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Population genetic structure of *Myzus persicae nicotianae* (Hemiptera: Aphididae) in China by microsatellite analysis

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ABSTRACT. The tobacco aphid, *Myzus persicae nicotianae* (Hemiptera: Aphididae), is an important agricultural pest that feeds on host plants and transmits plant viruses in China. To effectively control this pest, we investigated the genetic variation and genetic structure of 54 populations of tobacco aphids collected in China, using five microsatellite loci. An average of 7 alleles with effective number ranging from 1.5 to 6.6 was detected using these five loci, and the average polymorphic information content (PIC) was 0.652, suggesting that the five selected microsatellite loci were polymorphic and suitable for the study of population genetics. The expected heterozygosities in the populations studied ranged from 0.128 and 0.653, with an average value of 0.464. However, the observed heterozygosities ranged from 0.250 and 0.942 (average = 0.735), revealing

a high genetic variability and heterozygosity excess in the Chinese tobacco aphid populations. The global fixation index (F_{ST}) and mean gene flow (N_m) were 0.34 (P < 0.0001) and 0.50, respectively, suggesting the high genetic differentiation among Chinese populations. The 54 populations of tobacco aphids were classified into two groups. The populations did not cluster geographically, as populations from the same provinces were usually present in different clusters. This was also confirmed by the Mantel test, which showed no significant correlation between the genetic distance and geographical distance or altitude. Long distance migration might be responsible for the lack of distance-related isolation.

Key words: *Myzus persicae nicotianae*; Genetic diversity; Genetic differentiation; Parthenogenesis; Genetic structure

INTRODUCTION

Green peach aphid (*Myzus persicae*) is a highly polyphagous pest with a host range of >400 species, including many economically important crop plants (Blackman and Eastop, 2000). This pest is distributed worldwide and accounts for a large percentage of plant economic losses (Blackman and Eastop, 2000). *M. persicae nicotianae* causes direct damage by feeding on plant sap and spreads a variety of secondary pathogens and plant viruses like other aphids, resulting in serious losses (Gray and Gildow, 2003; Mowry, 2005).

Previous studies have shown that host-plant adaptation could drive the genetic differentiation of green peach aphid, such as that seen in *Brassica*-type and tobacco-type *M. persicae* in China (Xie, 1992). Yang et al. (1999) showed that the *M. persicae* affecting peach trees were more diverse than the ones on oilseed rape or tobacco. Green peach aphids also show differences in host preference; for example, the fecundity of *M. persicae* is higher in lilies and cabbage than in beans and chrysanthemums (Cao et al., 2004). Moreover, the life-cycle of red and green biotypes of the tobacco aphid feeding on oriental tobacco differed significantly from those growing on Virginia tobacco (Goundoudaki et al., 2003). For example, the subspecies *M. persicae nicotianae* had a shorter life cycle and higher reproductive rate than *M. persicae* (Blackman and Eastop, 2007). However, genetic differentiation in aphids affecting tobacco plants remains to be explained.

In the previous decade, entomologists focused on the study of population genetics in crop pests to facilitate insect pest management; these studies extensively used microsatellites as the biomarkers (Kim and Sappington, 2013; Wu et al., 2015). For example, Sun et al. (2015) reported the lack of significant differences in genetic structure in the small brown planthopper *Laodelphax striatellus* in China, as well as highlighting its high dispersal ability. Ascunce et al. (2011) used a large number of SSRs to investigate the global invasion route of the fire ant *Solenopsis invicta*. Microsatellite analysis has also been widely used to investigate the genetic structure of the *M. persicae* population. Bayesian clustering and admixture analyses helped classify green peach aphid genotypes into three groups, corresponding to the common *persicae*, the bisexual *nicotianae*, and unisexual *nicotianae* (Blackman et al., 2007). Angela (2006) classified 18 geographic populations of green peach aphids in Italy into two categories, and the genetic distance between these populations was positively correlated to the geographic distance.

However, Liu et al. (2010) showed no correlation between the genetic distance and geographic distance in different populations of aphids in the Gansu Province of China using microsatellite markers. Previous studies have also shown that the reproduction pattern of *M. persicae* is closely related to genotypic variability, population heterozygosity, and genetic differentiation (Simon et al., 1999; Delmotte et al., 2002; Guillemaud et al., 2003). At geographic distances greater than 50 km, *M. persicae* populations in Australia showed high genetic differentiation (Wilson et al., 2002).

However, there have been no reports on the population genetic structure of *M. persicae nicotianae* in China. In this study, we focused on the genetic differentiation in tobacco aphid and analyzed the genetic diversity of 54 geographic populations of the tobacco aphid using samples from 15 tobacco-cultivating regions in China.

MATERIAL AND METHODS

Sample collection

Tobacco aphid populations were collected from 54 tobacco-cultivating regions in 15 provinces in China between 2011 and 2012. Each population was collected from tobacco fields in the same town. Each tobacco aphid adult was collected from at least 5 m distance from the neighboring aphid to avoid collecting descendants of the same parthenogenetic aphid. The collected samples were transferred to 1.5 mL centrifuge tubes, immersed in anhydrous ethanol, and stored at -20°C in the Tobacco Research Institute of Chinese Academy of Agriculture Sciences. The sample collection procedure is detailed in Table 1.

DNA extraction, amplification of microsatellite loci, and electrophoresis

Approximately 24 well-preserved samples of tobacco aphid from each geographic population were selected for DNA extraction. The samples were taken out of the anhydrous ethanol solution, rinsed with distilled water, and blotted onto absorbent paper. DNA was extracted from each single aphid using the TIANamp Genomic DNA Kit (Tiangen, Beijing, China) according to the manufacturer protocols with slight modification, and stored at -20°C.

Microsatellite amplification was performed using five primer pairs described by Sloane et al. (2001) (Table 2). A 10-μM solution of each primer was prepared and stored at -20°C. Each Polymerase chain reaction (PCR) mixture contained 2 μL DNA template, 1 μL of each primer, 12.5 μL DreamTaq Green PCR Master Mix (2X) (Thermo Fisher Scientific Inc., Waltham, MA, USA), and 8.5 μL ddH_2O, for a final 25-μL volume. The reactions were performed in an Applied Biosystems PCR machine (Applied Biosystems, Foster City, CA, USA) using a procedure described by Sunnucks et al. (1996).

Electrophoresis was conducted on a DYY-12C electrophoresis apparatus with a DYCZ-20D electrophoresis tank (Liuyi Instrument Factory, Beijing, China). A pre-electrophoresis run was performed in an 8% non-denaturing polyacrylamide gel under constant voltage (850 V) for 15 min. Approximately 2 μL PCR product was then loaded into the gel, and a continuous electrophoresis performed for another 1.5 h. Silver staining was conducted as previously described by Han and Chang (2006). Target amplified products were selected based on a 90-200 bp fragment size range with clear and stable bands, and images of the results were obtained and stored.

Table 1. Collection details, observed heterozygotes (H_O), and expected heterozygotes (H_E) of *Myzus persicae nicotianae* populations in China.

Population code	Sampling site	Geographical coordinates	Altitude (m)	Collection date	Number of sample	H_O	H_E
JX-GX	Jingxi, Guangxi	106°22'E 23°11'N	727	2011.7	24	0.800	0.409
LC-GX	Luocheng, Guangxi	108°54'E 24°46'N	483	2012.7	24	0.800	0.409
HT-YN	Hongta, Yunnan	102°32'E 24°21'N	1646	2011.8	24	0.725	0.590
HX-YN	Huxi, Yunnan	103°45'E 24°31'N	1910	2011.7	24	0.742	0.419
SL-YN	Shilin, Yunnan	103°17'E 24°46'N	1732	2011.7	24	0.800	0.409
LL-YN	Luliang, Yunnan	103°41'E 24°50'N	1998	2011.7	24	0.400	0.204
CX-YN	Chuxiong, Yunnan	101°31'E 25°02'N	1785	2011.7	24	0.875	0.536
ZY-YN	Zhaoyang, Yunnan	103°42'E 27°19'N	1921	2011.7	24	0.800	0.409
ZX-YN	Zhenxiong, Yunnan	104°52'E 27°26'N	1662	2011.7	24	0.783	0.565
ZF-GZ	Zhenfeng, Guizhou	105°38'E 25°23'N	1135	2011.8	24	0.850	0.453
PX-GZ	Panxian, Guizhou	104°28'E 25°42'N	1817	2011.9	24	0.833	0.462
ZY-GZ	Ziyun, Guizhou	106°05'E 25°45'N	1160	2011.9	24	0.800	0.409
FQ-GZ	Fuquan, Guizhou	107°31'E 26°40'N	986	2011.9	24	0.800	0.409
WN-GZ	Weining, Guizhou	104°01'E 26°44'N	2268	2011.8	24	0.400	0.204
QX-GZ	Qianxi, Guizhou	106°02'E 27°01'N	1239	2011.9	24	0.800	0.409
JS-GZ	Jinsha, Guizhou	106°13'E 27°27'N	973	2011.9	24	0.833	0.585
YH-JX	Yihuang, Jiangxi	116°10'E 27°27'N	104	2011.6	24	0.742	0.588
HY-HN	Hengyang, Hunan	111°59'E 25°50'N	79	2011.6	24	0.800	0.409
CL-HN	Cili, Hunan	110°55'E 29°25'N	632	2011.6	24	0.667	0.498
HL-SC	Huili, Sichuan	102°14'E 26°39'N	2074	2011.6	24	0.683	0.588
MY-SC	Miyi, Sichuan	102°13'E 27°19'N	1159	2011.7	24	0.775	0.500
DC-SC	Dechang, Sichuan	102°10'E 27°24'N	1412	2011.6	24	0.800	0.409
GL-SC	Gulin, Sichuan	105°48'E 27°49'N	1007	2011.7	24	0.850	0.509
HD-SC	Huidong, Sichuan	106°36'E 27°54'N	2135	2011.7	24	0.800	0.409
MN-SC	Mianning, Sichuan	102°10'E 28°32'N	2302	2011.7	24	0.667	0.544
YB-SC	Yibin, Sichuan	104°38'E 28°45'N	308	2011.7	24	0.942	0.618
XY-SC	Xuyong, Sichuan	109°09'E 29°40'N	1035	2011.6	24	0.800	0.409
XH-SC	Xuanhan, Sichuan	107°43'E 31°21'N	472	2011.7	24	0.783	0.620
JG-SC	Jiange, Sichuan	105°31'E 32°17'N	924	2011.7	24	0.600	0.329
NC-CQ	Nanchuan, Chongqing	107°06'E 29°09'N	625	2011.7	24	0.633	0.513
WS-CQ	Wushan, Chongqing	109°52'E 31°04'N	896	2011.7	24	0.700	0.552
WL-CQ	Wulong, Chongqing	109°26'E 31°18'N	1192	2011.7	24	0.800	0.409
WX-CQ	Wuxi, Chongqing	109°34'E 31°24'N	820	2011.7	24	0.800	0.409
JS-HB	Jianshi, Hubei	109°43'E 30°36'N	641	2.11.8	24	0.800	0.632
XF-HB	Xianfeng, Hubei	109°55'E 31°42'N	778	2011.8	24	0.825	0.432
ZX-HB	Zhuxi, Hubei	109°49'E 31°43'N	850	2011.7	24	0.800	0.613
YX-HB	Yunxi, Hubei	110°25'E 32°59'N	299	2011.7	24	0.833	0.490
QC-AH	Qiaocheng, Anhui	105°31'E 32°17'N	44	2011.8	24	0.708	0.491
NNW-SX	Nanniwan, Shanxi	117°14'E 30°24'N	1119	2011.8	24	0.875	0.551
LN-SX	Luonan, Shanxi	109°21'E 32°50'N	968	2011.9	24	0.675	0.407
YL-SX	Yangling, Shanxi	108°05'E 34°16'N	470	2011.9	24	0.708	0.653
XY-SX	Xunyang, Shanxi	109°39'E 36°19'N	271	2011.8	24	0.758	0.608
XC-HN	Xuchang, Henan	113°51'E 34°05'N	71	2012.8	24	0.850	0.598
JX-SD	Juxian, Shandong	118°50'E 35°34'N	111	2011.7	24	0.542	0.407
YS-SD	Yishui, Shandong	118°37'E 35°47'N	169	2011.7	24	0.583	0.479
JM-SD	Jimo, Shandong	120°26'E 36°23'N	51	2011.6	24	0.800	0.409
PD-SD	Pingdu, Shandong	119°59'E 36°46'N	59	2011.7	24	0.800	0.409
FC-LN	Fengcheng, Liaoning	124°04'E 40°27'N	268	2011.7	24	0.692	0.520
KD-LN	Kuandian, Liaoning	124°47'E 40°43'N	552	2011.7	24	0.742	0.541
JP-LN	Jianping, Liaoning	119°36'E 41°24'N	529	2011.7	24	0.800	0.409
FM-LN	Fumeng, Liaoning	121°45'E 42°04'N	166	2011.7	24	0.650	0.370
CT-LN	Changtu, Liaoning	123°43'E 43°22'N	117	2011.7	24	0.400	0.204
YB-JL	Yanbian, Jilin	129°30'E 42°42'N	176	2011.7	24	0.725	0.511
ML-HLJ	Muling, Heilongjiang	129°37'E 44°33'N	573	2011.7	24	0.250	0.128

Data processing

Deviations from Hardy-Weinberg equilibrium (HWE) in all loci of each sample and linkage disequilibrium between pairs of loci were assessed using Genepop 4.0.10 (Rousset, 2008).

HWE and linkage disequilibrium were determined using Fisher's test and GENEPOP's default Markov chain parameters (1000 dememorizations, 100 batches, and 1000 iterations). PopGene32 software was used to analyze the genetic diversity and calculate the diversity indicators, including the effective number of alleles, *F*-Statistics, gene flow, and genetic distance. The Google Maps Distance Calculator (http://www.Daftlogic.Com/projects-google-maps-distance-calculator.htm) was used to obtain the geographic distances between each sampling point. Google Earth was used to obtain the altitude of each sampling point and calculate the altitude differences between sampling points. NTSYS-pc2.1 software was used for Mantel test analysis of the genetic distance, geographic distance, and the altitude difference.

Table 2. Locus name, repeat motif, sequence, size, and GenBank accession number of five microsatellite loci.

Locus	Repeat motif	Primer sequence	Size range (bp)	GenBank accession No.
$M37^{A}$	$(AC)_{16}$	F: GTGTGAGTAAGTCGTATTG R: TTGTATTATGTACCTGTGC	178-198	AF233241
$M40^{A}$	$(AC)_{17}$	F: ACACGCATACAAGAATAGGG R: AGAGGAGGCAGAGGTGAAAC	123-135	AF233242
$M86^{X}$	$(CA)_{23}$	F: TCCACTAAGACCTCAAACAC R: ATTTATTATGTCGTTCCGCC	97-141	AF233248
$M63^{A}$	$(AC)_{29}$	F: GCGGTTTTCTTTGTATTTTCG R: GATTATGGTGCTCGGTGG	163-207	AF233246
$M35^{A}$	$(AT)_{9}$~$(AC)_{22}$impure	F: GGCAATAAAGATTAGCGATG R: TGTGTGTATAGATAGGATTTGTG	178-198	AF233240

An analysis of molecular variation (AMOVA) implemented in Arlequin 3.5 was used to test the hierarchical genetic structure of the populations (Excoffier and Lischer, 2010). Significance in AMOVA analysis was ascertained using 10,000 permutations. Structure 2.3.3 (Pritchard et al., 2000) was used with a non-spatial algorithm to further assess the degree of population differentiation using microsatellite data. An initial range of potential genotype clusters (K) from 1 to 10 in the admixed model and the assumption of correlated allele frequencies among populations was specified. For each value of K, 3 runs were performed with 100,000 iterations (discarded as burn-in), followed by an additional 100,000 iterations. To estimate the most likely K value, we utilized both the log likelihood [lnPr(X/K)] method as recommended by Pritchard et al. (2000) and the ΔK statistic of Evanno et al. (2005).

RESULTS

Genetic diversity and differentiation in tobacco aphid populations

The number of alleles of the five microsatellite loci in the tobacco aphid populations ranged from 5 to 9 (Table 3), with an average of 7. The average effective number of alleles was at a range of 1.5-6.6 (average = 4.0). The $M40^{A}$ locus had the lowest number of alleles and the lowest effective number of alleles. The effective number of alleles for all loci was lower than the total number of alleles, indicating an uneven distribution of alleles in the tobacco aphid populations, with the possibility that several rare alleles also existed. The $M86^{X}$ locus had the greatest polymorphic information content (*PIC*; 0.820), whereas the $M40^{A}$ locus had the smallest PIC value of 0.319 (average *PIC* = 0.652). The observed heterozygosities ranged from 0.327 to 0.972, whereas the range of expected heterozygosities was 0.335-0.848. In general, the five microsatellite loci selected

in this study were highly polymorphic. The linkage disequilibrium (LD) test revealed that these 5 loci were significantly linked to each other, which may be attributed to the specific reproduction mode of aphids (parthenogenesis), which results in heterozygosity excess, as discussed below. Hence, all 5 loci were used in this analysis.

Table 3. Genetic variation among 5 microsatellite loci of *Myzus persicae nicotianae* in China.

Locus	N_A	N_E	H_O	H_E	PIC	F_{IS}	F_{ST}	N_m
M37^A	6	4.0	0.833	0.750	0.711	-0.60	0.30	0.57
M40^A	5	1.5	0.327	0.335	0.319	-0.51	0.35	0.46
M86^X	9	6.6	0.934	0.848	0.830	-0.67	0.36	0.45
M63^A	7	3.1	0.622	0.678	0.630	-0.52	0.40	0.38
M35^A	8	5.0	0.972	0.799	0.769	-0.70	0.28	0.64
Mean	7	4.0	0.738	0.682	0.652	-0.60	0.34	0.50

N_A = number of alleles; N_E = effective number of alleles; H_O = observed heterozygotes; H_E = expected heterozygotes; PIC = polymorphism information content; F_{IS} = inbreeding coefficient; F_{ST} = coefficient of gene differentiation; N_m = gene flow.

GENEPOP analysis revealed that all populations deviated from Hardy-Weinberg equilibrium (HWE) with respect to the five loci, which might be due to heterozygosity excess. The observed heterozygosities in the 54 geographic populations ranged from 0.250 (in Muling, Heilongjiang (ML-HLJ)) to 0.942 (in Yibin, Sichuan (YB-SC)), while the expected heterozygosities ranged between 0.128 (Muling, Heilongjiang (ML-HLJ)) and 0.653 (Yangling, Shanxi (YL-SX)) (Table 3). The observed heterozygosity in each population was greater than the expected heterozygosity, suggesting the existence of an excess of heterozygotes and absence of random mates in these populations. The inbreeding coefficients of the five microsatellite loci in all 54 tobacco aphid populations were negative, also indicating an excess of heterozygotes (Table 3). No geographical distinction was observed in the genetic variation in these populations (Table 3).

The coefficients of genetic differentiations among populations were between 0.28 and 0.40, while the gene flow ranged from 0.38 to 0.64.

Correlation analysis of geographic distance, altitude difference, and genetic distance

The software NTSYS-pc2.1 was used to determine the correlations between the genetic distance matrix and altitude difference/geographic distance matrix, calculated using Google Earth and Google Maps Distance Calculator, respectively. Two correlation graphs (Figure 1) were obtained. The data points were scattered and distributed irregularly. The Mantel test showed that the genetic distance and altitude difference were not significantly correlated (r = 0.1809; P = 0.9999). Similarly, the genetic distance and geographic distance were not significantly correlated (r = 0.2407; P = 0.9998). The genetic differentiation among populations did not fit the model of geographic isolation.

Cluster analysis of the geographic populations of tobacco aphids

Bayesian clustering analysis revealed the presence of two distinct clusters (Figure 2). One hypothetical cluster included populations from Guangxi, Yunnan, Guizhou, Jiangxi, Hunan,

Sichuan, Chongqing, Hubei, Shanxi, and Liaoning. The other cluster includes populations from Yunnan, Guizhou, Hunan, Sichuan, Hubei, Shanxi, Henan, Shandong, Liaoning, Jilin, and Heilongjiang. Populations from the same provinces were often separated into two clusters, such as the populations from Yunnan, Guizhou, and Liaoning. The high genetic differentiation between these two groups was also confirmed by the AMOVA analysis (Table 4).

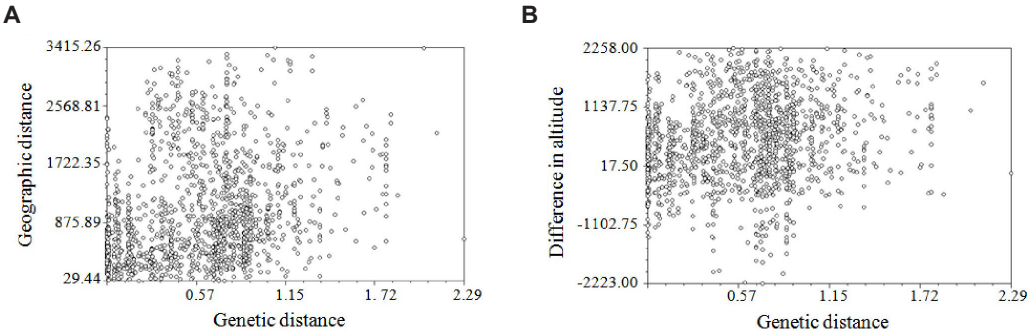

Figure 1. Mantel test scattergrams were built by matrix comparison plot (MxComp) in NTSYSpc. **A.** Correlation based on genetic and geographic distance between populations. **B.** Correlation based on genetic distance and the difference in altitudes between populations. The sampling points were scattered and distributed irregularly in the scattergrams.

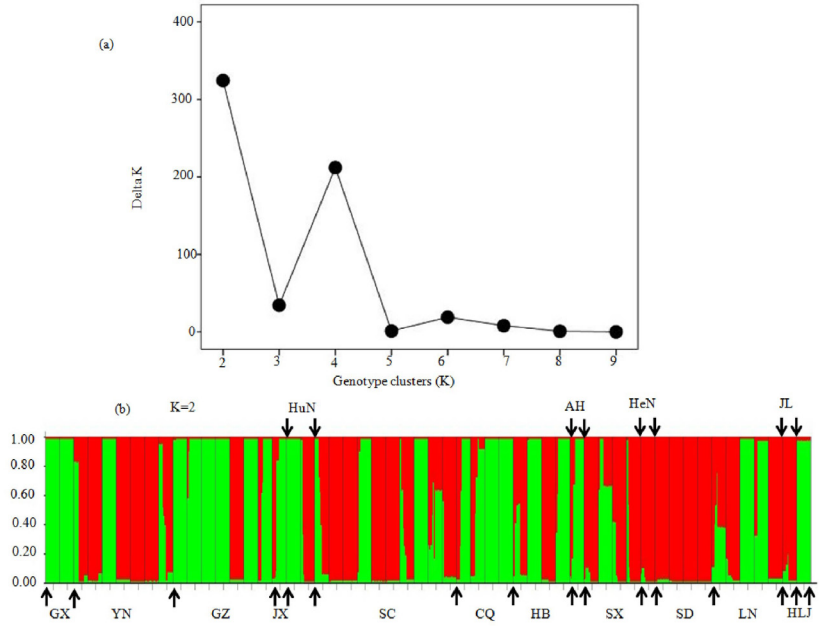

Figure 2. Bayesian analysis using STRUCTURE. **A.** Inference of the number of genetic clusters (K) from STRUCTURE. **B.** Bayesian clustering analysis using whole datasets from 54 locations indicating the presence of two clusters. The 54 blocks strand for the samples from 54 different locations as shown in Table 1. GX = Guangxi, YN = Yunnan, GZ = Guangzhou, JX = Jiangxi, HuN = Hunan, SC = Sichuan, CQ = Chongqing, HB = Hebei, AH = Anhui, SX = Shanxi, HeN = Henan, SD = Shandong, LN = Liaoning, JL = Jilin, and HLJ = Heilongjiang.

Table 4. Results of AMOVA test on mitochondrial and microsatellite markers.

Source of variation	Variation (%)	Fixation indices
Among groups	9.78	FCT = 0.098 (P < 0.001)
Among populations within groups	25.41	FSC = 0.282 (P < 0.001)
Within populations	64.80	FST = 0.352 (P < 0.001)

DISCUSSION

In this study, we investigated the population structure of *Myzus persicae nicotianae* in 54 Chinese populations. Our results showed that the Chinese tobacco aphid populations had a high level of genetic diversity and were highly differentiated.

Genetic diversity within the tobacco aphid populations

The five microsatellite loci selected in this study were all highly or moderately polymorphic, with a PIC value greater than 50%, except for $M40^A$, which had a PIC of 31.86%. The $M40^A$ locus in most populations expressed only one allele, resulting in a significantly lower *PIC* value compared to that in other loci. This might be because the samples used in this study were collected from tobacco plants in the summer, and allowed to reproduce by parthenogenesis, a form of asexual reproduction where offspring were produced without fertilization. The specific reproduction mode of parthenogenesis might also result in the deviation of HWE among the studied populations.

The average observed heterozygosity (H_O) in our study was 0.735, which was consistent with the results obtained in a previous study of tobacco aphids in the Guizhou Province (Lv et al., 2013). However, the expected heterozygosity was higher than the H_O, suggesting a heterozygosity excess, which could be attributed to the specific mode of asexual reproduction (parthenogenesis). Delmotte et al. (2002) proposed that sexual populations display a heterozygosity deficit and a high allelic diversity, whereas asexual populations show heterozygosity excess and a low level of allelic diversity. Some studies have also shown that sexual reproduction may remove gene mutations that occur during parthenogenetic periods (Simon et al., 2002), thereby reducing the possibility of heterozygosity excess. However, the observed heterozygosity was low in some populations, such as the Muling aphid population (0.25), suggesting a high genetic identity within the population. These results could be attributed to the specificity of the geography and climate in Heilongjiang Province, where the aphids usually underwent sexual reproduction under conditions of high latitude and low temperature (Zhang and Zhong, 1983), or the location of Muling near the northeastern region of China, where the peripheral population often displays low diversity. Chinese tobacco aphid populations showed greater genetic variability than the *Aphis nerii* populations seen in the Southern United States (Harrison and Mondor, 2011).

Genetic differentiation of tobacco aphid populations

The F_{IS} values of the five microsatellite loci evaluated in this study were all less than 0, which indicated an excess of heterozygotes, which in turn reflected a severe imbalance in the sex ratio of the population (Weir and Cockerham, 1984). In this study, the global F_{ST} value for the five microsatellites in the 54 tobacco aphid populations was 0.3353, indicating a high degree of genetic differentiation among these populations (Wright, 1978). Lv et al. (2013) detected a moderate to

high level of differentiation among the 25 geographic populations of *M. persicae* in the Guizhou Province using microsatellite marker analysis, which was consistent with the results of our study. However, another study observed a weak spatial population genetic structure in the rosy apple aphid, *Dysaphis plantaginea*, in French apple orchards (Guillemaud et al., 2011).

Previous studies have shown that the genetic distance usually increases with the increase in geographic distance (Wei et al., 2005). The geographical distances among sampling localities ranged from 30 to 3400 km in this study, with altitude differences ranging from 50 to 2300 m. However, the results of the Mantel test showed that the genetic distance was not correlated with the geographical distance or altitude difference, consistent with the results obtained by Liu et al. (2010). These results might reflect the occurrence of long distance dispersion in China. Additionally, we theorized that a short distance step-by-step dispersion also occurred in the same or the neighboring provinces simultaneously. Long distance range expansion was also observed in some invasive species like the Russian wheat aphid, *Diuraphis noxia (Z*hang et al., 2014). The average gene flow of the five microsatellite loci tested in this study was lower than 1.0, suggesting an inter-population gene flow. This hypothesis was also confirmed by the fact that tobacco aphid populations from the same province displayed lower genetic differentiation than the populations from different provinces, usually clustering together, excluding the populations from Liaoning, Guizhou, Yunnan, and Sichuan. This low gene flow might result in a genetic drift, causing differentiation within the population (Slatkin, 1987). The population genetic structure was often closely related to the location and climate (Sandrock et al., 2011), as well as other factors, such as the gene flow level and reproduction mode. The low gene flow despite a lack of isolation (by distance) among these populations implies a long distance spread of this pest caused by anthropogenic influences. The genetic differentiation of tobacco aphid populations is a result of multiple factors, and our understanding of this matter can only be improved by conducting a multi-factorial analysis.

The number of microsatellite loci selected in this study was relatively low; moreover, future studies should include more polymorphic microsatellite loci. Nuclear genes, including ITS and 28SrDNA, can better represent the evolutionary status of different species because of its higher degree of conservation than microsatellite loci. Future studies on the genetic diversity of the tobacco aphid population must include an analysis of nuclear genes to better understand the evolutionary status of local populations.

CONCLUSION

This study revealed that majority of the tobacco aphid populations had high genetic diversity; however, these populations were not randomly mated, which might be because the observed heterozygosity was higher than the expected heterozygosity in most populations. The excess of heterozygosity in the tobacco aphid populations might be related to its reproduction pattern (parthenogenesis). The tobacco aphid populations in China expressed diverse reproduction patterns and significant genetic differentiation. The genetic differentiation in tobacco aphid populations is closely related to the geographic environment and climate; however, a geographic isolation was not observed. Tobacco aphid populations from the same province were clustered together, except for those from the Liaoning, Guizhou, Yunnan, and the Sichuan Provinces.

Conflicts of interest

The authors declare no conflict of interest.

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