

PRIMER NOTE

Isolation and characterization of 10 microsatellite loci in the magnetic termite, *Amitermes meridionalis* (Isoptera: Termitidae)

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Abstract

The elongated mounds of the 'magnetic termite', *Amitermes meridionalis* are a prominent feature of the Northern Territory in Australia. They are restricted to habitat patches of seasonally flooded plains which are largely isolated from each other. To investigate the population structure of *A. meridionalis*, we developed 10 polymorphic microsatellite loci. We tested the variability of the markers on at least 20 individuals from two populations. We found three to 12 alleles per locus with a level of heterozygosity at each locus ranging from 0.05 to 0.74.

Keywords: *Amitermes meridionalis*, Australia, Isoptera, microsatellites, social insects, termites

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The mounds built by *Amitermes meridionalis* in the tropical savannahs of northern Australia are unique in shape and orientation (Grigg & Underwood 1977; Korb 2003). The thin, wall-like shape and the astonishing alignment according to the north–south axis of the earth's magnetic field has ever since risen the interest of explorers and scientists (Jack 1897). The distribution of *A. meridionalis* is limited to seasonally flooded plains, which can be separated from each other by several kilometres (Jacklyn 1991). As the dispersal abilities of the winged termites are poor (Nutting 1969) gene flow is likely to be restricted.

To investigate the population structure of such island populations, neutral markers such as microsatellites are ideal means. As there are no genetical markers available for *A. meridionalis*, we developed 10 microsatellite markers.

Specimens were collected from mounds and stored immediately in 100% ethanol. For 60 workers or soldiers, the head and thorax were ground individually in liquid nitrogen. DNA was extracted following a modified cetyltrimethyl ammonium bromide (CTAB) protocol (Sambrook & Russell 2001) and pooled. Approximately 50 µg DNA was digested with TSP 509 I and two adaptors (Tsp AD short and Tsp AD long; Tenzer *et al.* 1999) were ligated to the DNA, resulting in blunt-ended fragments. After purification

with Ultrafree-4 spinning columns (Millipore), the ligation product was amplified using the adaptor Tsp AD short as a primer. Forty polymerase chain reactions (PCR) were carried out in a final volume of 25 µL each, containing 0.5 µL ligation product, 0.25 µL *Taq* DNA polymerase (5 U/µL; MBI Fermentas) and a final concentration of 1 µM Tsp AD short, 1× buffer, 2.5 mM and 250 µM of each dNTP. We used a Biometra T1 thermocycler (Whatman) and the following temperature profile: initially 72 °C for 5 min to synthesize the nick between the linker and the genomic DNA followed by 20 cycles of initial denaturation at 93 °C for 1 min, primer annealing at 55 °C for 1 min and extension at 72 °C for 1 min. The PCR products were pooled, purified and concentrated using Centrifuge Filter Columns (Genomics). To enrich repeat motifs, the purified amplification product was hybridized with biotinylated oligonucleotides of either (AC)₁₃ or (AG)₁₃ linked to streptavidin-coated magnetic beads (Dynabeads M-280 Streptavidin; Dynal). Hybridization was carried out following Tenzer *et al.* (1999). Enriched DNA was recovered from the beads and amplified again using the same conditions as before without the initial extension step. The PCR was performed directly with 1 µL of bead solution. The enriched and amplified fragments were cloned in the plasmid pCR 2.1 and xL1 blue cells (TA Cloning Kit; Invitrogen) were transformed with these plasmids. Clones with an insert were dot-blotted on nylon

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Table 1 Primer sequences and characterization of 10 microsatellite loci in the termite *Amitermes meridionalis*

Locus	Primer (5'–3')	Repeat motif	Label forward primer	Insert size (bp)	T_a (°C)	n	+A	N_A	Size of mca (bp) (range)	Frequency of mca	H_O	H_E	HWE P value	Accession no. (EMBL)
AG1-B10	F: TTAGAAAAACATGTGATTAG R: GACCAGATTTGGGGCTCTTAC	(AG) ₃₂ AA(AG) ₃	FAM	382	55	27	–	9	119 (101–123)	0.37	0.48	0.80	0.0002	AM 420321
AC2-G1	F: TAAACCTGATGTCAGTAC R: ACGTAGTCACTTTTCATTGCC	(AC) ₂₇ TCCG(TC) ₅	TET	372	55	24	–	8	135 (124–141)	0.33	0.67	0.81	0.1	AM 420322
AC2-H5	F: GCAGTCTGCTCACCATTAGC R: GTGAGTTAAAATGCACATAC	(AG) ₁₁ (TG) ₄₀	TET	321	59	20	–	10	181 (162–197)	0.28	0.45	0.85	0.0002	AM 420323
AC2-H10	F: GAAGTAGCAGTTTATCACTT R: GAACTGCTGTCTTAAACCAAC	(AC) ₃₈	TET	384	59	26	–	11	141 (125–158)	0.44	0.69	0.78	0.3	AM 420324
AG1-B11	F: TTAGGGTGGACATATGGA R: CCTACAGAGCAACGACCT	(AG) ₂₃ TG(AG) ₂	FAM	603	55	26	–	5	133 (127–135)	0.56	0.54	0.65	0.2	AM 420325
AG2-C12	F: TACAGAAGGACTCACGACGC R: TGTATCTGTGACTAGGTCGC	(AG) ₃₁	HEX	370	59–65	21	+	8	173 (169–183)	0.31	0.67	0.82	0.12	AM 420326
AC2-F4	F: ATAGGCATATATAGCCAT R: ACTGTGAACCTCCCGGTGAC	(AC) ₅ AT(AC) ₂₁	FAM	307	55–58	20	–	3	128 (109–128)	0.55	0.05	0.57	0	AM 420327
AG1-A2	F: GGAAGGTAGCATGCTAAAGC R: CAACGAATATGACTAGCGTC	(AG) ₁₈ AT(AG) ₅ GG(AG) ₁₅	TET	343	55	20	+	12	202/237 (202–239)	0.18	0.75	0.90	0.0814	AM 420328
AG1-H9	F: ATCTCCATGTTCTGAACTGC R: GGACAACCTTAGAGACACCTA	(AG) ₃₃	HEX	387	55	26	–	13	108 (109–162)	0.19	0.65	0.91	0.0004	AM 420329
AG2-F4	F: AACATTCACCTTAAGTTCGC R: CAGTCAACATTAATAATG	(AG) ₂₉	HEX	431	50–55	20	+	11	126 (120–157)	0.25	0.65	0.88	0.0072	AM 420330

T_a , annealing temperature; n , sample size; +A-effect, PCR was carried out with 1.8–2.0 μ L MgCl₂ (25 mM) and with 30-min final extension; N_A , number of alleles; mca, most common allele; range, observed range of alleles; H_O , observed heterozygosity; H_E , expected heterozygosity, HWE, P values of the test on Hardy–Weinberg-Equilibrium.

membranes (Hybond – N⁺ Amersham) and probed with the oligonucleotides (AC)₁₃ and (AG)₁₃ labelled with fluorescein-11-dUTP (MWG Biotech).

Positive clones (140) were identified, of which 99 were successfully sequenced with the Big Dye Cycle Sequencing version 1.1 Ready Reaction kit (PE Biosystems) and T7 and M13 reverse primers (MWG Biotech) in an ABI PRISM 310 Genetic Analyser (PE Biosystems). Clones (61) contained repeat motifs and primer pairs were designed for 37 of these. Of the primer pairs, 20 yielded a single fragment and therefore the forward primer was labelled with FAM, HEX or TET at the 5'-end.

To assess the variability of the microsatellites, DNA from one to five individuals from 20 colonies from two different sites, that is populations, was extracted and the microsatellites were amplified using the following standard PCR protocol: a final volume of 20 µL contained 1–50 ng DNA template, 0.025 U/µL *Taq* polymerase, 0.5 µM labelled forward and reverse primer, 1× *Taq* buffer, 1× Enhancer, 1.6–1.8 µL from a 25 mM MgCl₂ solution and 200 µM per dNTP (MBI Fermentas). The initial denaturation step was set at 95 °C for 2 min, followed by 34 cycles of denaturation at 95 °C for 45 s, locus-specific annealing temperature for 45 s and extension at 72 °C for 45 s, followed by a final extension at 72 °C for 10–30 min. Because some loci showed an +A-effect that made interpretation ambiguous, MgCl₂ concentration was increased as well as the final extension time of up to 30 min. This increased the +A-effect strongly and simplified interpretation. The labelled products were diluted with water, mixed with Genescan-500 (Tamra) size standard and scored on the ABI.

Ten of the 20 primers were polymorphic, with three to 12 alleles (Table 1). Heterozygosities were calculated using HW-QUICKCHECK (Kalinowski 2006). The observed heterozygosity was lower than expected for all loci and significant deviation from Hardy–Weinberg equilibrium was detected for loci AG1-B10, AC2-H5, AG2-F4, AC2-F4 and AG1-H9. This indicates population substructuring or inbreeding. There was no evidence for null alleles (exception: AC2-F4 which had only three alleles), large allele dropouts or stuttering (MICRO-CHECKER, Van Oosterhout

et al. 2004). No linkage disequilibrium (GENEPOP Raymond & Rousset 1995) was found, except for AG1-B10 and AC2-H10 loci pair that showed a significant deviation from random association ($P = 0.03$; Bonferroni corrected). Therefore, at least eight loci had sufficient variability to analyse population genetics of *Amitermes meridionalis*.

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References

- Grigg GC, Underwood AJ (1977) An analysis of the orientation of 'magnetic termite' mounds. *Australian Journal of Zoology*, **25**, 87–94.
- Jack RL (1897) Note on the 'meridional anthills' of the Cape York Peninsula. *Proceedings of the Royal Society of Queensland*, **12**, 99–100.
- Jacklyn PM (1991) Evidence for adaptive variation in the orientation of *Amitermes* (Isoptera: Termitinae) mounds from Northern Territory. *Australian Journal of Zoology*, **39**, 569–577.
- Kalinowski ST (2006) HW-QUICKCHECK: an easy-to-use computer program for checking genotypes for agreement with Hardy–Weinberg expectations. *Molecular Ecology Notes*, **6**, 974–979.
- Korb J (2003) The shape of compass termite mounds and its biological significance. *Insectes Sociaux*, **50**, 218–221.
- Nutting W (1969) Flight and colony foundation. In: *Biology of Termites I* (eds Krishna K, Weesner FM), pp. 233–282. Academic Press, New York.
- Raymond M, Rousset F (1995) GENEPOP (version 2.1): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.
- Sambrook J, Russell DW (2001) Preparation and analysis of eukaryotic genomic DNA. In: *Molecular Cloning: a Laboratory Manual* (eds Sambrook J, Russell DW), 3rd edn, chapter 6. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
- Tenzer I, degli Ivanissevich S, Morgante M *et al.* (1999) Identification of microsatellite markers and their application to population genetics of *Venturia inaequalis*. *Phytopathology*, **89**, 748–753.
- Van Oosterhout C, Hutchinson WF, Wills DPM *et al.* (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, **4**, 535–538.