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PRIMER NOTE

Polymorphic microsatellite loci for Diaprepes root weevil (*Diaprepes abbreviatus* L.)

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Abstract

***Diaprepes abbreviatus* (L.) is an insect pest of the US agriculture that originated from the Caribbean islands. Larvae are of economic importance in both nursery and commercial citrus plantings due to root feeding. Eight polymorphic microsatellite markers were developed from a $(CA)_n$-enriched genomic library of Diaprepes root weevil. Three to eight alleles were observed for each locus during screening of 17 to 25 individuals. Observed and expected heterozygosities ranged from 0.182 to 0.864 and 0.587 to 0.835, respectively. These markers will be useful to characterize the genetic variability and track the migration patterns of populations of *D. abbreviatus* (L.).**

Keywords: citrus pest, *Diaprepes abbreviatus*, Florida, microsatellite

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Diaprepes abbreviatus (L.) is an important insect pest for Florida and US agriculture. Adults feed on leaves and roots, and larvae feed on many agronomic and native host plants in Florida and several Caribbean island nations (Jones 1915; Wolcott 1933, 1934, 1936; Fennah 1942; Blackwelder 1947; Woodruff 1968; Simpson *et al*. 1996). In the United States, economically important hosts include citrus, corn, cotton, potatoes, tobacco, sugar cane, soybeans and many ornamental plants (Simpson *et al*. 1996). This species has now been identified in California, Minnesota and Texas (S.E. Simpson, FLDACS, personal communication). As part of a project to investigate the genetic variability and population structure of the current Florida distribution of Diaprepes root weevil, we have isolated eight polymorphic microsatellite loci from *D. abbreviatus*.

DNA was isolated from 25 specimens originating from seven localities in Florida using the DNeasy Tissue Kit (QIAGEN) following the protocol for animal tissue. An enriched genomic DNA library was prepared with DNA extracted from one specimen using protocols modified from Kandpal *et al*. (1994) and Fleischer & Loew (1996). Briefly, genomic DNA was digested with *Sau*3AI restriction enzyme. Fragments within 400–1500 bp were excised and purified from a low melt agarose gel, and ligated to *Sau*3AI linkers (*Sau*-L-A: 5′-GCGGTACCCGGGAAGCTTGG-3′; and *Sau*-L-B: 5′-GATCCCA-AGCTTCCCGGGTACCGC-3′). Excess linkers were removed using an Ultrafree–MC Centrifugal Filter Unit (Millipore). Linker-ligated fragments were then amplified by polymerase chain reaction (PCR) using the *Sau*3AI linker: Sau-L-A.

A library enriched for $(CA)_n$ repeats was created by initially hybridizing the DNA fragments to a biotinylated nucleic acid probe [5′-$(CA)_{15}$TATAAGATA-biotin], followed by capture of probe-targeted fragments using VECTREX Avidin D (Vector Laboratories). Genomic fragments enriched for $(CA)_n$ repeats were PCR-amplified using *Sau*3AI linker primers, ligated into a TOPO TA pCR 2.1 vector (Invitrogen) and transformed into One Shot *Escherichia coli* cells (Invitrogen). Colonies were screened on magnagraph nylon transfer membranes (Osmonics Laboratory Products) and hybridized with a chemiluminescent $(CA)_n$ probe (LifeCodes). Detection of colonies containing $(CA)_n$ repeats was accomplished using Lumi-Phos 480 (LifeCodes). Colonies containing repeats were grown overnight in LidBac Safe-Lock tubes (Eppendorf). Plasmid DNA was purified using a QIAprep Spin Miniprep kit (QIAGEN) and sent to the Interdisciplinary Center for Biotechnology Research (ICBR) DNA Sequencing Core of the University of Florida to be sequenced on an ABI 377 automated sequencer.

Thirty-seven recombinant colonies were sequenced and 19 pairs of primers were designed from flanking sequences

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using OLIGO version 6.0 software (Molecular Biology Insights Inc.). Eight pairs of primers were found to be polymorphic. For these primers, final PCR amplifications were done in 10 μL reactions containing 1× PCR buffer, 0.24 μM of each primer, 200 μM each dNTP, 0.4 U *Taq* (Sigma Jumpstart), at least 20 ng template DNA, and various $MgCl_2$ concentrations indicated in Table 1. All PCRs began with a 94 °C denaturization (5 min), followed by a primer-specific number of cycles at 94 °C (30 s), primer-specific annealing temperature (30 s), 72 °C (50 s) and a final extension of 72 °C (20 min). The number of PCR cycles and the annealing temperatures are presented in Table 1. PCR products were separated on a 6% denaturing acrylamide gel to determine polymorphism of each locus. The upper primer of each polymorphic pair was 5′-fluorescently labelled with either FAM or HEX (MWG Biotech) (Table 1). All PCR products were run on an ABI 377 automated sequencer using ROX 500 size standard and analysed using GENESCAN AND GENOTYPER software (Applied Biosystems). Each polymorphic locus was screened with a minimum of two individuals per locality with a minimum total of 17 individuals. Characteristics of the polymorphic loci were calculated using ARLEQUIN version 2 (Schneider *et al*. 2000) and are included in Table 1. The number of alleles at each locus ranged from three to eight and observed heterozygosities ranged from 0.182 to 0.864 (Table 1). When pooling all the individuals from different Floridian populations, departure from Hardy–Weinberg equilibrium (HWE) was observed in four out of eight cases due to a heterozygote deficit. Since the tested individuals originated from different localities, we performed HWE analysis per population using only the sites with data from four or more individuals. Two localities showed departure from HWE, one at loci DP-G2 and DP-G8 and another at locus DP-K3. Linkage equilibrium (LD) was tested per population, in this case, only one locality rejected LD for one out of 15 pairwise exact tests between loci DP-A12 and DP-E2 ($P > 0.05$). Since four to eight individuals were used in both HWE and LD analyses, these results should be considered preliminary.

The microsatellite loci characterized here will allow the investigation of the genetic variability and population structure of the Diaprepes root weevil in Florida, its possible geographical origin(s) from the Caribbean and its pattern of invasion(s) (e.g. one or multiple invasions). In addition, data from these microsatellite loci will contribute to the generation of information needed to design appropriate management protocols and will allow monitoring the expansion of this pest into western and northern areas of the United States.

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Table 1 Characterization of eight polymorphic *Diaprepes abbreviatus* microsatellite loci

Locus (GenBank ID)	Primer sequences (5′–3′)	Repeated motif	T_a (°C)	No. of cycles	$MgCl_2$ (mM)	Sample size	No. of alleles	Allele size range	H_O / H_E	HW
DP-A12	F: FTCTCTTGGTTTGTTTCGTGG	$(GT)_{45}$	53.6	35	2.5	25	5	306–338	0.6400	Yes
(AY788999)	R: CGCATACTCCGCACTTAG								0.6955	
DP-E1	F: FGTTGAGCCTTGTGTTGATATGT	$(GT)_{48}$	58	35	3.0	17	6	295–327	0.3529	No
(AY789000)	R: AATAACCCTTCATTAGAACC								0.6881	
DP-E2	F: FGCATCATTTACGCCTGTT	$(CA)_{29}CCTA(CA)_6$	61.4	35	2.5	22	8	313–329	0.8636	Yes
(AY789001)	R: CATTTTCTTGAAACCGACG	$CCTA(CA)_3AA(CA)_{21}$							0.8351	
DP-E5	F: HTACGTCATAAGTTCAGTTCA	$(CA)_{15}CG(CA)_6$	58.3	35	3.0	18	7	315–351	0.3889	No
(AY789002)	R: CGAAAAGAAAACAGATGTAA	$CGCAA(GCAC)_{11}$							0.8286	
DP-G2	F: HCTAGCAGGGCAAGTACATTA	$(GT)_{22}$	58.3	35	3.0	18	4	380–390	0.2222	No
(AY789003)	R: TTTTGCGACCGAACTTATC								0.6794	
DP-G8	F: HCGAGTTTTGCGTTCTTATT	$(CA)_{19}CCATTA(CA)_4$	57.8	35	1.5	23	7	430–458	0.4348	Yes
(AY789004)	R: ATGGAATCAATGGACGACC	$CT(CA)_4$							0.6512	
DP-H1	F: HTTTAACAAACGGTGACTGG	$(GT)_{15}$	53/57.2*	5/35*	2.5	20	3	309–331	0.2500	Yes
(AY789005)	R: AGATACTTCCGATTGGTTAGA								0.5731	
DP-K3	F: FCAGCGAAAGTTGGATTAAG	$(GT)_{28}(GA)_{15}$	62	35	3.0	22	8	223–269	0.1818	No
(AY789006)	R: CCGTATGATTTCGTCAGTGGA								0.7590	

Fluorescent primer labels: F, FAM; H, HEX; T_a, annealing temperature; * indicates loci where a 'touchdown' cycling profile was used with initial annealing temperature of first set of cycles/annealing temperature of second set of cycles. Number of cycles during the first/and second set of cycles; H_O, observed heterozygosity; H_E, expected heterozygosity. Heterozygosity values were estimated based on Nei (1987); HW, compliance with Hardy–Weinberg equilibrium estimated based on Guo & Thompson (1992) and Levene (1949), test including all Floridian individuals.

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