	-	-	-	-
13	GH	0.38	0.55	0.93	-	-	-	-	-	-
14	MY	-	-	-	0.00	1.00	1.00	-	-	-
15	AD	0.34	0.58	0.91	-	-	-	-	-	-
16	BUS	0.26	0.74	1.00	0.50	0.45	0.95	-	-	-
17	JJ	-	-		0.30	0.69	0.99	0.38	0.58	0.95

Chapter Ⅴ.

General conclusion

This study provides the first comprehensive genetic structure of *B. tabaci* MED from the long-term and large-scale analysis throughout Korea only commercial tomato greenhouses based on microsatellite markers. The results of genetic structure and diversity analysis show high genetic diversity *B. tabaci* MED in Korea based on genetic diversity analyses and classified into two differentiated genetic clusters. Interestingly, we found that its genetic cluster converged into one genetic cluster during a short period in many populations. Between 2016 and 2017, genetic cluster changes were observed in six of the populations (CC, PT, SJ, BS, SC, and MY population). This similar phenomenon of *B. tabaci* has been reported previously in Australia and China. However, there have been no follow-up studies that delve into causes. There was no exact experimental basis for just assumptions. Therefore, to find this evidence for the rapid genetic cluster change, we conducted several followed-up studies.

The results of chapter Ⅲ and Ⅳ were confirmed strong evidence that the life history characteristics and insecticide resistance trait are one of the evidence for rapid genetic cluster change in the *B. tabaci* MED populations. In conclusion, the rapid convergence of genetic clusters in *B. tabaci* MED populations is strongly related to

their different life history characteristics and insecticide resistances. If this phenomenon continues, one out of the two *B. tabaci* MED genetic clusters in Korea might become the dominant species in the future in most tomato greenhouses.

In *B. tabaci* populations, genetic diversity and genetic structure can be affected by various factors. There are several possibilities except for life history characteristics and insecticide resistances. This phenomenon may be associated with the multiple route introductions of other *B. tabaci* populations from out of greenhouses as a consequence of natural dispersal or human activities as like as commercial trading and material shipments. Representative possibilities include diverse nursery routes, different crops are cultivated for each season, difference cropping systems of practices by greenhouses, crops are left in the greenhouses after the season ends, and the types of crops in the surrounding greenhouses. Also, *B. tabaci* which were inhabiting nearby various weeds may enter the greenhouses in winter. Because in most cases, the *B. tabaci* moves back to the crops in greenhouses for overwintering when becoming low temperature in fields. A greater comprehension of the factors influencing *B. tabaci* MED population dynamics may improve predictions of. population fluctuations and identify potential sources of

individual dispersal in the greenhouses. However, tracking these dynamics are very difficult to track and verify in reality.

Therefore, other possibilities are plant virus transmission rates and endosymbionts that can be verified through experiments. Because, begomoviruses genus can be transmitted by *B. tabaci* (Czosnek & Laterrot, 1997) and endosymbionts can affect the biology and physiology of their host about survival and reproduction of *B. tabaci* (Kikuchi et al., 2012).

However, when TYLCV that a representative virus mediated by *B. tabaci* in tomatoes was tested, there was no association with the changed genetic clusters. Also, the endosymbionts were tested but the association with the changed genetic cluster phenomenon was not found. Besides, we had investigated those other environments (tomato varieties by greenhouses, the temperature and humidity change in tomato greenhouses, etc.), but this was also not related to the changed genetic cluster phenomenon.

This study help for better understanding the population genetic structure of *B. tabaci* MED in Korea and turning of genetic cluster patterns. Understanding the accurate cause and consequence of the rapid change in genetic cluster information is likely to more influence controlling the *B. tabaci* MED in commercial tomato greenhouses. As

well as this study may help improve understanding of the biology, ecology, and genetics of *B. tabaci* on tomato greenhouses in Korea.

In particular, if further research is conducted on these various possibilities for much more greenhouses around the PT population where genetic cluster 1 is still maintained, it is thought that more exact conclusions can be obtained. Because the PT population was genetic cluster 1 in 2016, but cluster 2 in 2017 and cluster 1 again in 2018. Furthermore, if it observes more populations across the country and over a longer period using other diverse molecular marker methods, it will be a more accurate and useful analysis.

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List of Appendix

Chapter Ⅱ.

Chapter Ⅲ.

Chapter Ⅳ.

Abstract in Korean

국내 담배가루이 (노린재목: 가루이과)의 유전적 구조와 생활사 특성 및 살충제 저항성 비교

서울대학교 대학원

농생명공학부 곤충학전공

박 유 정

담배가루이 (노린재목: 가루이과)는 1889년 그리스에서 담배 해충으로 최초 보고된 이후, 국제 무역으로 인해 지중해 지역을 시작으로 점차 확산되어 현재까지 전 세계적으로 약 1,000종 이상의 작물에 심각한 경제적 피해를 일으키는 주요 해충이다. 본 연구는

(1) 8개의 초위성체 마커를 이용하여 국내 상용 토마토 온실에서 담배가루이 개체군의 유전적 구조와 다양성을 확인하고, (2) 서로 다른 유전적 구조를 가지는 대표적인 개체군의 생활사의 특성 차이를 비교하고, 이들 개체군의 개체군 내, 개체군 간의 단일 및 교차 교배 실험을 통해 이들의 생활사 특성과 유전적 구조와의 상관관계를 확인하고, (3) 채집 지역의 대표적인 살충제 종류의 저항성 발달 수준 확인 및 서로 다른 유전적 구조를 가지는 대표적인 개체군의 생물 검정을 통한 저항성 발달 차이와 유전적 구조와의 상관관계를 확인하기 위해 수행되었다.

국내 지역 별 담배가루이의 유전적 구조 및 다양성을 확인하기 위해 2016년부터 2018년까지 국내 총 35개 지역의 상용 토마토 온실(2016년 17개 지역, 2017년 13개 지역, 2018년 5개 지역)에서 1,145 마리의 담배가루이 암컷 성충을 채집하였고, 8개의 초위성체 마커를 선정하여 실험을 진행하였다. 그 결과 35개 지역

평균 기대 이형접합도는 0.218에서 0.600, 평균 관측 이형접합도는 0.061에서 0.580, 평균 근친 교배 계수는 -0.391에서 0.872의 값으로 확인되었다. 유전적 거리와 지리적 거리와의 상관관계(IBD) 분석에서는 유의미한 상관관계가 확인되었고, 집단 간의 변이가 높은 것으로 확인되었다. 전체 유전적 클러스터 분석 결과, 국내 35개 지역 담배가루이의 유전적 구조는 총 두 가지(구조1, 구조2)로 확인되었으며, 3년 동안 전체적으로 이 두 가지의 유전적 클러스터가 한가지의 유전적 구조(구조2)로 점점 변해가는 양상이 확인되었다.

따라서 이러한 현상의 원인을 확인하기 위해 두 가지의 유전적 구조를 가지는 대표적인 개체군을 실내 사육 후 개체군 내, 개체군 간의 단일 및 교차 교배 실험을 통해 생활사 특성 차이를 비교하기 위한 실험을 진행하였다. 그 결과 구조 2의 개체들이 구조 1에 비해 총 산란 수, 암컷의 비율, 몸무게 및 몸 길이, 발육 기간, 내적 증가율 값의 부분에서 매우 우세한 것을 확인할 수 있었다.

실험 결과를 통해 국내 담배가루이의 유전적 구조의 변화의 원인 중 하나가 서로 다른 두 가지의 유전적 구조의 생활사의 특성 때문인 것을 확인할 수 있었다.

담배가루이는 특히 다양한 종류의 살충제에 대한 저항성이 매우 빠르게 발달하는 종으로 보고가 되어오고 있는데, 이러한 살충제 저항성의 발달 정도와 유전적 구조의 변화와 밀접한 연관이 있을 것으로 가정되어 실제 토마토 시설의 담배가루이 방제에 많이 사용되고 있는 대표적인 살충제 세 종류 (유기인계 : 아세페이트, 피레스로이드계 : 비펜트린, 네오니코티노이드계 : 치아메톡삼)를 선정하여 3개년도 각 지역 별 저항성 정도를 확인하였다. 그 결과 각 지역 별 저항성 대립 유전자와 유전적 구조와의 상관관계는 없었지만, 생물검정 결과 구조 2의 개체들이 구조 1의 개체들에 비해 세 종류의 살충제에 대한 저항성이 상당히 높은 것으로 확인되었으며, 특히 치아메톡삼에 대한 저항성의 차이는 사이토크롬

활성에서 비롯된 것으로 확인되었다. 따라서 살충제에 대한 저항성 또한 국내 담배가루이의 유전적 구조의 변화의 원인 중 하나로 확인되었다.

검색어: 담배가루이, 집단유전학, 초위성체 마커, 생활사 특성, 살충제 저항성

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감사의 글

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