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PRIMER NOTE

Twelve polymorphic microsatellite loci from the Asian citrus psyllid, *Diaphorina citri* Kuwayama, the vector for citrus greening disease, huanglongbing

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Abstract

Twelve polymorphic microsatellite markers were developed from microsatellite-enriched DNA libraries and mined from an expressed sequence tags library of *Diaphorina citri*, the vector of the citrus greening disease (huanglongbing). Analysis of 288 individuals from Florida, Texas, and Brazil showed that allelic diversity ranged from three to eight alleles per locus and observed and expected heterozygosities ranged from 0.014 to 0.569 and from 0.052 to 0.653, respectively. These variable microsatellite loci can provide means for assessing overall genetic variation and migration patterns for this agriculturally important pest species. This information can be used to aid in developing successful management strategies.

Keywords: citrus greening disease, citrus, *Diaphorina citri*, huanglongbing, microsatellite, psyllid

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The Asian citrus psyllid (*Diaphorina citri* Kuwayama, Hemiptera: Psyllidae) is a phloem-feeding insect native to Asia and the Far East (Halbert & Manjunath 2004). In Asia, the current range of *D. citri* includes China, India, Myanmar, Taiwan, the Philippines, Malaysia, Indonesia, Sri Lanka, Thailand, Nepal, Cecum, Hong Kong, and the Ryukyu Islands. In the Middle East, *D. citri* has been reported in Afghanistan, Pakistan and Saudi Arabia. There are also reports of this species on the Indian Ocean islands of Reunion and Mauritius (Catling 1973). Through human movement of infested plant material among countries, the pest now has an expanded range that includes North, Central and South America and the Caribbean (Núñez 2004). Its discovery in the continental USA occurred in 1998 (Bové 2006).

The Asian citrus psyllid is a serious pest of citrus production worldwide because it vectors phloem-limited bacterial citrus pathogens, *Candidatus* Liberibacter *asiaticus*, *C.* L. *africanus*, and *C.* L. *americanus*, which cause citrus greening or huanglongbing (Capoor *et al*. 1967; Martinez & Wallace 1967). The invasive movement throughout the world and the horticultural importance of this insect has spurred research on *D. citri* that includes plant host range, parasitoid interactions and vector–pathogen interactions. All of these areas may be influenced by the genetic diversity of *D. citri*; however, almost no information is available regarding *D. citri* genetic structure. Understanding genetic diversity would provide basic information about the nature of its global spread and determine whether '*Candidatus* Liberibacter' vectoring or parasitoid interactions are influenced by underlying genetic differences among *D. citri* populations.

In an attempt to characterize the worldwide genetic diversity of *D. citri*, we have obtained *D. citri* microsatellite markers through two methods. First, six microsatellites were identified from a microsatellite-enriched library constructed following the procedure of Carleton *et al*. (2002) using primers for the isolation of CA, ATG, and GATA repeats. Second, six more microsatellites were mined from an expressed sequence tags (EST) database available in GenBank (Hunter *et al*. 2007).

DNA was extracted from whole individual psyllids using Cartagen's (www.cartagen.com) rapid homogenization

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for plant leaf DNA amplification (catalogue no. 20700-500). The first six microsatellites listed in Table 1 were identified from the *D. citri* EST library using the MISA PERL SCRIPT [Thiel *et al*. 2003; MISA (microsatellite identification tool) available at http://pgrc.ipk-gatersleben.de/misa]. PRIMER 3 (Rozen & Skaletsky 2000) was then used to design primer sites for each of the 12 loci (Table 1). The polymerase chain reactions (PCRs) were composed of 6.25 μL Immomix (Bioline, catalogue no. BIO-25019), 5.25 μL water, 0.25 μL forward primer (10 pmol), 0.25 μL reverse primer (10 pmol), and 1 μL DNA template. A fluorescein-derivative (FAM, MWG Biotech) labelled primer was incorporated for genotyping reactions. For primers 1–6 the forward primer was labelled and for primers 7–12 the reverse primer was labelled. The 13 μL PCRs used a thermal regime of 94 °C for 7 min followed by 35 cycles of 30 s at 94 °C, 30 s at optimal annealing temperature (Table 1), 1 min at 72 °C, and a final step of 72 °C for 1 h. One microlitre of the FAM-labelled PCR product was added to a mix of 13.75 μL formamide (Ameresco, code size K295–100 mL) and 0.25 μL GENESCAN 500 ROX Size Standard (part no. 401734) and loaded onto an Applied Biosystems 3730XL DNA analyser.

Individuals from 20 collection sites were included from throughout Florida, one population from Texas and one population from Brazil. Two hundred and eighty-eight individual psyllids were genotyped and their amplicons were sized and characterized using GENEMAPPER 4.0 (Applied Biosystems).

Observed and expected heterozygosity values ranged from 0.014 to 0.569 and from 0.052 to 0.653, respectively, for 288 individuals. The statistical test for Hardy–Weinberg equilibrium was carried out in GENEPOP version 3.3 (Raymond & Rousset 1995). Loci Dci01, Dci02, Dci03, Dci05, Dci10, Dci12 (Palm Beach County, Florida, $N = 20$, Table 1) exhibit significant deviations from Hardy–Weinberg expectations ($P < 0.05$) after sequential Bonferroni correction for multiple tests. All loci deviated significantly from Hardy–Weinberg equilibrium when 288 individuals were included. All departures from Hardy–Weinberg equilibrium indicate heterozygote deficit consistent with the presence of null alleles. Genotypic linkage disequilibrium between all pairs of loci was checked by contingency exact test of a clone-corrected material using GENEPOP version 3.4 (Raymond & Rousset 1995). No significant departure from the null hypothesis was detected.

Table 1 Characteristics of microsatellite loci in *Diaphorina citri*. †indicates FAM labelled primer; imp, imperfect repeat; T_a, optimized annealing temperature; N_a, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity, P_{HW}, Hardy–Weinberg equilibrium P values. Results are presented first from a single population from Palm Beach County, Florida ($N = 20$) and then $N = 288$ from all individuals included from all locations. The asterisk indicates deviation from Hardy–Weinberg equilibrium

Locus	Primer sequence	Genbank Accession no.	T_a	Repeat sequence	Size range	Clone size	$N_{a\,(20)}$	$H_{O\,(20)}$	$H_{E\,(20)}$	$P_{HW\,(20)}$	N_a	H_O	H_E
Dci01	F: TTTCGAAGACCCAAACAACC†	EF186921	54	$(TTTA)_3(TTA)_6$	197–222	200	5	0.676	0.500	0.031*	8	0.569	0.587
	R: TCCTGCCTTCCATCTCTCTT												
Dci02	F: GGTGAACGAAAACAAAGGAGA†	EF786922	54	$(CTATT)_4$	190–200	200	3	0.527	0.250	0.008*	3	0.292	0.528
	R: CGGGGTGATAGGTCTCTAGC												
Dci03	F: GAAGGATGCCAAGAAAGCAC†	EF186923	54	$(GCT)_8N_{21}(GCT)_4$	209–227	230	4	0.627	0.150	0.000*	6	0.392	0.472
	R: TCGGCACATTCTTCTTCACA												
Dci04	F: CCAGCGTGCTAAAACTCAAA†	EF186924	47	$(CTT)_4$	294–304	300	4	0.528	0.400	0.160	6	0.424	0.188
	R: TTGATGCAAAAAGGAACAAAAA												
Dci05	F: CCCCCAAGAGACAAGTTCAA†	EF186920	45	$(TGA)_5$	330–340	338	3	0.487	0.750	0.018*	5	0.538	0.361
	R: TCCTTGTTCAACGACCATGA												
Dci06	F: GGCAATCAATATGGATGCTCA†	EF186925	45	$(ATT)_{13}$	283–300	300	3	0.415	0.150	0.000*	7	0.139	0.347
	R: CAACCTGTTTTGTTGGTTGG												
Dci07	F: CGGCAGTCCCAGTAGGTAAG	EF185169	60	$(TAGG)_3$	263–275	272	3	0.388	0.250	0.138	4	0.313	0.495
	R: GAATTCGTCGCTTCCCAATA†												
Dci08	F: AAGGAAGGACGGGCTAAAAA	EF186915	55	$(CAA)_{15}$	160–288	288	5	0.739	0.600	0.198	6	0.542	0.653
	R: AATCCAGGAACAGCCATTCA†												
Dci09	F: CATCCAAAGGAGCGACACTT	EF186916	60	$(GAA)_{24}$	174–186	184	3	0.089	0.050	0.112	4	0.014	0.090
	R: TCCTTTTCCCCTTCTCCTGT†												
Dci10	F: GAAGAAAGAGGGGAAGAGGAA	EF186917	60	$(GAA)_{26}$imp	210–262	253	3	0.529	0.400	0.029*	5	0.233	0.410
	R: CGACTTCACCAGGAGAGAAAG†												
Dci11	F: GGTCTGCCAACTTGTCCATT	EF186918	45	$(GA)_{10}(GGGA)_2(GA)_4$	242–260	239	3	0.094	0.050	0.113	3	0.028	0.052
	R:CCCCCTCTTACCTCGTCTCT†												
Dci12	F: GGTGAGTGCTCTCCTTTCCA	EF186919	60	$(CT)_4$	211–223	211	3	0.564	0.150	0.000*	6	0.178	0.521
	R: CTGCAGACATCCTGCATACAA†												

The microsatellite markers are currently being used to investigate population genetics and phylogeography of *D. citri* from global collections. One aim is to track possible geographical origin(s) of the introductions (e.g. one or multiple invasions) into the USA, especially Florida. This information will be used in research targeting the design of appropriate management strategies and monitoring of any expansion of the range of this agricultural pest.

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