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PERMANENT GENETIC RESOURCES

Identification and characterization of 15 polymorphic microsatellite loci in the western drywood termite, *Incisitermes minor* (Hagen)

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Abstract

Despite recognition of the western drywood termite, *Incisitermes minor* (Hagen), as one of the most economically important and destructive termite species in the USA, both its population and colony breeding structure genetically remain unclear. Here, we present primer sequences and initial characterization for 15 polymorphic microsatellite loci. In a sample of 30 individuals, representing six geographically distinct locations collected in California, USA, three to 15 alleles were detected segregating per locus. Within a single population observed heterozygosity ranged from 0.050 to 0.866.

Keywords: dinucleotide microsatellite, drywood termite, *Incisitermes*, tetranucleotide microsatellite, trinucleotide microsatellite

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The western drywood termite, *Incisitermes minor* (Hagen), is recognized as one of the most economically important and destructive termite species in the USA (Su & Scheffrahn 1990). Although native from northwest Mexico to southwest USA (Light 1934), due to the ease of both intra- and intercontinental movement of infested furniture and timbers, infestations have been documented outside their native range (Hathorne *et al*. 2000; Xie *et al*. 2000; Indrayani *et al*. 2005). Given the vast economic impact of this species (Rust *et al*. 1988), the understanding of population genetic structure and breeding systems is fundamental to the formulation of effective management strategies. Here, we present primer sequences, polymerase chain reaction (PCR) conditions, and initial characterization of the genetic variation for 15 microsatellite markers.

The protocol employed for microsatellite isolation essentially followed that described by Dopman *et al*. (2004), with minor modifications. Linker-ligated genomic DNA fragments were enriched for microsatellite loci using biotinylated dimer, trimer and tetramer repeat motif probes, described by Perera *et al*. (2007). Following capture of microsatellite-enriched fragments and subsequent PCR amplification using the SNX forward primer, PCR products were then cloned using the TOPO TA cloning kit (Invitrogen) and colonies containing recombinant DNA identified by the disruption of β-galactosidase activity. Recombinants were individually picked and transferred to Falcon 48-well cell culture plates (Becton Dickson) and incubated in LB broth at 37 °C for 48 h. To confirm the presence of insert DNA from a single clone, 1.5 μL of DNA from the bacterial cell culture was then used as template and PCR-amplified using the vector M13 forward and reverse primers following the protocol described by Glenn & Schable (2005). A total of 55 clones containing inserts within the desired size range (200–600 bp) were sequenced using the M13 forward primer at the Genome Research Laboratory at North Carolina State University. PCR primers were designed for 27 loci using the GENEFISHER software (Giegerich *et al*. 1996).

Primer pairs were optimized using five individual adult *I. minor* collected from each of six sample locations (North Hollywood; Santa Cruz; Los Angeles; Oakland; Irvine; Fresno) across California, USA. PCRs were carried out in 12 μL volumes, each containing 1× PCR buffer, 2.5 mM $MgCl_2$, 100 μM dNTPs, 50 ng DNA template, 0.3 U *Taq* DNA Polymerase (Apex), and double-distilled water to 12 μL. Primer concentration varied between 0.1 to 2.5 pM with the forward primer of each end-labelled with an M13F-29 IRDye tag (LI-COR). Annealing temperatures ranged from 48 to 65 °C (see Table 1

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Table 1 Characteristics of 15 microsatellite DNA loci developed for the drywood termite (*Incisitermes minor*) and screened for a total of 30 specimens collected in California, USA: locus designation (GenBank Accession nos EU365818–EU365832), primer sequences, repeat motif, PCR conditions, number of alleles observed (N_a), average expected (H_E) and observed (H_O) heterozygosities, and range of PCR product sizes in (bp)

					Individuals collected at one location (n = 23)			Individuals (N = 30) collected across six locations (N = 5/location)		
Locus	Primer sequences (5′–3′)	Repeat motif	Annealing temperature (°C)	μM each primer	N_a	H_E	H_O	N_a	Size (bp)	GenBank Accession no.
DW-2	F: TGACAACGTATCAGCTGA R: ATCCCCCGTCCAAGGA	$(TC)_7$	60	0.5	2	0.200	0.200	3	241–247	EU365818
DW-3	F: CAGTCACTTCCTCTTCAGA R: GTAGCAGAAACTGTCAGA	$(CAT)_9$	60	0.25	3	0.529	0.750	8	325–352	EU365819
DW-4	F: CATGGAAGGCACAACTTGAGA R: CTGCCGATCTGCCTGTCA	$(GA)_{12}$	60	0.1	5	0.733	0.666	9	171–187	EU365820
DW-7	F: ATCTGGTCATGAGCCCTA R: TTGAACGGTCGCAGGA	$(ACTG)_8$ ... $(ACTG)_5$	60	0.25	2	0.446	0.238	4	223–235	EU365821
DW-8	F: TTCTGCTGCCAATGCA R: CGCCAATGACAACGACA	$(GTTGAG)_2(GTT)_{10}(GTC)_8$ $(GTT)_2$ ... $(GTC)_3$ ... $(GTC)_1$	65	0.5	5	0.866	0.866	9	301–348	EU365822
DW-11	F: TCTTAGTCCGTACGCAA R: CCCGATAATTCCGTCC	$(CTTT)_2(GTT)_9$	52	0.5	6	0.775	0.619	6	289–319	EU365823
DW-12	F: CTTGTACAGTGGGAGCAA R: TCGGTAGGACTACTTCGA	$(ATAG)_5ATAA(ATAG)_{12}$	55	0.5	7	0.823	0.750	15	209–305	EU365824
DW-14	F: CAAGCGCAGACTAACAGA R: TTTGCCGCGGTCACA	$(ACAT)_8$	57	0.25	4	0.681	0.739	4	202–214	EU365825
DW-20	F: GAGACAGCGAATGCAGA R: CTGGGTGCTTTAACTCCA	$(GAA)_{17}$	48	2.5	4	0.383	0.508	4	158–290	EU365826
DW-21	F: GCTCTTGTGAACACGGTA R: CTGTTCACAGTCTGGTGA	$(TGAG)_2(TG)_2(TGAG)_{10}$	55	0.5	6	0.659	0.681	8	170–238	EU365827
DW-27	F: CTTTTCACGGATGCCCTA R: ATTGCATAGCGGAGCAA	$(GAA)_6$	52	1.0	8	0.827	0.789	8	310–339	EU365828
DW-38	F: GGTGGAAAGCTACAGAGA R: ATTCGCTCCCTCACTCA	$(GAGT)_4$	52	2.5	2	0.050	0.050	4	189–213	EU365829
DW-39	F: AGTGAAGAAGGGGCAGA R: GACGGAATTCCACAACCA	$(GAA)_{14}AAG(AGG)_6$	52	2.5	8	0.787	0.316	15	141–210	EU365830
DW-40	F: GGGTTAAACACCCTTTCC R: GCCATCTTGTCAGTACTG	$(CTGT)_6$	52	0.25	2	0.487	0.522	4	147–163	EU365831
DW-46	F: TGGGCGATCTTCAGCA R: AGAAGCCCAAGGTCCAA	$(TCTA)_7CA(TGTC)_5$	55	1.5	6	0.740	0.565	10	145–185	EU365832

for details). PCR cycling conditions were comprised of an initial denaturation stage of 3 min at 95 °C, followed by 35 cycles each consisting of 30 s at 95 °C, 30 s at the ideal temperature for each primer set, and 30 s at 72 °C, with a subsequent terminal extension at 72 °C for 3 min, carried out using ABI 2720 thermal cyclers (Applied Biosystems). Following PCR, 4 μL of stop solution (95% Formamide, 20 mM EDTA, Bromophenol blue) was added to each 12 μL reaction. Reactions were subsequently denatured at 90 °C for 4 min, and 1 μL was loaded onto 25 cm 6% 1× TBE polyacrylamide gels, mounted on a LI-COR 4300 automated DNA sequencer. Loci were sized using a 70–400 bp standard (Microstep-20a, Microzone). Gels were run at a constant power of 40 W at 50 °C for 2 h. Results were analysed using GENEPROFILERsoftware (Scanalytics).

Fifteen primer pairs produced unambiguous PCR products demonstrating allelic variation. Locus characteristics are provided in Table 1. All loci were polymorphic in *I. minor*, with three to 15 alleles per locus observed across the six sample locations (Table 1). Observed heterozygosities ranged from 0.050 to 0.866 within a single population sampled in Fresno, California (n = 23). When tested across this single population, no evidence for linkage disequilibrium (GENEPOP version 3.3; Raymond & Rousset 1995) was detected among the 105 possible pairwise locus comparisons. Within this same population, loci did not deviate from Hardy–Weinberg equilibrium (GENEPOP).

Preliminary results confirm these markers yield sufficient within- and between-colony/population polymorphism for the resolution of patterns of dispersal, gene flow and colony breeding structure in *I. minor*.

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