

Isolation and Characterization of Nine Microsatellite Loci from the Sycamore Lace Bug Corythucha ciliata (Hemiptera: Tingidae)

Authors: Yang, Wen-Yan, Tang, Xiao-Tian, Cai, Li, Dong, Chang-Sheng, and Du, Yu-Zhou

Source: Florida Entomologist, 97(3): 1070-1074

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.097.0310

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at <u>www.bioone.org/terms-of-use</u>.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

ISOLATION AND CHARACTERIZATION OF NINE MICROSATELLITE LOCI FROM THE SYCAMORE LACE BUG CORYTHUCHA CILIATA (HEMIPTERA: TINGIDAE)

WEN-YAN YANG¹, XIAO-TIAN TANG¹, LI CAI¹, CHANG-SHENG DONG² AND YU-ZHOU DU¹* ¹School of Horticulture and Plant Protection & Institute of Applied Entomology, Yangzhou University, Yangzhou 225009, China

²Guangling Agricultural Technology Extension and Service Center, Yangzhou 225009, China

*Corresponding author; E-mail: yzdu@yzu.edu.cn

ABSTRACT

The sycamore lace bug, Corythucha ciliata (Say) (Hemiptera: Tingididae) of North America, is an invasive pest of plane and sycamore trees (*Platanus* spp.) (Proteales: Plantanaceae), and has invaded many countries. To explore the population genetic structure and the invasion route by which C. ciliata reached China, we developed 9 highly polymorphic microsatellites loci by the FIASCO method. Polymorphism of the 9 loci was assessed in 48 individuals from 2 populations (Guiyang and Nanjing) in China. The number of alleles per locus ranged from 2 to 13. The observed $(H_{\rm o})$ and expected $(H_{\rm g})$ heterozygosities varied from 0.146 to 0.958 and 0.290 to 0.849, respectively, in Guiyang population. Likewise $H_{\rm o}$ and $H_{\rm g}$ varied from 0.483 to 0.739 and 0.443 to 0.865, respectively, in Nanjing population. Two loci (CA15 and GA365) showed significant deviations from the Hardy-Weinberg equilibrium (HWE) in Nanjing population. Moreover, loci CA200>26, GT26&TG100, and TG100&GA365 showed significant linkage disequilibria (LD) in the Guiyang population (P < 0.01), and loci GT26 and GA5 (P < 0.01) showed significant linkage disequilibria (LD) in the Nanjing population. Finally, we found 2 types of mutational events that could generate the new alleles, but the main mutation mechanism for the newly developed microsatellites was slippage in the repeat motif and in the flanking region. In future work, the nine loci identified here will be used to study the population genetic structures of C. ciliata populations in China and in putative regions of their origin, and investigate the probable route by which the pest reached China.

Key Words: microsatellite, Corythucha ciliata, population genetics, invasive routes

RESUMEN

El chinche encaje del sicómoro, Corythucha ciliata (Say) (Hemiptera: Tingididae) de América del Norte, es una plaga invasora de plataneros y sicómoros (Platanus spp.) (Proteales: Plantanaceae), y ha invadido muchos países. Para explorar la estructura genética de la población y la ruta de invasión por el cual C. ciliata llegó a China, hemos desarrollado 9 loci de microsatélites altamente polimórficos por el método FIASCO. Se evaluó el polimorfismo de los 9 loci en 48 individuos de 2 poblaciones (Guiyang y Nanjing) en China. El número de alelos por locus varió de 2 a 13. El heterocigosis observado (H_0) y el esperado (H_E) varian de 0.146 a 0.958 y de 0.290 a 0.849, respectivamente, en la población de Guiyang. Del mismo modo el H_0 y $H_{\rm p}$ variaron de 0.483 a 0.739 y 0.443-0.865, respectivamente, en la población de Nanjing. Dos loci (CA15 y GA365) mostraron desviaciones significativas del equilibrio de Hardy-Weinberg (EHW) en la población de Nanjing. Por otra parte, loci CA200 y GT26, GT26 y TG100 y TG100 y GA365 mostraron un desequilibrio de ligamiento (DL) significativo en la población de Guiyang (P < 0.01), y loci GT26 y GA5 (P < 0.01) mostraron un desequilibrio de ligamiento (DL) significativo en la población de Nanjing. Por último, encontramos 2 clases de eventos mutacionales que podrían generar los nuevos alelos, pero el principal mecanismo de la mutación de los microsatélites desarrollados recientemente fue el deslizamiento en la repetición de motivos y en la región de flanqueo. En trabajos futuros, los nueve loci identificados aquí se utilizarán para estudiar la estructura genética de la población de de C. ciliata en China y en regiones putativas de su origen, e investigar la ruta probable por el cual la plaga llegó a China.

Palabras Clave: microsatélites, *Corythucha ciliata*, genética de poblaciones, rutas invasoras

The sycamore lace bug, *Corythucha ciliata* (Say) (Hemiptera: Tingidae) is a new invasive insect species in China from North America (Halbert et al. 1998) that previously had invaded many countries including France, Germany, Chile, Korea, Japan (Wulf et al. 1987; Prado 1990; Chung et al. 1996; D' Aguilar et al. 1997; Tokihiro et al. 2003). In invaded locations, *C. ciliata* feeds primarily on leaves of plane and sycamore trees (*Platanus* spp.; Proteales: Plantanaceae), reducing photosynthesis and promoting diseases, which may be followed by death of the foliage.

Although the physiology and ecology of C. *ciliata* and its control with pesticides have been investigated (Yoon et al. 2008; Ju et al. 2010, 2011a, 2011b), its population genetics is unknown. To better control this pest, an increased understanding of its population genetics and invasion routes is needed. Microsatellites markers can help identify the origins of newly established populations of invasive species, as well as their genetic makeup and their routes of migration (Behura 2006; Ascunce et al. 2011). However, the microsatellite loci of C. ciliata have not been reported and few expressed sequence tags (ESTs) of *C. ciliata* are available in the public domain, hindering the study of the population genetics and routes of migration of this species. Consequently, we used the FIASCO method (fast isolation by AFLP of sequences containing repeats) with slight modifications to develop an enriched library of microsatellite loci (Zane et al. 2002) to screen polymorphic loci. Here, we present sequences of 9 microsatellite loci for C. ciliata.

MATERIALS AND METHODS

Samples and DNA extraction

Adult *C. ciliata* samples were collected in 2010 from Guiyang, Guizhou Province and Nanjing, Jiangsu Province, China. Samples were identified and preserved in 100% ethanol, and then stored at -20 °C. Genomic DNA was extracted from individual samples using Axy-Prep Multisource Genomic DNA Miniprep Kit and stored at -20 °C until needed for PCR.

Isolation of Microsatellite Markers

We used the FIASCO (fast isolation by AFLP of sequences containing repeats) method with slight modifications to develop an enriched library of microsatellite loci (Zane et al. 2002). The genomic DNA was first digested with the restriction enzyme MseI (BioLabs, Beijing, China) and ligated to MseI AFLP adaptor (5'-TACTCAG-GACTCAT-3'/5'-GACGATGAGTCCTGAG-3') in a total volume of 25 µL containing 250 ng of

adaptor-specific primers (5'-GATGAGTCCT-GAGTAAN-3', MseI-N) in 20 µL reactions containing $1 \times PCR$ buffer (10 mM Tris-HCl, pH 9.0, 50 mM KCl), MgCl₂ 1.5 mM, dNTPs 250 uM, MseI-N 0.5 uM, 1 U of Taq DNA polymerase (TaKaRa, Dalian, China) and 5 µL diluted digestion-ligation DNA. The PCR conditions were 5 min at 94 °C followed by 26 cycles of 30 s at 94 °C, 1 min at 53 °C, 1 min at 72 °C with a final extension time of 10 min at 72 °C. After denaturation at 95 °C for 5 min, the PCR products were hybridized for 1 h at 68 °C with biotinylated (GA)₁₂, (GT)₁₂, (CA)₂₅ and (TG)₁₈, respectively. The DNA fragments hybridized to biotinylated probes were selectively captured by streptavidin coated magnetic beads (Streptavidin Magnesphere Paramagnetic Particles, Promega, Shanghai, China). The microsatellite-enriched DNA fragments were purified using PCR Cleanup Kit (Axygen, www.axygen. com)and then amplified by MseI-N primers for the following PCRconditions: 3 min at 95 °C followed by 26 cycles of 30 s at 95 °C, 30 s at 53 °C, 45 s at 72 °C with a final extension time of 30 min at 72 °C. The purified PCR products were ligated into pGEMT Easy vectors (Promega, www. promega.com) and then transformed into Escherichia coli strain (DH5a). The positive clones were identified by PCR using M13 primers and visualized by agarose gel electrophoresis. All positive clones were sequenced in both directions using the BigDye Terminator Sequencing Kit (Applied BioSystems) and the ABI 3730XL Genetic Analyzer (PE Applied Biosystems, San Francisco, California, USA) with 2 vector-specific primers and internal primers for primer walking. Microsatellite sequences were identified by software SSRHunter 1.3 (Li & Wan 2005). Primer pairs for each microsatellite locus were designed by Primer Premier 5.0 software (http://www.premierbiosoft.com/primerdesign/). PCR Amplification and Genotyping

genomic DNA, 1 × OnePhorAll buffer, 5.0 mM DTT, 50 µg/mL BSA, 1.0 µM adaptor, 200 µM

ATP, 2.5 U of MseI (NEB), and 1.0 U of T4 DNA

ligase (Promega). The reaction was then incu-

bated at 37 °C for 3 h. The digestion-ligation

products was diluted (1:10) and amplified with

Each primer pair was screened against 48 individuals of *C. ciliata*. Three kinds of fluorophores (FAM, HEX and TAMRA) were tagged with forward primers at the 5'end of each pair. PCR amplifications were conducted in 25 μ L volumes including 1 × PCR buffer (10 mM TrisHCl, pH 9.0, 50 mM KCl), 1.5 mM MgCl2, 200 FM of each dNTPs, 50 ng genomic DNA, 0.75 U of Taq DNA polymerase (TaKaRa), 4 pmol of each primer. Conditions for PCR amplification were

Mercession is beind is not intersectionRepeat is not intersectionSize is NAMathMathMathMathKr80816CAUIIFrancess (5.3)Fluorophor<	-i	CHARACTERIS: OF ALLELES (P-HW).	TICS OF NIN. (\mathbf{N}_{A}) , OBERS	e microsatellite loci of <i>Corythuch</i> erved heterozygosity (H _o), expectei	<i>ia cillata</i> : Gene d heterozygos	JANK ACCESSIO ITY $(H_{_{ m E}})$ AND VA	N NUMBER LUE FROM	, REPEAT MO THE TEST F	TIF, ALLEL ROM THE E	E SIZE RANC (ACT TEST]	E, ANNEAL FOR THE H ₁	ARDY-WEI	RATURE (TA NBERG EG	(), NUMBER JUILIBRIUM
KF6031D5 Cuyant Endinate Guyant Nanjing Guyant		Accession no	Repeat motif	Primer sequences (5'-3')	Fluorophore	Size range (bp) Ta	4	AA	H	0	H	5	P-F	M
KF803195 CA)11 F:CACTATTGGTCTCGTCT FM 176-233 52 10 13 0.636 0.6349 0.735 0.0474 0.0903 KF803194 CA)11 R:CCATTTACTCCACTTTC HEX 344-357 58 7 0.642 0.543 0.6473 0.0474 0.0903 KF803194 CA)11 R:CCATTTACTCCACTTTC TAMRA 1173-195 5 4 0.625 0.542 0.542 0.642 0.543 0.0143 0.0903 KF803198 GT)10 F:CCATTTACTCCACTTTC TAMRA 173-195 3 0 0.542 0.542 0.543 0.4136 0.0203 KF803201 GT)10 F:ACCACTCCACCTGACTTTC TAMRA 179-195 3 0.552 0.552 0.556 0.556 0.542 0.563 0.6136 0.0101 KF803201 GT)11 F:TTCCTCGACCTGACTTTG TAMRA 179-195 3 0.542 0.563 0.542 0.566 0.6033 0.0131 KF803201 GT)11 F:							Guiyang	Nanjing	Guiyang	Nanjing	Guiyang	Nanjing	Guiyang	Nanjing
KF003194 (A)11 F:GAGGAGGGGAAGGGT HEX 344-357 5 4 0.625 0.542 0.643 0.8114 0.0233 KF003198 (GT)10 F:CCATTTACTCCATTTC TAMRA 1173-195 52 9 7 0.905 0.642 0.643 0.8114 0.0203 KF003198 (GT)10 F:CCATTTACTCCACTTCG TAMRA 173-195 52 9 7 0.905 0.650 0.762 0.3105 0.1910 KF003202 (CT)9 F:ACCACTCCACCTCCACCT TAMRA 179-195 5 3 0.541 0.630 0.443 0.1910 KF003201 (GT)11 F:ACCACTCCACCTCACCT TAMRA 192-202 5 0.550 0.643 0.4136 0.1910 KF003201 (GT)11 F:TTTCTCTGAGCATCATT FAM 192-202 5 0.550 0.643 0.6136 0.6136 0.0101 KF003201 (GT)11 F:TTTCTCTGAGCATCATT FAM 192-202 5 0.556 0.526 0.625 <td< td=""><td></td><td>KF803195</td><td>(CA)11</td><td>F.CACTATTTGGTCTCGTCGT R.CCATTTTACTCCACTTTC</td><td>FAM</td><td>176-233 52</td><td>10</td><td>13</td><td>0.958</td><td>0.636</td><td>0.849</td><td>0.735</td><td>0.0474</td><td>0.0990</td></td<>		KF803195	(CA)11	F.CACTATTTGGTCTCGTCGT R.CCATTTTACTCCACTTTC	FAM	176-233 52	10	13	0.958	0.636	0.849	0.735	0.0474	0.0990
KF803195 (F)10 F: CATTTACTCGATTC TAMRA 1173-195 52 0 0.556 0.828 0.762 0.3666 0.3005 KF803202 (CT)9 F: ATTGGTCTCGATGG TAMRA 179-195 55 3 0.541 0.433 0.4136 0.4101 KF803202 (CT)1 F: ATCGATCCACTCGAGG TAMRA 179-195 55 3 0.542 0.530 0.433 0.4136 0.1910 KF803201 (GT)1 F: TTCTCTGAGGCGAGCAACA FAM 192-202 56 2 0.542 0.583 0.4136 0.4136 0.1910 KF803201 (GT)1 F: TTCTCTGAGGCGAGCAACA FAM 192-202 56 2 0.553 0.542 0.690 0.6903 0.0131 KF803201 (GT)1 F: TTCTCTGAGCATCACACA FAM 233-269 5 3 0.146 0.433 0.0501 0.0101 KF80319 (AD)1 F: AGGCAGTAGCACA FAM 233-269 5 3 0.146 0.423 0.05		KF803194	(CA)11	F:GAGGATGGGGAAAGGGTT R:GCTGGTTTTGAGGGGGGGAC	HEX	344-357 58	ວ	4	0.625	0.542	0.642	0.543	0.8114	0.0239
KF803201 (CT)9 F:ACCATCACCTCCACCG TAMRA 179-195 55 3 0.542 0.630 0.443 0.4136 0.1010 KF803201 (GT)11 F:TTTCTCGAGGGAGGCAAGC FAM 192-202 56 2 5 0.550 0.642 0.696 0.60603 0.0131 KF803201 (GT)11 F:TTTCTCGAGCATCATT FAM 192-202 56 2 0.550 0.625 0.696 0.0603 0.0131 KF803201 (GT)1 F:TGCCGTTCACCACC FAM 233-269 5 3 0.146 0.483 0.696 0.0603 0.0131 KF803191 (GJ)12 F:AGCCGTGGGTAGGAAC TAMRA 347-362 3 11 0.543 0.739 0.865 0.9041 0.0084 KF803191 (GJ)12 F:AGCCGTGGGTAGGAAC TAMRA 347-362 5 9 0.753 0.865 0.9041 0.0384 KF803191 (GJ)12 F:AGCCGTGGGTAGCAC TAMRA 168-219 5 0 0.753		KF803198	(GT)10	F: CCATTTTACTCCACTTTC R: ATTGGTCTCGTCGTGATG	TAMRA	1173-195 52	6	7	0.905	0.556	0.828	0.762	0.3666	0.0202
KF803201 (TTTCTCTGAAGACTGTGA FAM 192-202 56 0.250 0.625 0.422 0.696 0.0603 0.0131 R: GGCTGGCTAGCATCTTT R: GGCTGGCTAGCATCTTT FAM 192-202 56 3 0.146 0.422 0.696 0.0603 0.0131 KF803200 (AC)7 F: AGGATCTTCATCACCATC FAM 233-269 55 3 0.146 0.483 0.590 0.582 0.0901 0.000* KF803197 (CM)12 F: AGCGTGGCGTGGGAGGAG TAMRA 347-362 54 3 11 0.542 0.793 0.9044 0.038* KF803199 (G3)26 F: AGCCGTGGGAGGGAG TAMRA 168-219 5 9 0.775 0.793 0.794 0.038* KF803199 (G3)26 F: CCGACTCAGTACCCGG TAMRA 168-219 5 9 0.775 0.794 0.038* KF803196 (TC)13 F: CCGACTCAGTACCCG TAMRA 168-219 5 9 0.775 0.7793 0.8758 0.039* </td <td></td> <td>KF803202</td> <td>(CT)9</td> <td>F: ACCACTCCACCTCCACCG R:AGTAAGGGCGAAGCAACA</td> <td>TAMRA</td> <td>179-195 55</td> <td>co</td> <td>က</td> <td>0.542</td> <td>0.583</td> <td>0.630</td> <td>0.443</td> <td>0.4136</td> <td>0.1910</td>		KF803202	(CT)9	F: ACCACTCCACCTCCACCG R:AGTAAGGGCGAAGCAACA	TAMRA	179-195 55	co	က	0.542	0.583	0.630	0.443	0.4136	0.1910
KF803200 (AC)7 F:AGGAATCTTCATCACATC FAM 233-269 55 3 0.146 0.483 0.290 0.582 0.0901 0.0000* R: TGACGATTCTTGACGAG A 337-369 55 3 0.146 0.483 0.590 0.582 0.0901 0.0001* KF803197 (CA)12 F:AGCGTGGGAGGGAGG TAMRA 347-362 54 3 11 0.542 0.739 0.565 0.9041 0.0385 KF803199 (AG)26 F:CCGACTCAGTAGCGAGG TAMRA 168-219 5 9 0.750 0.733 0.793 0.8758 0.039* KF803196 (TC)13 F:CCGACTCAGTACCCCG TAMRA 168-219 5 9 0.7750 0.733 0.793 0.8758 0.039* KF803196 (TC)13 F:CCGACTCAGTACCCCG TAMRA 168-219 5 9 0.7750 0.733 0.793 0.8758 0.039* KF803196 (TC)13 F:CCGACTCAGGAGGTCATT HEX 310-369 5 9<		KF803201	(GT)11	F: TTTCTCTGAAGACTGTGA R: GGGTGGGTAGCATCATTT	FAM	192-202 56	2	ũ	0.250	0.625	0.422	0.696	0.0603	0.0131
KF803197 (CA)12 F:AGCCGTGCGGTAGCGAAT TAMRA 347-362 54 3 11 0.542 0.739 0.563 0.865 0.9044 0.0388 KF803199 (AG)26 F: CCGACTCAGTACCACCGA TAMRA 168-219 54 5 9 0.750 0.563 0.865 0.09044 0.0388 KF803199 (AG)26 F: CCGACTCAGTACCACCGA TAMRA 168-219 54 5 9 0.7700 0.7733 0.8758 0.039* KF803196 (TC)13 F: CCCAGCCTAGAGTCATT HEX 310-369 57 4 9 0.391 0.727 0.849 1.0000 0.0383 KF803196 (TC)13 F: CCCAGCCTAGAGTCATT HEX 310-369 57 4 9 0.391 0.727 0.849 1.0000 0.0383 KF803196 (TC)13 F: CCCAGCCTAGAGTCATT HEX 310-369 57 4 9 0.391 0.727 0.849 1.0000 0.0383		KF803200	(AC)7	F: AGGAATCTTCATCACATC R: TGACCGTTTCTTGACCAG	FAM	233-269 55	°	က	0.146	0.483	0.290	0.582	0.0901	0.0000*
KF803199 (AG)26 F: CCGACTCAGTACCACCGA TAMRA 168-219 54 5 9 0.750 0.733 0.793 0.8758 0.0039* R: GACTTCATAATGTCTCCC R: GACTTCATAATGTCTCCC HEX 310-369 57 4 9 0.391 0.727 0.849 1.0000 0.0383 KF803196 (TC)13 F: CCCAGCCTAGAGTCATT HEX 310-369 57 4 9 0.391 0.727 0.849 1.0000 0.0383 R: GCACCTCCTCTTTTCA HEX 310-369 57 4 9 0.391 0.727 0.849 1.0000 0.0383		KF803197	(CA)12	F.: AGCCGTGCGGTAGCGAAT R: TTTGCGTTTTTGAAGGAGC	TAMRA	347-362 54	co	11	0.542	0.739	0.583	0.865	0.9044	0.0388
KF803196 (TC)13 F: CCCAGCCCTAGAGTCATT HEX 310-369 57 4 9 0.391 0.727 0.397 0.849 1.0000 0.0383 R: GCACCTCCTTCTTTTCA R: GCACCTCCTTCTTTTCA R 9 0.391 0.727 0.397 0.849 1.0000 0.0383		KF803199	(AG)26	F: CCGACTCAGTACCACCGA R: GACTTCATAATGTCTCCC	TAMRA	168-219 54	ວ	6	0.750	0.500	0.733	0.793	0.8758	0.0039^{*}
		KF803196	(TC)13	F: CCCAGCCCTAGAGTCATT R: GCACCTCCTCCTTTTCA	HEX	310-369 57	4	6	0.391	0.727	0.397	0.849	1.0000	0.0383

Downloaded From: https://bioone.org/journals/Florida-Entomologist on 03 Dec 2021 Terms of Use: https://bioone.org/terms-of-use

** ***********************************	CTCGGGTCCGC	AAAGCGTGGTGA AAAGCGTGGTGAI	****** ***** GTGTGG-GTGTG GTGTGGTGTGTG	******************** FGTGTGTGTGCTGTGTG FGTGTGTGTGCTGTGTG	**************************************	************** CGAGAGAGGGTTC. CGAGAGAGGGGTTC.	A
1	.20	3040. ********		60 ***************	.7080 ** *******	*************	
TATATGAACTCTTCCC TATATGAACTCTTCCC TATATGAACTCTTCCC	GAGAGAGAGAGA GAGAGAGAGAGA GAGAGAGAGAGA	JAGAGAGAGAGAGA JAGAGAGAGAGAGA JAGAGAGAGA	AGAGAGAGAGAGAGA AGAGAGAGAGAGAGA AGAGAGAGAGAGAG	GAGAGAGAGAGAGA GAGAGAGAGAGAGAGA GAGAGAGAGAGAGAGA	GAGAGAGAAAAAAAAA GAGAGAGAAAAAAAAA GAAAAAAAAA	AAGTITAGTITGCG AAGTITAGTITGCG AAGTITAGTITGCG	B
440			480	490		.520	
ACTAAAQITTTTTTTTT ACTAAAQITTTTTTTTTT ACTAAAQITTTTTTTTTT ACTAAAQITTTTTTTTTTTTTTT ACTAAAQITTTTTTTTTTT	********** TCTCTCTCTCT TCTCTCTCTCTCT -CTCTCTCTCTCT	CTETETETETETET CTETETETETETETE CTETETETE	CTCTCTCTCTCTCT CTCTCTCTCTCTCTCT CT	TCTCTCTCTCTCTCTC TCTCTCTCTCTCTCTCTC TC	** ******** TCAGGGAAGA ICTCTCAGGGAAGA ICTCTC-AGGGAAGA ICTCTCTCAGGGAAGA	GTTCATATAATTCT GTTCATATAATTCT GTTCATATAATTCT GTTCATATAATTCT GTTCATATAATTCT	С

Fig. 1. Mutational events of *Corythucha ciliata* clone microsatellite sequences. A and B are the first type of mutational pattern; C is the second type of mutational pattern.

as follows: an initial denaturing at 94 °C for 4 min, followed by 42 cycles of 50 s at 94 °C, 50 s at 51–58 °C depending on the primer pair (Table 1), and 1min at 72 °C, followed by a final extension for 10 min at 72 °C. The PCR products of 3 fluorophores (FAM, HEX and TAMRA) were mixed in a ratio dependent on the brightness of bands visualized by agarose gel electrophoresis, respectively. The PCR products were run on an ABI 3730XL DNA sequencer and the electropherograms drawn through Gene Scan 4.0 were used to extract DNA fragment sizing details using Gene Mapper 4.0 software by Sangon Biotech (Shanghai) Co., Ltd.

Statistical Analysis

The number of alleles (N_A) , observed heterozygosity (H_o) , expected heterozygosity (H_E) , and polymorphism information content (PIC) were calculated for each locus using Cervus version 3.0 (Marshall et al. 1998). The Hardy-Weinberg equilibrium (HWE) and genotypic linkage disequilibrium between pairs of microsatellites were tested by Genepop 3.4 (Raymond et al. 1995). Null allele frequencies were measured by MICRO-CHECKER 2.2.3 (van Oosterhout et al. 2004). All P-values were adjusted for multiple tests using the sequential Bonferroni method (Rice 1989).

RESULTS AND DISCUSSION

Characteristics of Microsatellite Loci

Thirty five primers were designed based on 163 clones that contained a microsatellite sequence, but 9 microsatellite loci showed high polymorphisms (PIC > 0.5) (Table 1). The number of alleles per locus ranged from 2 to 13, with an average of 6 alleles per locus. The observed (H_0) and expected (H_E) heterozygosities varied from 0.146 to 0.958 and 0.290 to 0.849,

respectively, in Guiyang population. The observed (H_0) and expected (H_E) heterozygosities varied from 0.483 to 0.739 and 0.443 to 0.865, respectively, in Nanjing population. The null allele frequency was lower than 0.1 in at least 1 population. After sequential Bonferroni corrections, 2 loci (CA15 and GA365) showed significant deviations from the Hardy-Weinberg equilibrium (HWE) in Nanjing population. This phenomenon may be caused by the Wahlund effect, the effect of evolutionary pressure during the process of invasion or the existence of a null allele. Loci CA200>26, GT26&TG100, TG100&GA365 showed significant linkage disequilibria (LD) in the Guiyang population (P <0.01), and loci GT26 and GA5 ($\overline{P} < 0.01$), showed significant linkage disequilibrium in Nanjing population. This phenomenon may be caused by genetic drift of the C. ciliata population after invasion. Consequently, the 9 loci can be useful for population genetics studies and explore invasive routes of C. ciliata.

Mutations of Microsatellite Sequences

In total, we obtained 1,385 bacterial colonies from the enriched library of microsatellite loci that used the biotinylated probes: (GA)₁₂, (GT)₁₂, (CA)₂₅ and (TG)₁₈. Two hundred and four recombinant clones were sequenced, but only 163 were successful. We analyzed these microsatellite sequences to determine the mutational model. We found 2 types of mutational events that could have generated the new alleles. First, the differences in numbers of repeat motifs caused size variation of alleles (Figs. 1A and 1B). Secondly, 2 different repeat motifs contributed to the allele-size variation (Fig. 1C). However, slippages in the repeat motif and flanking region were the main mutation mechanism for the newly developed microsatellites.

The findings of this study will allow us to elucidate genetic structure of the *C. ciliata*

populations in China and in putative regions of their origin, and thereby investigate the probable route by which the pest reached China, as well as its subsequent spread in China.

ACKNOWLEDGMENTS

Special thanks to Y. Ji and J. Xu, students at Yangzhou University. This work was supported by Science & Technology Program of Yangzhou (YZ2010064) and the National Natural Science Foundation of China (31300467).

References Cited

- ASCUNCE, M. S., YANG, C. C., OAKEY, J., CAICATERRA, L., WU, W. J., SHIH, C. J., GOUDET, J., ROSS, K. G., AND SHOEMAKER, D. 2011. Global invasion history of the fire ant *Solenopsis invita*. Science 331: 1066-1068.
- BEHURA, S. K. 2006. Molecular marker systems in insects: current trends and future avenues. Mol. Ecol.15: 3087-3113.
- CHUNG, Y. J., KWON, T. S., YEO, W. H., BYUN, B. K., AND PARK, C. H. 1996. Occurrence of the sycamore lace bug, *Corythucha ciliata* (Say) (Hemiptera: Tingidae) in Korea. Korean J. Appl. Entomol. 35: 137-139.
- D' AGUILAR, J., PRALAVORIO, R., AND PABASSE, J. M. 1977. Introduction into France of the plane tree lace bug: *Corythucha ciliata* (Say) (Heteroptera, Tingidae). Bull. Soc. Entomol. France 82: 2-6.
- DOMINIAK, B. C., GILLESPIE, P. S., WORSLEY, P., AND LOCKER, H. 2008. Survey for sycamore lace bug Corythucha ciliata (Say) (Hemiptera: Tingidae) in New South Wales during. 2007. Gen. Appl. Entomol. 37: 27-30.
- HALLBERT, S. E., AND MEEKER, J. R. 1998. The sycamore lace bug, Corythucha ciliata (Say) (Hemiptera: Tingidae). Entomology Circular (Gainesville), 387: 2. Florida Dept. Agric. & Consumer Serv., Div. Plant Ind. http://www.fl-dof.com/publications/fh_pdfs/Sycamore%20Lace%20Bug.pdf.
- HALBERT, S. E., AND MEEKER, J. R. 2007. The sycamore lace bug, *Corythucha ciliata* (Say) (Hemiptera: Tingidae). Entomology Circular (Gainesville) EENY-190, University of Florida. 2 pp. Available at http://entomology.ifas.ufl.edu/creatures.
- JU, R. T., WANG, F., AND LI, B. 2011a. Effects of temperature on the development and population growth of the sycamore lace bug, *Corythucha ciliata*. J. Insect Sci. 11, 16 available online: insectscience.org/11.16.

- JU, R. T., XIAO, Y. Y., AND LI, B. 2011b. Rapid cold hardening increases cold and chilling tolerances more than acclimation in the adults of the sycamore lace bug, *Corythucha ciliata* (Say) (Hemiptera: Tingidae). J. Insect Physiol. 57: 1577-1582.
- JU, R. T., XIAO, Y. Y., WANG, F., AND LI, B. 2010. Supercooling capacity and cold hardiness of the adults of the sycamore lace bug, *Corythucha ciliata* (Hemiptera: Tingidae). CryoLetters 31: 445-453.
- LI, Q., AND WAN, J. M. 2005. SSRHunter: development of a local searching software for SSR sites. Hereditas 27: 808-810.
- MARSHALL, T. C., SLATE, J., KRUUK, L. E. B., AND PEM-BERTON, J. M. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. Mol. Ecol. 7: 639-655.
- MAZZON, L., AND GIROLAMI, V. 2000. The sycamore lace bug. Sherwood-Foresteed Alberi Oggi 6: 27-28.
- PELLIZZARI, G., AND MONTA, L. D. 1997. The insect pests introduced into Italy between 1945 and 1995. Informatore Fitopatologico 47(10): 4-12.
- PRADO, C. E. 1990. Presence in Chile of Corythucha ciliata (Say) (Hemiptera: Heteroptera: Tingidae). Rev. Chilena Entomol. 18: 53-55.
- RAYMOND, M., AND ROUSSET, F. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J. Heredity 86: 248-249.
- RICE, W. R. 1989. Analyzing tables of statistical tests. Evol. 43: 223-225.
- STREITO, J. C. 2006. Note sur quelques espèces envahissantes de Tingidae: Corythucha ciliata (Say, 1932), Stephanitis pyrioides (Scott, 1874) et Stephanitis takeyai Drake & Maa, 1955 (Hemiptera Tingidae). Entomologiste 62: 31-36.
- TOKIHIRO, G., TANAKA, K., AND KONDO, K. 2003. Occurrence of the sycamore lace bug, *Corythucha ciliata* (Say) (Heteroptera: Tingidae) in Japan. Res. Bull. Plant Prot. Serv. 39: 85-87.
- VAN OOSTERHOUT, C., HUTCHINSON, W. F., WILLS, D. P. M., AND SHIPLEY, P. 2004. MICRO-CHECKER: Software for identifying and correcting genotyping errors in microsatellite data. Mol. Ecol. Notes 4: 535-538.
- WULF, A., AND BUTIN, H. 1987. Diseases and pests of plane tree. Nachrichtenblatt des Deutschen Pflanzenschutzdienstes 39: 145-148.
- YOON, C., YANG, J. O., KANG, S. H., AND KIM, G. H. 2008. Insecticidal properties of bistrifluron against sycamore lace bug, *Corythucha ciliata* (Hemiptera: Tingidae) J. Pesticide Sci. 33: 44-50.
- ZANE, L., BARGELLONI, L., AND PATARNELLO, T. 2002. Strategies for microsatellite isolation: a review. Mol. Ecol. 11: 116.