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Short Communication

Isolation and characterization of polymorphic microsatellite loci in *Aleurodicus dispersus* (Hemiptera, Aleyrodidae)

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ABSTRACT. Ten microsatellite markers were isolated and characterized from *Aleurodicus dispersus*, the spiraling whitefly, an exotic pest species that is considered to be one of the most serious agricultural pests on Hainan Island, China. The polymorphism of these loci was examined in individual whiteflies from Hainan Island and from the Canary Islands. All loci were polymorphic, with two to four alleles per locus. Mean observed and expected heterozygosity values were 0.773 and 0.585, respectively. These microsatellite markers provide powerful tools for ecological, epidemiological and population genetic studies on this highly invasive insect. Thirty insects were collected

and studied at each location. There were no differences between the two locations.

Key words: *Aleurodicus dispersus*; Microsatellite loci; Population genetics; Invasive insect

The whitefly, *Aleurodicus dispersus* (Russell, 1965), is an insect pest of many tropical and sub-tropical crops. It lays eggs in a typical spiral pattern, so it is more commonly known worldwide as 'spiralling whitefly'. This whitefly is native to the Caribbean region and Central America (Russell, 1965). The insect is highly polyphagous, and has been recorded on more than 100 plants belonging to 38 genera and 27 families, including many vegetables, ornamental and fruit crops (Waterhouse and Norris, 1989; Charati et al., 2003). In 2006, the spiralling whitefly was first detected in Lingshui, Hainan Province, China (E 110°02'26.8", N18°31'52.4") (Yu et al., 2007). Since then, the whitefly has spread throughout Hainan Island and is considered to be one of the world's major agricultural pest groups (Han et al., 2008). Adults and nymphs of the whitefly cause direct feeding damage by piercing and sucking of sap from foliage. The "honeydew" excreted is a substrate for a sooty-mold fungus that interferes with photosynthesis, ultimately causing leaf shedding and reduced growth rate (Alam et al., 1998). The spiralling whitefly has a dramatic negative effect on the agricultural industry, causing a significant amount of monetary loss to these enterprises. Here, we report on the development 10 polymorphic microsatellite loci isolated from *A. dispersus*, which will be used to analyze the population genetics of *A. dispersus*.

The specimens of *A. dispers*us were sampled from Hainan Island and the Canary Islands. Adults were collected from the plants *Pterocarpus indicus* and *Cocoloba uvifera* and transferred to 1.5-mL microcentrifuge tubes containing 95% alcohol, then taken to the laboratory and preserved at -20°C. To avoid microsatellite hemizygotes on the X-chromosome, and amplification of Y-chromosomal rDNA, only females were selected for DNA extraction and subsequent analyses.

The method used for DNA extraction from whitefly individuals was described by Sambrook and Russell. A subgenomic library, enriched for microsatellites, was generated using a modified protocol described by Bloor et al. (2001). Total genomic DNA extracted from 15 whitefly individuals was digested with the restriction enzyme *Sau*3AI. Digested fragments were size-selected between 400 and 1000 bp and were purified from a 1.2% TAE agarose gel using the Axygen Gel Extraction Kit. Double-stranded linkers (adaptorA: 5'-GGCCAGAGACCCCAAGCTTCG-3'; phosphorylated adaptorB: 5'-PO_4GATCC GAAGCTTGGGGTCTCTGGCC-3') were ligated to the digested DNA fragments. Biotinylated nucleotide probes $(AC)_{12}$, $(AG)_{12}$ and $(TTC)_8$ (Sangon) were hybridized to the linker-ligated DNA. DNA containing nucleotide microsatellites were selectively selected using Streptavidin MagneSphere Paramagnetic Particles (Promega). Enriched fragments were recovered via polymerase chain reaction (PCR). Each PCR consisted of approximately 10 ng microsatellite-enriched genomic DNA, 10X PCR buffer (200 μM KCl, 100 μM Tris), 2.5 mM each dNTP, 25 mM $MgCl_2$, 10 μM linker-F as primer, and 5 U Taq DNA polymerase (TaKaRa). PCR amplifications consisted of 95°C for 5 min, followed by 30 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 1 min, and a final cycle of 10 min at 72°C. PCR products were ligated into a pMD18-T vector (TaKaRa) and used to transform DH5α competent cells.

Of 480 screened colonies, 212 (44.2%) were positive for nucleotide repeats. A large number of redundant clones were observed when sequencing all positive clones. Microsatellite primers were designed based on nucleotide sequence regions flanking microsatellites using the Oligo 6 software. A total of 32 PCR primer pairs were developed with 10 successfully amplifying microsatellite loci. These loci were tested in whitefly to determine if a polymorphic product could be reliably amplified and to determine the optimum annealing temperature. Fifteen-microliter PCRs consisted of 1-10 ng genomic DNA, 10X PCR buffer, 2.5 mM of each dNTP, 25 mM $MgCl_2$, 10 µm of each primer, and 5 U Taq DNA polymerase (TaKaRa). The PCR amplification program was 95°C for 5 min, followed by 30 cycles of 95°C for 30 s, 50-60°C for 30 s, 72°C for 1 min, and a final extension of 72°C for 7 min. PCR products were resolved on 8% denaturing polyacrylamide gels (Wu et al., 2008) and 11 microsatellite loci were proved to be polymorphic (Table 1).

Table 1. Characterization of microsatellite markers for *Aleurodicus dispersus*.

Locus	Primer sequences (5'-3')	Repeat motif	T_a (°C)	Size range alleles (bp)	No. of alleles	H_O	H_E	GenBank No.
AD2	F: CTCCATGCTGTTCTTGAT R: CAGGCACCTATAAACCG	$(CT)_{16}$	53	251-275	3	0.867	0.660	HQ243664
AD3	F: CGACGATTTATACGAACGCA R: ACACGAATTGAAGTTGAGGG	$(TC)_{20}$	53	241-261	3	0.800	0.659	HQ243665
AD5	F: CGTCTATTCTTACAGCCACA R: ACCTGCCAGTAGTTTTGA	$(CT)_{22}$	53	248-252	2	0.933	0.541	HQ243666
AD12	F: TCACCAGACCCCACCCACCGAC R: CACAAATGCTCCCAATACC	$(AC)_{11}$	53	242-246	3	0.867	0.572	HQ243667
AD13	F: CGACAACAGGAAACAACGGT R: AAACTGGCAAAGGCGGAC	$(AG)_{11}$GGAGG $(GA)_{10}$	55	308-320	2	0.833	0.541	HQ243668
AD15	F: CATTGAGTGGGTCCATTGTT R: CGGGAAATGATGTCAGGAGG	$(TC)_{11}C(TC)_7$	55	278-290	4	0.767	0.767	HQ243669
AD20	F: TGCGGGCTCCAACTATGT R: TGTGGTCGGCAGGATTTA	$(CT)_{11}$	53	174-194	4	0.767	0.771	HQ243670
AD21	F: CGTTGAATCCCTCTACTCT R: GCTGCCATCTGTGAAATA	$(CT)_{20}$	53	149-151	2	0.933	0.541	HQ243671
AD23	F: GTAATGACCGTGCTAAGT R: CTTTGAGATTTTGCGAGC	$(TCT)_{10}$	53	141-147	3	0.867	0.669	HQ243672
AD26	F: TTAAATTGCTCGCATGGC R: TAAAATAGGCTTCAGACCC	$(TTC)_{14}$	53	191-194	2	0.100	0.129	HQ243673

T_a = annealing temperature; H_O and H_E = observed and expected heterozygosities, respectively.

Genepop version 3.4 (Raymond and Rousset, 1995) was used to estimate Hardy-Weinberg equilibrium (HWE) and linkage-disequilibrium of the 10 microsatellite loci. The observed heterozygosity (H_O) and expected heterozygosity (H_E) of each polymorphic locus were calculated using the CERVUS 2.0 program (Marshall et al., 1998). Furthermore, we used MicroChecker version 2.2.3 (van Oosterhout et al., 2004) to test for the presence of null alleles.

The number of alleles per locus ranged from 2 to 4, with an average of 2.8 alleles per locus. The H_O ranged from 0.100 to 0.933 (mean = 0.773), and the H_E from 0.129 to 0.771 (mean = 0.585) (Table 1). This low level of genetic variability may reflect the bottleneck effect associated with the introduction of this species to Hainan Island. Alternatively, insecticides used against *A. dispersus* could be implicated in selection pressure imposed by widespread use. After sequential Bonferroni's correction, significant deviation from HWE was not found in all loci. With the exception of AD2-AD3, AD15-AD20 and AD23-AD26 ($P < 0.05$), none of the loci had significant linkage disequilibrium.

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