

## 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

Received 21 January 2007; revision accepted 13 April 2007

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, Ceylon, 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  $\mu$ L Immomix (Bioline, catalogue no. BIO-25019), 5.25  $\mu$ L water, 0.25  $\mu$ L forward primer (10 pmol), 0.25  $\mu$ L reverse primer (10 pmol), and 1  $\mu$ L 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  $\mu$ L 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  $\mu$ L formamide (Amersco, code size K295–100 mL) and 0.25  $\mu$ L 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	$N_a$ ( $N=20$ )	$H_O$ ( $N=20$ )	$H_E$ ( $N=20$ )	$P_{HW}$ ( $N=20$ )	$N_a$	$H_O$	$H_E$
Dci01	F: TTTCGAAGACCCAAACAACCT† R: TCCTGCCTTCCATCTCTCTT	EF186921	54	(TTTA) <sub>3</sub> (TTA) <sub>6</sub>	197–222	200	5	0.676	0.500	0.031*	8	0.569	0.587
Dci02	F: GGTGAACGAAAACAAAGGAGA† R: CGGGGTGATAGGTCTCTAGC	EF786922	54	(CTATT) <sub>4</sub>	190–200	200	3	0.527	0.250	0.008*	3	0.292	0.528
Dci03	F: GAAGGATGCCAAGAAAGCAC† R: TCGGCACATCTCTTTCACA	EF186923	54	(GCT) <sub>8</sub> N <sub>21</sub> (GCT) <sub>4</sub>	209–227	230	4	0.627	0.150	0.000*	6	0.392	0.472
Dci04	F: CCAGCGTGCTAAAACTCAAA† R: TTGATGCAAAAAGGAACAAAA	EF186924	47	(CTT) <sub>4</sub>	294–304	300	4	0.528	0.400	0.160	6	0.424	0.188
Dci05	F: CCCCCAAGAGACAAGTTCAA† R: TCCTTGTTC AACGACCATGA	EF186920	45	(TGA) <sub>5</sub>	330–340	338	3	0.487	0.750	0.018*	5	0.538	0.361
Dci06	F: GGCAATCAATATGGATGCTCA† R: CAACCTGTTTTGTTGGTTGG	EF186925	45	(ATT) <sub>13</sub>	283–300	300	3	0.415	0.150	0.000*	7	0.139	0.347
Dci07	F: CGGCAGTCCCAGTAGGTAAG† R: GAATTCGTTCGCTTCCCAATA†	EF185169	60	(TAGG) <sub>3</sub>	263–275	272	3	0.388	0.250	0.138	4	0.313	0.495
Dci08	F: AAGGAAGGACGGGCTAAAA† R: AATCCAGGAACAGCCATTCA†	EF186915	55	(CAA) <sub>15</sub>	160–288	288	5	0.739	0.600	0.198	6	0.542	0.653
Dci09	F: CATCCAAAGGAGGACACTT† R: TCCTTTTCCCTTCTCTCTGT†	EF186916	60	(GAA) <sub>24</sub>	174–186	184	3	0.089	0.050	0.112	4	0.014	0.090
Dci10	F: GAAGAAAGAGGGGAGAGGAA† R: CGACTTCACCAGGAGAGAAAG†	EF186917	60	(GAA) <sub>26</sub> imp	210–262	253	3	0.529	0.400	0.029*	5	0.233	0.410
Dci11	F: GGTCTGCCAACTTGTCCATT† R: CCCCCTCTTACCTCGTCTCT†	EF186918	45	(GA) <sub>10</sub> (GGGA) <sub>2</sub> (GA) <sub>4</sub>	242–260	239	3	0.094	0.050	0.113	3	0.028	0.052
Dci12	F: GGTGAGTGTCTCTCTTCCA† R: CTGCAGACATCTGCATACAA†	EF186919	60	(CT) <sub>4</sub>	211–223	211	3	0.564	0.150	0.000*	6	0.178	0.521

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.

### Acknowledgements

The authors wish to thank Paula Hall, Bryan Baclaski, Laurie Keidong and John Prokop for technical assistance. Fernando L. Consoli, Mathew Hentz, Chris Knox, and Kathy Moulton for collections of *Diaphorina citri*. The University of St Thomas Cullen Trust–Harry K. Smith Chair in Biology and University of St Thomas Faculty Development Grant provided financial support for R.C.R., R.A.B. and D.R.F.

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