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PRIMER NOTE

Microsatellite markers in the primitive termite *Mastotermes darwiniensis*

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Abstract

We developed 10 microsatellite loci in the primitive termite *Mastotermes darwiniensis*. The number of alleles per locus ranged from four to 15, and the expected heterozygosites spanned from 0.21 to 0.90, in a sample of 40 workers collected from the Northern Territory, Australia. We also determined that only two loci amplified in five other termite species. The low frequency of cross-amplification probably resulted from the high level of phylogenetic divergence between *M. darwiniensis* and the other taxa. Thus, although the loci are not widely applicable, they should prove effective in elucidating the genetic structure of *M. darwiniensis* populations.

Keywords: gene flow, Isoptera, population genetic structure, relatedness, single locus DNA markers, social insect

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The termite *Mastotermes darwiniensis* is the sole surviving representative of the family Mastotermitidae and is considered to be one of the most primitive members of the Isoptera (Watson & Gay 1991). *M. darwiniensis* colonies are typically headed by many secondary reproductives, which are presumed to mate with relatives in the nest. Consequently, the genetic structure of colonies is likely to be complex. Molecular markers would be beneficial in elucidating the structure of colonies, as well as understanding the extent of gene flow among larger geographical regions. In this study we report the development and characterization of 10 microsatellite loci, which should prove useful in studying the genetic structure of *M. darwiniensis* at these levels.

DNA was extracted from the heads and skins of 20 frozen termites from four colonies collected in the Northern Territory, Australia. Detection of microsatellites followed the protocol detailed by Oke *et al*. (1999) with slight modification. Briefly, approximately 11 μg of genomic DNA was digested with the restriction enzymes *Sau*3AI and *Alu*I, and fragments between 300 and 800 bp in length were purified and ligated into the *Bam*HI and *Hin*cII restriction sites of the plasmid pUC19. *Escherichia coli* JM109 cells were transformed with the plasmids and plated onto nutrient agar, resulting in approximately 6500 colonies. The colonies were then lifted onto nylon membranes and probed with the oligonucleotides $(AG)_{10}$, $(TG)_{10}$, $(CAC)_5CA$, $CT(CCT)_5$, $CT(ATCT)_6$ $(TGTA)_6TG$, which were end-labelled with [γ^{32}P]-dATP. Thirty-nine positive clones were identified as potentially containing microsatellite sequences based on their hybridization signal. Polymerase chain reaction (PCR) primers were developed for 15 of the 39 clones.

M. darwiniensis termites used for screening the variability of the markers were obtained from 40 trees in the Northern Territory, Australia. A single worker was sampled from each of 1–3 trees from 15 sites located along a 350-km long transect. DNA for the population variation screen was obtained from single heads of termites using a variation of the Chelex® protocol as described by Crozier *et al*. (1999). To visualize microsatellites, the forward PCR primer for amplifying each locus was first end-labelled with [γ^{33}P]-ATP. PCRs were then carried out in a final volume of 10 μL containing 2 μL genomic DNA, 5 μg bovine serum albumin (New England Biolabs), and 0.4 U *Taq* DNA polymerase (Promega), and a final concentration of 167 μM dNTPs, 0.4 μM of unlabelled reverse primer, 0.1 μM of unlabelled

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Table 1 Characteristics of microsatellite loci in the termite *Mastotermes darwiniensis*

Locus*	Repeat motif	T_a (°C)	Size range (bp)	Primer sequence (5′→3′)	n	N_A	H_O	H_E
Mdar2	$(AG)_{12}(TG)_{14}$	55	152–188	F: GGTAATTTCTGTAAATTGTAACGG R: GGTAATTTGTCATTTAATCTTGG	40	15	0.50	0.87
Mdar3	$(CG)_5(CA)_{13}$	55	283–305	F: TGAACACGATAAAAGCCGAGG R: CCATAATAATAATAAATGCTCACTG	37	13	0.51	0.83
Mdar4	$(CA)_7$	61	155–165	F: GATGTTCAGGAACGTGTATGACC R: CCAGGTCGCGCCATGCCGTGT	40	6	0.20	0.38
Mdar5	$(TATG)_{14}$	57	265–333	F: ATAAGTTCTGAAATCGAAACAGG R: CAACGTAAACGTGATGAGAGG	40	15	0.80	0.88
Mdar6	$(TG)_{11}(TATG)_9$	61	370†	F: TTGTTCATCGTTTGATGCAACGTC R: GACGGTCTTGAAATGACATAATGA	37	14	0.73	0.90
Mdar7	$(TG)_9G_8$	50	214–222	F: CGGATGACAAATATACAAGG R: TGTAGCCTGATGTAAATAGG	38	8	0.53	0.77
Mdar8	$(AG)_8$	55	175–185	F: TCTTACTCTTCGGTGTGTG R: GAACAGAGCACATTTCACT	40	6	0.73	0.71
Mdar11	$(AG)_8$	55	181–190	F: CATCTACTTGACAATTTCC R: TCGGTATGAATACTTTGCC	40	5	0.25	0.29
Mdar12	$(GT)_8$	57	245–250	F: ATCCTCTGTTTTACGACTGAG R: GAGCCGTTCTGGACGTGGT	40	4	0.13	0.21
Mdar13	$(TATG)_9 \ldots (TG)_6$	51	244–266	F: ACTGTCACTTCAGCGACC R: TCTAGGATGAAATGTTTACC	32	8	0.50	0.66

*GenBank accession numbers AF361240–AF361249; T_a, annealing temperature; n, number of individuals assayed; N_A, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; †size of cloned allele, range not determined.

forward primer, 0.03 μM of radioactively end-labelled forward primer, and 1× Promega Buffer (with 1.5 mM $MgCl_2$). PCR cycling profiles were carried out in a Perkin-Elmer 9700 thermocycler and began with an initial denaturation at 94 °C for 2 min, and then proceeded with 30 or 35 cycles of 93 °C for 30 s, the locus-specific annealing temperature (Table 1) for 30 s, and 72 °C for 30 s, followed by a final extension of 72 °C for 10 min. PCR products were run on 5% denaturing polyacrylamide gels at a constant power of 65 W for 3–5 h and then scored after exposing the dried gel to Kodak film.

Of the 15 primer pairs tested, 12 produced scorable patterns, and 10 of these 12 loci proved polymorphic. Observed heterozygosites were almost always lower than expected heterozygosities (Table 1), because of population structuring exhibited by *M. darwiniensis* in this region (MAD Goodisman and RH Crozier, unpublished results). In addition to characterizing the variability of the 10 loci in *M. darwiniensis*, we also attempted to amplify the loci in five other termites: *Coptotermes lacteus* (Rhinotermitidae), *Cryptotermes dudleyi* and *Neotermes insolaris* (Kalotermitidae), *Hodotermes mozambicus* (Hodotermitidae), and *Porotermes adamsoni* (Termopsidae). Given the high divergence between *M. darwiniensis* and these other termites (Watson & Gay 1991), it was not surprising that few of the priming sites were sufficiently conserved to allow cross-amplification. Only loci Mdar2 and Mdar4 amplified in all five species, and locus Mdar7 amplified in *C. dudleyi* only and Mdar10 amplified in *N. insolaris* only. Thus, the loci developed in this study are probably insufficient for studying other Isoptera. However, they should prove very useful in understanding the colony and population genetic structure of *M. darwiniensis*.

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