EUROPEAN JOURNAL OF ENTOMOLOGY
ISSN (online): 1802-8829
http://www.eje.cz
Eur. J. Entomol. **116**: 221–228, 2019
doi: 10.14411/eje.2019.024
ORIGINAL ARTICLE

Host-based genetic divergence in populations of an exotic spiralling whitefly, *Aleurodicus dispersus* (Hemiptera: Aleyrodidae)

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Key words. Hemiptera, Aleyrodidae, *Aleurodicus dispersus*, genetic variability, host plants, India, microsatellite markers, polymorphism

Abstract. The morphology, physiology, behaviour and ecology of spiralling whitefly, *Aleurodicus dispersus* Russell (Hemiptera: Aleyrodidae) on different host plants differ greatly. The genetic differences between the *A. dispersus* populations on 17 host plants were evaluated in the current study. Microsatellite markers were used to identify the presence of host-related genetic variation among *A. dispersus* populations. Our research clearly shows that there is a significant amount of genetic divergence among the *A. dispersus* populations on 17 host plants in India. The spiralling whitefly on acalypha and calotropis were genetically more distinct than whiteflies on other host plants. Various population genetic parameters, like heterozygosity, Nei's genetic distance, fixation indices (F_{ST}), source of genetic variation in AMOVA, etc. indicate that populations of spiralling whiteflies differ greatly genetically, probably because the spiralling whitefly populations on the Indian sub-continent came from multiple sources. The results of this study have implications for the quarantine protection strategy against this invasive pest.

INTRODUCTION

The spiralling whitefly, *Aleurodicus dispersus* Russell (Hemiptera: Aleyrodidae: Aleurodicinae) was first reported in 1993 at Thiruvananthapuram (Kerala, India) on cassava (Palaniswami et al., 1995) and has spread across Southern and North-eastern India since 1993 and has subsequently become a major pest of agricultural and horticultural crops (Srinivasa, 2000; Boopathi et al., 2013, 2015a, b). This species was possibly introduced into India from neighbouring countries like Sri Lanka (Ranjith et al., 1996), Maldives (Muniappan, 1996) and Myanmar (Boopathi, 2008; Boopathi et al., 2014). The spiralling whitefly has had a dramatic negative effect on the agricultural industry and resulted in significant monetary losses (Alam et al., 1998). Woets & van Lenteren (1976) attribute the recorded differences in the fecundity, lifespan and development rate of whitefly populations to the host plants. Geetha (2000) and Boopathi (2013) report that the fecundity, egg hatchability, incubation period, total developmental period, pupal period, percentage adult emergence, sex ratio (female : male) and adult longevity varies on the different host species. Mayer et al. (2010) report that the type and level of damage varies depending on the species and condition of the plant.

Dense populations of this polyphagous pest causes premature leaf drop and the honeydew they produce serves as a substratum for the growth of sooty mould, leading to the crops being abandoned (Akinlosotu et al., 1993; Boopathi et al., 2014). A four month long infestation of guava by *A. dispersus* can result in 80% fruit yield loss (Wen et al., 1995). Banana production is also reduced by an *A. dispersus* infestation (Ranjith et al., 1996). *A. dispersus* is also one of the yield limiting factors in cassava cultivation

causing up to 53% loss of yield (Geetha, 2000). This pest has a serious negative effect on the agricultural industry, resulting in significant monetary losses (Alam et al., 1998; Boopathi et al., 2016, 2017a). *Aleurodicus dispersus* is a recent economic pest in both southern India and Eastern Himalayan regions of India (Boopathi et al., 2016, 2017a), for example, it is a pest of cassava, on which up to 580 insects per leaf are reported (Palaniswami et al., 1995). In India, Boopathi (2013) records 56 host plants as new hosts for *A. dispersus*. The range in the susceptibility of crops has been catalogued for India (Andaman Nicobar, Andhra Pradesh, Himachal Pradesh, Karnataka, Kerala, Manipur, Meghalaya, Mizoram, and Tamil Nadu) by Ranjith et al. (1996) and Boopathi (2013). Banana, castor, chili, citrus, cocoa, coconut, cashew nut, eggplant, guava, jack fruit, okra, papaya, pea pigeon, pepper, sapota and tomato are breeding and feeding hosts for *A. dispersus* (Srinivasa, 2000; Boopathi, 2013; Boopathi et al., 2014).

Analysis of microsatellite and allozyme markers of *Acyrthosiphon pisum* (Harris) from alfalfa, pea and clover indicates the existence of genetically different individuals on these plants (Simon et al., 2003). While much of the information on population diversity is for different locations and regions, there is little molecular information on the genetic diversity associated with different host plants. In addition, there is no clear evidence as to whether host specialization preceded or followed the introduction of this species into India. Moreover, the level of genetic divergence among host plant specific *A. dispersus* populations is uncertain because in earlier studies the populations were analysed using markers with different resolutions. Therefore, in this study, we analysed the level of genetic differentiation between *A. dispersus* populations on 17 host plants. Microsatellite or simple sequence repeat (SSR) markers are one of the most effective tools for population genetic studies (Jarne & Lagoda, 1996). This study addresses the hypothesis that the elucidation of the host specificity of different whitefly populations infesting many different plants in the same environment would provide an insight into mechanisms involved in the development of biotypes/new races. Thus, the SSR-PCR technique was used in this study to identify the existence of host-related genetic variability among populations of *A. dispersus* from 17 different host plants in India.

MATERIAL AND METHODS

Spiralling whitefly collections and sampling method

The collection of spiralling whitefly adults was made in areas where agriculture is important and biologically diverse. If different host plants occurred at the same location, samples were randomly collected from plants at least 500 m apart. Adult spiralling whiteflies were collected from 17 species of plants (10 plants/species) (Table 1). The "leaf turning" technique, aspirator and camel hair brush were used to collect adults from the lower and upper parts of each host plant (Boopathi et al., 2015a). The adults collected from the 17 host plants were preserved in 90% ethanol until required for the extraction of DNA. Adult females were identified based on the taxonomic description of Russell (1965). Adult females do not have pores, whereas males have numerous circular pores on their abdomens (Russell, 1965). Twenty-five adult females were collected from each host plant at each site.

Isolation of DNA

The DNA was isolated using the CTAB (cetyl trimethylammonium bromide) method adopted by Gawel & Jarrett (1991). Before extraction, one adult female was placed in a micro-centrifuge tube (1.5 mL) containing 50 µL of preheated DNA extraction buffer and a pestle used to pulverize the sample. The buffer for the DNA extraction contained 2% CTAB, 100 mM Tris (pH 8), 20 mM EDTA and 1.4 M NaCl. A one-tenth volume of RNase (10 mg mL^{-1}) and 1 µL proteinase K (25 mg mL^{-1}) were added to each micro-centrifuge tube containing a sample and incubated at 65°C for 45 min. Equal volume of chloroform: isoamyl alcohol mixture (24 : 1) was added and reversed for 15 min by inversion. The tubes were centrifuged at 12,000 rpm for 15 min at 4°C (Eppendorf Centrifuges Model No.: 5430R). The clear aqueous phase was transferred to a new sterile micro-centrifuge tube (1.5 mL). One-third of the volume of ice cold iso-propanol was added and gently mixed by inverting the tubes and the DNA was precipitated overnight by incubating the tubes at –20°C. It was then centrifuged at 12,000 rpm for 20 min at 4°C in order to produce a pellet of DNA. The pellet of DNA was washed with 70% ethanol and then air dried. The DNA was dissolved in 20 µL of 1 × TE buffer (pH 8.0) depending on the pellet size.

Table 1. *Aleurodicus dispersus* DNA populations for assessing genetic divergence from 17 host plants in India.

Population code	Host plants	Locations	Latitude	Longitude	Altitude (m)
EGG	Eggplant (*Solanum melongena* L.)	Coimbatore, Tamil Nadu	11°0′47.39″N	76°56′14.19″E	434
PAP	Papaya (*Carica papaya* L.)	Trissur, Kerala	10°31′34.94″N	76°12′53.79″E	26
CAS	Cassava (*Manihot esculenta* Crantz)	Coimbatore, Tamil Nadu	11°11′29.21″N	77°16′7.98″E	313
BAN	Banana (*Musa paradisiaca* L.)	Pudukottai, Tamil Nadu	10°39′33.30″N	79°3′6.46″E	112
GUA	Guava (*Psidium guajava* L.)	Kolasib, Mizoram	24°12′39.57″N	92°40′35.18″E	635
COC	Coconut (*Cocos nucifera* L.)	Coimbatore, Tamil Nadu	11°0′47.39″N	76°56′14.19″E	434
COT	Cotton (*Gossypium hirsutum* L.)	Coimbatore, Tamil Nadu	11°0′47.39″N	76°56′14.19″E	434
CHI	Chilli (*Capsicum annuum* L.)	Namakkal, Tamil Nadu	11°31′14.08″N	78° 5′14.13″E	270
MUL	Mulberry (*Morus alba* L.)	Coimbatore, Tamil Nadu	11°0′47.39″N	76°56′14.19″E	434
TOM	Tomato (*Solanum lycopersicum* L.)	Coimbatore, Tamil Nadu	11°0′47.39″N	76°56′14.19″E	434
ALM	Almond (*Terminalia catappa* L.)	Bengaluru, Karanataka	13°1′36.90″N	77°35′5.07″E	928
TEA	Teak (*Tectona grandis* L.f.)	Dindugal, Tamil Nadu	10°46′38.06″N	77°54′40.91″E	156
ACA	Acalypha (*Acalypha indica* L.)	Coimbatore, Tamil Nadu	11°0′47.39″N	76°56′14.19″E	434
SAI	Saimarupa (*Saimarupa gluaca* DC.)	Coimbatore, Tamil Nadu	11°0′47.39″N	76°56′14.19″E	434
ROS	Rose (*Rosa* spp.)	Umiam, Meghalaya	25°41′24.46″N	91°55′20.47″E	965
CAL	Calotropis (*Calotropis gigantea* (L.) W.T. Aiton)	Coimbatore, Tamil Nadu	11°0′47.39″N	76°56′14.19″E	434
AVO	Avocado (*Persea americana* Mill.)	Mettupalayam, Tamil Nadu	11°20′9.82″N	76°51′41.18″E	488

SSR-PCR (polymerase chain reaction) analysis

DNA samples from spiralling whitefly populations from 17 host plants were amplified using a set of 45 SSR primers comprising 10 SSR primers of spiralling whitefly (Ma et al., 2011) and 35 SSR primers of sweet potato whitefly, *Bemisia tabaci* (Gennadius) (De Barro et al., 2003; Tsagkarakou et al., 2007; Gauthier et al., 2008), as detailed in Table 2. Preliminary studies were carried out using 35 *B. tabaci* SSR primers to determine the genetic variation among the 17 *A. dispersus* populations. Polymorphic SSR primers from the *B. tabaci* genome were used to enhance the genome coverage for better results in the analysis of diversity. In the SSR amplifications, 14 of the 45 primers screened produced clear bands and were used in the analysis.

The polymerase chain reactions were carried out using a thermocycler (BIORAD DNA engine). The reactions were carried out in a volume of 25.0 containing buffer µL (10 mM Tris-HCl pH 9, 50 mM KCl), 200 µM dNTPs, 1.5 mM MgCl2, 10 pmol of forward and reverse primers, 1 unit *Taq* DNA polymerase and 4 ng of genomic DNA. The PCR cycle program included an initial 5.0 min of pre-denaturation at 95°C, 40 amplification cycles (1.0 min of denaturation at 95°C; 1.0 min of annealing ranging from 50.0 to 59.8°C for the various SSR primers listed in Table 2; 1.0 min of extension at 72°C) and 5.0 min of post-extension at 72°C.

Gel electrophoresis

The electrophoresis of amplified products was carried out using 3% agarose gel, at 80 V. Gels were stained and documented using a Bio-Rad gel documentation system (Gel Doc XR System, model: 1708170EDU, Bio-Rad Laboratories, Hercules, CA, USA). In polyacrylamide gel electrophoresis (PAGE), amplification of products of Primers with lower fragment resolution were

Table 2. SSR markers used for characterizing the *Aleurodicus dispersus* populations from 17 host plants.

Primer name	Sequences (5´–3´)		Annealing temperatures (°C)
	Forward	Reverse	
1. *Bemisia tabaci* primers (35)			
Btls1-2	CTT ACC TTC CAT TCA CC	ATC CCG AGT CTT ATG TTG	54.0
Btls1-4	GAA ATC CTC ACA CTG GC	CAC TGA TGT GAC CTG G	56.0
Btls1-6	GAA TCA TTG ACT AGC AAC AG	GTC CAA CTA CCA TAC TCG T	57.0
Btls1-9#	GTG TTT GAG GAG GTG GG	CTA TTT ATC TAT TTG GGT CA	55.0
Btls1-12	AAT CCT CAC ACT GGC TG	CCT GAT GTC AGA CAT GG	57.0
Btls1-17	GTG ACC GAG TAA GGC CA	CAC GTC CGC TCT CTA TG	59.0
Btls2-4	CAT CTG GCA TTC TTT CTC	ACT CAT ACC TCC TCC ATC T	56.0
Btls2-3	CAG AAC GAC AGG TCG AG	CAA AAT TAA TGG TAT TGA CTC	55.0
AY145452	TTA CAC TTA ACA CCA GAA CT	GAT GGC TTA TGT TAT AAT ACT A	50.0
AY145453	TTC AAT GAT GCT TTC CTG AC	CAA ATA AAT ACA CCA TTT ACA	50.0
AY145454	CTA TCG TTG ACT GAT TTT TG	AAA TTA CCT ACA CCT GCC T	50.0
AY145455	AGA AGT CAG ACA ATA TGT G	ATT AAC CCT CTC TCA TCG C	50.0
AY145456#	AGC AGC ATC AAC AGG CTC	CTA GAT TCT GCT TGA AAG G	50.0
AY145457	TCA GGA TTC GGC CGG CTA	CAT TCA TTT GCG CTG TAG ATT	55.0
AY145458#	GTC TTT GGG AGA GCC AGA AT	AAC AAG ACG GTG GCA GCG A	56.0
AY145459	ATC AAG CAG TCG CTA CAC CT	ATG CAC TAG TTG TTA GTG CG	55.0
AY145460	GGC CCT AGT TAG AAG GTA GTA	CTA ATC GGT CAA GAG ATT TGG	55.0
AY145461	CGG AGC TTG CGC CTT AGT C	CGG CTT TAT CAT AGC TCT CGT	55.0
AY145462	AAG TAT CAA CAA ATT AAT CGT G	TGA AGA ATA AGA ATA AAG AAG G	50.0
AY145463	AAG AAC TAG CCA GGG ACA AAC	GTC ATT TCT GGA TTC TCA GCA	50.0
AY145464#	TGA CGA CCT GAG GCT GAG AG	TGC AAC GGC AAC AGC AAG CAA	59.8
AY145465	ACT CTA GGC TGT ATG CAT CCA	CGC ATG ACA AAT CGT ATC AAG	55.0
AY145466	TGG AGA ATG TTA TAA AGT GGA	GAA AGT GGA GAG TTT AGG TGA	50.0
AY183673#	GAG ATC ATA TCC CCA TTG TTT C	ATC ACG GGT CAT AGA TCA CG	50.2
AY183674	GAT GCC ACA GGT TGT CTG G	GCT TGC CAG GCA CTT TCT AG	60.0
AY183675#	AAA TTA ACT GCC GCT CAA CG	ATA TCG ATA CAA TCT TAC CCG	50.2
AY183676	GTT GCA TAC AGC AGA AAT TTG A	AAA GCG TTC ACG TCT TAC AAT G	60.0
AY183677	ACA TGA ATT TTG GTT AGC AAC A	ATT TGA CTA TTT CAC GTT TCC G	58.0
AY183678#	ATT CGG TTC GTC TTA GGG AC	ACG ATG TTT CCA AAC TGA GC	50.2
AY183679#	GCT CAA CCG AAT ACA TCC AC	AAG TCT AAA GGA AGC GTG GAA	50.2
AY183680	ACA TGT TTC CTT GTC GCA CT	ATT AGT GCT CGG TTC GAT TC	60.0
AY183681	ACT CCA TTT GGC TTA TGT GC	ATT ATC GTC TGA AAA CTG GTG G	58.0
AY183682#	ACG ACA CAA ATT GGC ATT ACA T	ACA AGT CAA CAT CCT CTA GGT A	50.2
2. *Aleurodicus dispersus* primers (10)			
AD2	CTC CAT GCT GTT CTT GAT	CAG GCA CCT ATA AAC CG	53.0
AD3#	CGA CGA TTT ATA CGA ACG CA	ACA CGA ATT GAA GTT GAG GG	53.0
AD5	CGT CTA TTC TTA CAG CCA CA	ACC TGC CAG TAG TTT TGA	53.0
AD12#	TCA CCA GAC CCC ACC CAC CGA C	CAC AAA TGC TCC CAA TAC C	55.3
AD13#	CGA CAA CAG GAA ACA ACG GT	AAA CTG GCA AAG GCG GAC	53.0
AD15#	CAT TGA GTG GGT CCA TTG TT	CGG GAA ATG ATG TCA GGA GG	53.0
AD20	TGC GGG CTC CAA CTA TGT	TGT GGT CGG CAG GAT TTA	53.0
AD21	CGT TGA ATC CCT CTA CTC T	GCT GCC ATC TGT GAA ATA	53.0
AD23	GTA ATG ACC GTG CTA AGT	CTT TGA GAT TTT GCG AGC	53.0
AD26#	TTA AAT TGC TCG CAT GGC	TAA AAT AGG CTT CAG ACC C	50.2

#Produced clear bands in the SSR amplifications and were used for the analysis of genetic divergence.

resolved. The electrophoresis of amplified products was carried out using 6% polyacrylamide gels at 70 W for 2 h. Gels were stained with silver nitrate (0.1% w/v silver nitrate) to visualize the fragments and photographed using a Nikon camera (model No. D5200).

Statistical analysis

Using a 100 bp ladder, molecular weights of bands were calculated and band homology determined based on the distance a band migrated in the gel. On the agarose and polyacrylamide gels, the SSR amplicons obtained for each population appeared as a single band and this data was used in the statistical analysis.

The polymorphism information content (PIC) value for each SSR locus was calculated as described by Botstein et al. (1980) and modified by Anderson et al. (1993). The PIC formula is given below:

$$\mathrm{PIC} = 1 - \sum_{j=1}^{n} \mathrm{P}_{ij}^2$$

where, P_{ij} is the frequency of the *j*th allele for the *i*th marker, summed over n alleles.

PopGene Version 1.32 software (Yeh et al., 2000) was used to estimate the various genetic diversity parameters. Graphical Genotyping 2 (GGT2) (van Berloo, 2008) was used to prepare the dendrogram based on Nei's genetic distance. To determine the optimal number of genetic clusters for spiralling whitefly, population structure was also determined using the Bayesian model-based approach of Pritchard et al. (2000) using STRUCTURE 2.3.4. This program partitions individuals into a number of clusters (K) based on the multi-locus genotypic data. As prior information on population differentiation was not available, the optimum population size (K) was estimated using the same method as used by Evanno et al. (2005). For obtaining the best K, the results from STRUCTURE with 15 replicates of each K (2-10) were used as the input data for the STRUCTURE HARVESTER server (Earl & VonHoldt, 2012). A burn-in period length of 10,000 with 100,000 Markov Chain Monte Carlo (MCMC) replications after burn-in was used. The best value of K obtained was 3. Finally, the STRUCTURE analysis was carried out using K = 3. Admixture model and correlated allele frequency models were used to identify availability of any subtle population structure. Finally, the data was subjected to an analysis of molecular variation (AMOVA) using Arlequin version 3.1 software (Excoffier et al., 2005). The genotypic data for the spiralling whiteflies collected form particular host plants were assigned to three different populations based on the STRUTURE analysis. The AMOVA based population structure is based on an analysis of the variance of gene frequencies, taking into account the number of mutational differences between molecular haplotypes. Fixation indices (Weir & Cockerham, 1984) and pairwise estimates of the correlation of alleles between populations (pairwise F_{ST} comparison) were also computed using Arlequin software.

Table 3. Summary of genetic variation and heterozygosity statistics for 14 loci.

Locus	PIC	NA_O	NA_E	SII	H_O	H_E	Nei H_E
Btls1-9	0.36	2	1.56	0.55	0.00	0.37	0.36
AY145456	0.63	3	2.70	1.04	0.00	0.65	0.63
AY145458	0.50	2	1.99	0.69	0.00	0.51	0.50
AY145464	0.66	3	2.93	1.09	0.06	0.68	0.66
AY183673	0.72	4	3.23	1.27	0.06	0.71	0.69
AY183675	0.21	2	1.26	0.36	0.00	0.21	0.21
AY183678	0.64	3	2.75	1.06	0.12	0.66	0.64
AY183679	0.62	3	2.65	1.03	0.00	0.64	0.62
AY183682	0.42	2	1.71	0.61	0.00	0.43	0.42
AD3	0.49	3	1.97	0.81	0.00	0.51	0.49
AD12	0.50	2	1.99	0.69	0.00	0.51	0.50
AD13	0.48	2	1.94	0.68	0.00	0.50	0.48
AD15	0.39	2	1.64	0.58	0.06	0.40	0.39
AD26	0.46	2	1.84	0.65	0.00	0.47	0.46
Mean		2.5	2.16	0.79	0.02	0.52	0.50
Standard Deviation		0.65	0.59	0.26	0.04	0.14	0.14

PIC – polymorphic information content, NA_O – observed number of alleles, NA_E – effective number of alleles, SII – Shannon's information index, H_O – observed heterozygosity, H_E – expected heterozygosity, Nei H_E – Nei (1973) expected heterozygosity.

RESULTS

All amplification products were consistent and reproducible in the SSR analysis. This revealed genetic variation among the populations of spiralling whiteflies collected from 17 host plants. In the SSR amplifications, fourteen of the 45 primers produced clear bands and were used in the analysis. For the 17 populations of spiralling whiteflies from 17 host plants, a total of 35 alleles were detected (Table 3). The number of alleles per locus varied from 2 to 4, with 2.5 alleles per locus on average. The PIC value ranged from 0.21 (AY183675) to 0.72 (AY183673), with a mean value of 0.51. The observed heterozygosity (H_O), the expected heterozygosity (H_E), and the Nei (1973) expected heterozygosity (Nei H_E) ranged from 0.00 to 0.12 (mean = 0.02), 0.21 to 0.71 (mean = 0.52) and 0.21 to 0.69 (mean = 0.50), respectively. It is noteworthy that the observed heterozygosity is much lower than the expected heterozygosity.

The simple matching, Neighbour Joining dendogram of *A. dispersus* populations from 17 host plants based on Nei's genetic distances revealed three major clusters with a few out group individuals (Fig. 1). The genetic distance coefficient based on 14 SSR markers ranged from 0.21 to 0.93. Spiralling whitefly populations from different hosts viz., eggplant, chilli, cotton and tomato grouped together, and those from papaya, cassava, banana, guava and coconut formed another group. Whiteflies from mulberry, calotropis and acalypha formed out groups close to one or another of the major clusters. The third major cluster consists of almond, teak, avocado, rose and saimarupa.

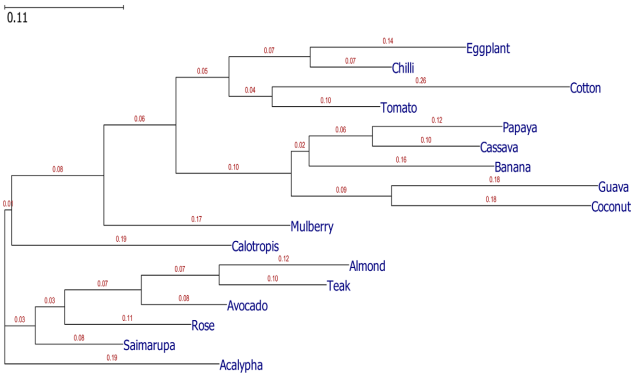

Fig. 1. Simple matching (SM), Neighbour Joining dendogram for populations of *Aleurodicus dispersus* collected from 17 host plants based on Nei's (1973) genetic distances generated using Graphical GenoType ver. 2 (GGT2).

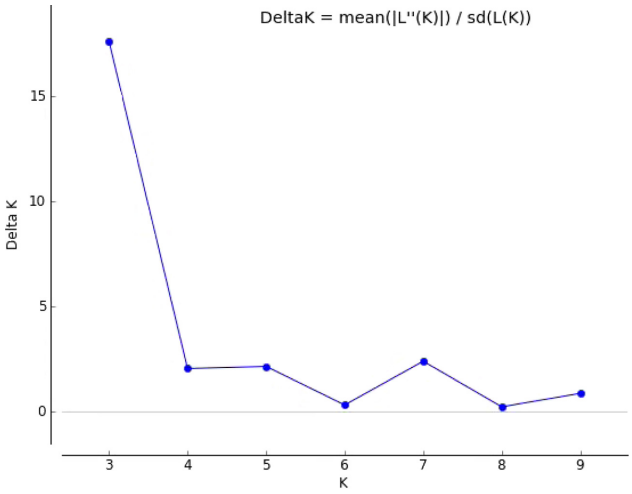

Fig. 2. Best K was determined using the Evanno et al. (2005) method. K value is plotted against ΔK. K value with the highest ΔK was selected as the best K, which is 3.

The optimal population size (K) estimated using Evanno et al.'s (2005) method and STRUCTURE ver. 2.3.4 was three (Fig. 2). STRUCTURE software was used to carry out a Bayesian model-based cluster analysis using background information for 35 alleles of 14 SSR loci from 25 individuals. It revealed very low to substantial amounts of genomic admixture present in almost all the individuals. Individuals from mulberry and calotropis had a very high level of admixture (Fig. 3). Comparison of the two clustering methods used, i.e. Neighbour-joining vs. Bayesian clustering, indicates that the results are similar. However, Neighbour-joining dendrogram shows the genetic relationship among individuals in terms of Nei' genetic distance with respect to their origin/ancestry, while STRUCTURE clustering is based on the genome admixture/level of common alleles among individuals.

Analysis of Molecular Variance (AMOVA) revealed partitioning of genetic diversity more among individuals (58. 32%) within populations (17 populations from 17 host plants) followed by among populations (38.32%) and least within individuals (3.45%) (Table 4). Pairwise F_{ST}, which estimates the correlation of alleles among hypothetical populations of spiralling whiteflies, indicated that population 1 is genetically closer to population 2 than the other population (Table 5).

Table 4. Analysis of Molecular Variance (AMOVA) and results based on the three K clusters identified by the STRUCTURE analysis.

Source of variation	*df*	Sum of squares	Variance components	% variation
Among populations	2	45.51	1.629 Va	38.32
Among individuals within populations	14	71.66	2.486 Vb	58.32
Within individuals	17	2.50	0.147 Vc	3.45
Total	33	119.68	4.262	
Fixation indices	F_{IS}: 0.944, F_{ST}: 0.382, F_{IT}: 0.965			

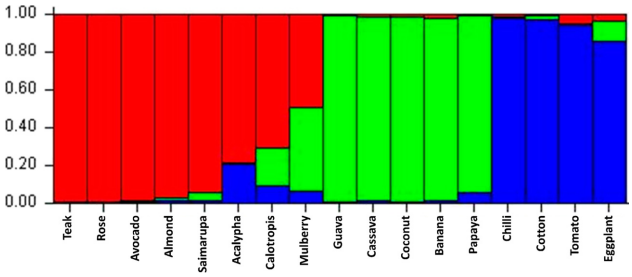

Fig. 3. Population structure of the 17 populations of spiralling whiteflies each collected from a different host plant for K = 3 in an individual line plot using STRUCTURE ver. 2.3.4 software. The three different colours represent the three sub-populations of spiralling whitefly identified, with each bar representing the estimated membership Q (0–1) for a single host in each of the three clusters.

DISCUSSION

Identification, validation and application of molecular markers are the first step towards a better understanding of insect ecology at the molecular level. The primer sequences for around 10 SSR markers are publicly available for studying the genome of the spiralling whitefly (Ma et al., 2011). SSR markers are used in the analysis of genetic divergence and geographical distribution of *A. dispersus* (Boopathi et al., 2014, 2017b). Earlier, the SSR markers were utilized for studying the genetic diversity of *B. tabaci* and differentiating the variants of *B. tabaci* and determining the genetic relationships of closely related populations (Gawel & Jarrett, 1991; De Barro et al., 2003; De Barro, 2005; Delatte et al., 2006; Tsagkarakou et al., 2007; Gauthier et al., 2008; Diaz et al., 2015). However, more advanced molecular tools have recently been used for understanding population dynamics of whitefly. For example, Elfekih et al. (2018b) report the genetic mixing between populations of the same invasive species within the *B. tabaci* complex from various geographical localities worldwide using dense array genome-wide SNPs. Elfekih et al. (2018a) have developed a useful molecular diagnostic toolkit for detecting invasive *B. tabaci* via sequence characterization of mtDNA haplotypes. The microsatellite analysis clearly demonstrated a substantial level of polymorphism among the populations of spiralling whiteflies; multiple alleles were identified for a few SSR loci. Average number of SSR alleles recorded are higher (2.5) than those previously reported for populations of spiralling whiteflies (Boopathi et al., 2014).

The polymorphism information content (PIC) of each SSR marker is a measure of its diversity. PIC gives an estimate of the discriminatory power of a locus considering not only the number of alleles expressed, but also the relative frequency of such alleles. Earlier, Boopathi et al.

Table 5. Pairwise F_{STs} correlation matrix for spiralling whitefly populations based on the three K clusters identified by the STRUCTURE analysis.

	Population 1	Population 2	Population 3
Population 1	0.000		
Population 2	0.470	0.000	
Population 3	0.414	0.408	0.000

(2014, 2017b) report that 66.0% and 54.7% of the microsatellite loci they studied are polymorphic in populations of spiralling whiteflies collected from different geographical regions of India. The use of 14 markers in the genetic variability analysis of whitefly populations revealed substantial variability among whitefly populations, with an observed mean heterozygosity (H_O) of 0.02 and average expected heterozygosity (H_E) of 0.52. In contrast, Ma et al. (2011) report, using microsatellite markers, a higher level of heterozygosity in populations of spiralling whiteflies in the Hainan and Canary Islands, which may be due to differences in the environment, population behaviour or shorter distances between samples. Expected heterozygosity is an estimate of the heterozygosity based on the assumption that the population is at Hardy-Weinberg equilibrium (HWE). Lower H_O than H_E indicates that the populations of spiralling whiteflies are smaller in size or have undergone a severe bottleneck in recent times. Another reason for this may be that the populations often do not outcross, which may vary from species to species. Substantial divergence among the populations of *A. dispersus* might be due to parallel multiple introductions of already diverged populations of the species from different sources and further divergence due to preferences for different host species.

AMOVA revealed greater diversity among individuals within populations (58.32) compared to that within any given population, which is expected for any natural population when polymorphic loci are present in individuals of a population. However, a substantial amount of variation (38.32) was recorded among populations, which indicates that populations are highly divergent. Earlier studies indicate that this species was first reported in 1993 on cassava (Palaniswami et al., 1995), which means that this species has had 25 years to adapt to the environment on the Indian sub-continent. Therefore, we speculate that the populations were pre-diverged and from multiple sources. The very low level of heterozygosity indicates little gene flow between host specific spiralling whiteflies. Low gene flow might be the result of restricted outcrossing between populations. Similar results are reported in earlier studies on whitefly populations from 18 districts in Tamil Nadu, India (Boopathi et al., 2017b) and distant geographic locations in India (Boopathi et al., 2014).

From the Neighbour Joining clustering pattern it is evident that the genetic distances between *A. dispersus* populations do not correlate with the taxonomic relations of their host plants. There is an exception; *A. dispersus* populations from eggplant, chili and tomato are in the same cluster and these plants all belong to the same family, the Solanaceae. However, the same cluster also includes whiteflies collected from cotton, which belongs to the family Malvaceae. Acalypha and calotropis are more different from other host species. Overall it was not possible to establish specific relationships between the different host plants and the genetic divergence among the different populations of spiralling whiteflies. However, the fixation indices based on the SSR loci data indicate that there is a significant level of divergence between the populations of spiralling whiteflies irrespective of the crops they infest. The fixation indices based on the genetic structure analysis of the populations assigned them to hypothetical populations. A low level of observed heterozygosity and high level of gene diversity (expected heterozygosity) indicate that the populations are not in HWE, which may be due to a high incidence of inbreeding and low incidence of intercrossing between individuals of distinct populations. This might be the result of sexual preferences between host plant specific populations and host plant-whitefly interactions. The divergence is further supported by the population structure analysis, which clearly grouped the populations into hypothetical groups. There is from very little to substantial levels of genetic admixture between individual populations (Fig. 3). This might be due to the coexistence of flies from two distinct populations on a single host species as genetic admixture resulting from intercrossing is not expected to occur (as discussed above).

The genetic data presented here and the biological data on performances and feeding behaviour presented in a parallel study (Palaniswami et al., 1995; Ranjith et al., 1996; Mani & Krishnamoorthy, 1999a, b) together demonstrate that the genetical divergence of Indian populations of *A. dispersus* is associated with differences in the host plants. Our results indicate that the genetic differentiation between spiralling whiteflies on sympatric plants of acalypha and calotropis is greater than that recorded for other host plants. The present study indicates that host specialization in spiralling whitefly may have preceded their introduction into India. A comparison of the genetic relatedness between and within host plants of *A. dispersus* in India is needed to test whether host specialization evolved once or many times. Although our genetic data also confirm earlier biological observations of host-associated populations of *A. dispersus* on several herbaceous families in India (Palaniswami et al., 1995; Ranjith et al., 1996; Mani & Krishnamoorthy, 1999a, b), they do not provide conclusive support for the taxonomic distinction of *A. dispersus* on calotropis.

CONCLUSIONS

The present study utilized SSR markers for identifying host correlated genetic variability amongst populations of *A. dispersus*. This study clearly demonstrates that Indian *A. dispersus* on 17 host plants differ substantially genetically and there is a minimum of intercrossing between these populations. The populations of *A. dispersus* from acalypha and calotropis were more distinct than those from other host plants. In order to shed light on the process of host-race formation in *A. dispersus* it is important to carry out phylogeographic studies. More studies on molecular sequences (mitochondrial and nuclear genomes), host phenotypes and mating compatibility, will significantly improve biosecurity preparedness and the quarantine protection against this invasive pest.

ACKNOWLEDGEMENTS. The authors are grateful to the Head of the Department of Agricultural Entomology and the Director of the Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore,-for providing facilities and support.

The assistance given by M. Govindaraj (Seed Science and Technology), M. Muthumariaapan, R. Govindaraj (Entomology), A. Mani and S. Arulnayagam in recording the observations is acknowledged.

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Received July 20, 2018; revised and accepted June 2, 2019
Published online June 27, 2019