

## PRIMER NOTE

# Polymorphic microsatellite loci in the European subterranean termite, *Reticulitermes santonensis* Feytaud

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## Abstract

We report on the identification and characterization of two dinucleotide, two trinucleotide and eight tetranucleotide microsatellite DNA loci isolated from the European subterranean termite *Reticulitermes santonensis*. We tested the loci on 51–92 individuals from 46 colonies from different regions of France. Eleven loci were polymorphic with 2–8 alleles per locus and low observed heterozygosities (0.10–0.48). We also tested the loci on 17–20 individuals from 10 colonies in the closely related North American species *R. flavipes* and found significantly more alleles (2–9 alleles per locus) and higher observed heterozygosities (0.15–0.80) than in *R. santonensis*. The lower observed heterozygosities in *R. santonensis* are consistent with higher levels of inbreeding in these colonies due to the presence of numerous inbred replacement reproductives.

**Keywords:** dinucleotide repeats, microsatellites, *R. flavipes*, *Reticulitermes santonensis*, tetranucleotide repeats, trinucleotide repeats

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*Reticulitermes santonensis* Feytaud is a European subterranean termite (Rhinotermitidae) that causes serious damage throughout western and southwestern France and in isolated locations in northern France. For at least 10 years, this species has been attacking live trees along streets in Paris (Lohou *et al.* 1997), probably connected to the adjacent, infested buildings via underground tunnels. Previous studies employing allozymes have found limited genetic variability in this species (Clément 1981). Polymorphic microsatellite markers would be useful for investigating colony breeding structure, for delineating colony foraging areas, and for identifying the colony of origin for groups of foragers. Results of preliminary studies employing microsatellite markers isolated by Vargo (2000) in the closely related North American species *R. flavipes*, suggested that colonies of *R. santonensis* in Paris were highly inbred with high numbers of replacement neotenic reproductives (Dronnet *et al.* 2002). However, because the *R. flavipes* microsatellite loci exhibited low variability in *R. santonensis*, analyses were limited in the latter species. We therefore developed

new variable micro-satellite markers in *R. santonensis* to provide greater sensitivity, especially for distinguishing among neighbouring colonies.

DNA was extracted from pooled samples of heads of *Reticulitermes santonensis* workers from two colonies, one from Paris and the other from Olonnes (Vendée, France) using the DNEasy Tissue Kit (Qiagen). Extracted DNA was sent to the Savannah River Ecology Laboratory, Aiken, SC, USA for enrichment of several microsatellite motifs. Details of the protocol are available from Travis Glenn ([glenn@srel.edu](mailto:glenn@srel.edu)). Briefly, as outlined by Hauswaldt & Glenn (2003), DNA was digested with *RsaI* and then ligated to SuperSNX linkers. DNA fragments were hybridized with biotinylated microsatellite oligonucleotides and captured with Dynabeads (DynaL Biotech Inc.), while unwanted DNA fragments were removed by washing. Hybridized DNA was recovered from the Dynabeads and amplified by polymerase chain reactions (PCR) with SuperSNX-f (5'-GTTTAAGGCCTAGCTAGCAGAATC-3'). The enriched fragments were cloned using the TOPO TA Cloning Kit (Invitrogen). Ninety-six white colonies were amplified using M13 primers. Fifty PCR products 500–1000 bp in length were sequenced using the ABI Prism® dRhodamine

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**Table 1** Primer sequences of 12 microsatellite loci in *Reticulitermes santonensis*. Repeat units and sizes refer to the sequenced alleles. F, forward; R, reverse

Locus	Primer sequence 5' (r) 3'	Repeat unit	Size (bp)	GenBank Accession no.
RS10	F: TCCGGCTGACAAATGACATA R: TATTACTGCTGTGGCGCTG	(TAG) <sub>3</sub> TAA(TAG) <sub>9</sub>	154	AY423583
RS13	F: GACTGACCCGAGCAAGACTC R: TGACTCCTGAAGATGGGACC	(GT) <sub>10</sub>	298	AY423584
RS15	F: GGTCTGTGGAGGTAGCTG R: ACAAAGGAGCGCCTTACAAA	(TAG) <sub>6</sub>	250	AY423585
RS16	F: CCATGACCCGAATACGGAC R: TTCCACACGAGATGAAGCTG	(GA) <sub>2</sub> GC(GA) <sub>8</sub>	288	AY423586
RS33	F: GCTTGTAGGCATCGCAAGTT R: GGAAGTATTGTCACGAGGA	(TTCA) <sub>5</sub>	248	AY423587
RS43	F: CGGACAGACAGGAAGGTAGG R: ACCTCACAAAAGCACCTTGC	(CAGA) <sub>6</sub>	219	AY423588
RS62	F: GTAGCGCATTGTCTCAACCA R: GAATCCCCAGCCAATATTCA	(GTTT) <sub>5</sub>	300	AY423590
RS68	F: ATCAAGGTGGATGTGGGAGA R: AATCCGGGGAATTTCTTGAC	(CTAA) <sub>5</sub>	161	AY423591
RS76	F: AATCCGGGGAATTTCTTGAC R: CTGCATAACGATGTCTGCGT	(AGTT) <sub>8</sub>	187	AY423592
RS78	F: GCTTCTCAAGAAGACTGTGTC R: GCCCAGTTGAGATATGGAA	(AGTT) <sub>7</sub>	178	AY423593
RS85	F: GCCATCATCAGGGTTGTTA R: CAACGTGCAGACTTCCTCCT	(TATG) <sub>5</sub>	245	AY423594
RS93	F: GGGAACTTCTTGCTAGCTG R: GACAGCTCTGTGCAGTGGAA	(AGTT) <sub>6</sub>	135	AY423595

Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems) on an ABI 377 sequencer. Primer pairs were designed for 21 clones containing five or more tandem repeats. These were initially tested using the PCR conditions and labelling methods described by Vargo (2000) in which the M13 forward sequence is attached to the 5' end of the forward primer and an IRD labelled M13 oligonucleotide is added to the PCR reaction. Thirteen primer pairs were found to yield strong products of the expected size, of which 12 were found to be variable in at least one of the two species tested (Table 1).

We screened each of the loci for variation by genotyping 1–2 individuals from 46 *R. santonensis* colonies located in several regions of France, including at least one colony from 11 departments (French subdivisions) and 17 colonies inside and around Paris (four departments). We also assessed variability of these markers in *R. flavipes* by genotyping 1–2 individuals from each of 10 colonies in the US, four from North Carolina, and three each from South Carolina and Virginia. DNA was extracted from whole bodies of workers by phenol-chloroform extraction (Sambrook *et al.* 1989). PCR amplifications were performed in a final volume of 10 µL containing about 1 ng DNA template, PCR buffer (10 mM Tris-HCl, 50 mM KCl and 0.1% Triton®X-100, 1.5 mM MgCl<sub>2</sub>), 0.2 µg/µL BSA, 200 µM each dNTP

(Euromedex), 2 µM unlabelled reverse primer, 0.5 µM labelled forward primer, 0.4 U *Taq* DNA polymerase (Qbiogene). Forward primers were labelled with a 5' IRD800 fluorescent modification (MWG-Biotech). PCR was performed on a Biometra T1 Thermocycler (Whatman) using the following program: initial denaturation step at 94 °C (5 min), followed by 40 cycles at 94 °C (1 min), 55 °C (1 min) and 72 °C (15 s), with a final extension step at 72 °C (5 min). PCR products were separated by electrophoresis on 6% polyacrylamide gels (1500 V, 25 cm), run on a Li-Cor 4000 L automated DNA sequencer, and sized using 50–350 bp IRDye800™ standard (Li-Cor, Inc.). Results were analysed using GENE-PROFILER™ software (Scanalytics, Inc.).

Eleven of the 12 microsatellite loci were polymorphic in *R. santonensis*, with 2–8 alleles per locus (Table 2). Observed heterozygosity was significantly less than the expected heterozygosity ( $P < 0.001$ , HW exact test in GENEPOP version 3.3, Raymond & Rousset 1995) in all loci except RS68 (Table 2). All 12 loci were polymorphic in *R. flavipes* (2–9 alleles per locus), while the observed heterozygosities differed from the expected in only two loci, RS16 and RS78 (Table 2). Although about four times more individuals and colonies were tested in *R. santonensis* than in *R. flavipes*, the latter species had significantly more alleles per locus ( $t_{11} = 2.25$ ,  $P < 0.05$ , paired test) and significantly greater observed

**Table 2** Characterization of 12 microsatellite loci in *R. santonensis* and *R. flavipes*. Number (*n*) of individuals examined (two individuals per colony), number of alleles (Na), size range in base pairs (bp), frequency and size of most common allele, observed and expected heterozygosity ( $H_O$  and  $H_E$ ) and *P*, the probability of HW exact tests are given

Locus	<i>R. santonensis</i>						<i>R. flavipes</i>							
	<i>n</i>	Na	Size range (bp)	Frequency and size (bp) of most common allele	$H_O$	$H_E$	<i>P</i>	<i>n</i>	Na	Size range (bp)	Frequency and size (bp) of most common allele	$H_O$	$H_E$	<i>P</i>
RS10	92	7	151–175	0.56 (154)	0.38	0.63	< 0.0001	20	7	124–163	0.50 (148)	0.65	0.70	0.71
RS13	92	5	290–298	0.43 (296)	0.20	0.69	< 0.0001	20	6	288–298	0.38 (296)	0.75	0.76	0.44
RS15	92	6	250–277	0.29 (250)	0.48	0.77	< 0.0001	20	9	247–297	0.25 (250)	0.75	0.86	0.58
RS16	92	5	284–292	0.41 (286)	0.29	0.68	< 0.0001	19	5	274–288	0.42 (286)	0.47	0.74	0.01
RS33	51	1	248	—	—	—		18	2	240–248	0.55 (248)	0.44	0.51	0.66
RS43	92	2	215–219	0.75 (215)	0.25	0.38	< 0.01	20	3	211–231	0.93 (215)	0.15	0.15	1.0
RS62	91	4	292–308	0.82 (300)	0.10	0.32	< 0.0001	20	3	296–304	0.63 (296)	0.55	0.53	0.57
RS68	92	4	149–165	0.83 (157)	0.25	0.30	0.07	20	7	149–177	0.40 (157)	0.80	0.77	0.90
RS76	92	8	167–195	0.53 (187)	0.34	0.62	< 0.0001	17	8	171–207	0.44 (179)	0.59	0.73	0.19
RS78	92	4	166–178	0.64 (174)	0.23	0.52	< 0.0001	18	8	158–186	0.22 (170)	0.72	0.85	0.04
RS85	92	5	237–253	0.58 (245)	0.27	0.60	< 0.0001	16	5	229–249	0.75 (241)	0.44	0.43	0.40
RS93	91	4	123–143	0.58 (123)	0.38	0.58	< 0.0001	18	4	119–135	0.67 (131)	0.39	0.51	0.08

heterozygosities ( $t_{10} = 14.8$ ,  $P < 0.0001$ , paired test). The lower levels of observed heterozygosities in *R. santonensis* are consistent with apparently greater levels of inbreeding in this species in France associated with much larger number of replacement reproductives than in *R. flavipes* in the southeastern U.S. (Dronnet *et al.* 2002; Vargo 2003). This set of microsatellite markers, with numerous polymorphic loci, should be useful for analysis and comparison of colony and population genetic structure in *R. santonensis* and *R. flavipes* and other related species.

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### References

Clément J-L (1981) Enzymatic polymorphism in the European populations of various *Reticulitermes* species. In: *Biosystematics of*

*Social Insects* (eds Clément J-L, Howse PE), pp. 49–61. Academic Press, London.

Dronnet S, Ohresser M, Vargo EL, Lohou C, Clément J-L, Bagnères A-G (2002) Colony studies of the subterranean termite, *Reticulitermes santonensis* (Isoptera: Rhinotermitidae), in the city of Paris. In: *Proceedings of the 4th International Conference on Urban Pests* (eds Jones SC, Zhai J, Robinson WmH), pp. 295–301. Pocahontas Press, Inc, VA, USA.

Hauswaldt JS, Glenn TC (2003) Microsatellite DNA loci from the Diamondback terrapin (*Malaclemys terrapin*). *Molecular Ecology Notes*, **3**, 174–176.

Lohou C, Burban G, Clément J-L, Jequel M, Leca J-L (1997) Protection des arbres d'alignement contre les termites souterrains. L'expérience menée à Paris. *Phytoma*, **492**, 42–44.

Raymond M, Rousset F (1995) GENEPOP Version 1.2.: population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor Laboratory Press, New York.

Vargo EL (2000) Polymorphism at trinucleotide microsatellite loci in the subterranean termite *Reticulitermes flavipes*. *Molecular Ecology*, **9**, 817–820.

Vargo EL (2003) Hierarchical analysis of colony and population genetic structure in the eastern subterranean termite, *Reticulitermes flavipes*, using two classes of molecular markers. *Evolution*, **57**, 2805–818.