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PRIMER NOTE

Characterization of polymorphic microsatellite loci in the red harvester ant, *Pogonomyrmex barbatus*

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Abstract

The red harvester ant, *Pogonomyrmex barbatus*, is a monogynous, polyandrous species: each ant colony is founded by a single queen that has mated with one or more males. To study levels of polyandry within a colony, as well as relationships among colonies, we developed 10 polymorphic microsatellite loci for *P. barbatus*. With the number of alleles per locus ranging from 3 to 39, and expected heterozygosities of 0.58–0.95, these markers promise to be useful in the study of colony and population genetic structure.

Keywords: harvester ant, microsatellite, *Pogonomyrmex barbatus*, polyandry, population structure, social insect

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The red harvester ant, *Pogonomyrmex barbatus*, is a monogynous, polyandrous species that reproduces by sending winged reproductive forms to an annual mating flight. Since each colony is founded by a single queen that has mated with one or more males, the offspring of a colony share the same matriline, but are from one or more patrilines. As a result, colonies vary in their levels of relatedness. Polymorphic genetic markers would provide a useful tool for establishing levels of polyandry among *P. barbatus* queens. In addition, highly variable genetic markers would allow us to characterize the relationships between *P. barbatus* colonies that have been part of an ongoing behavioural and demographic study for the past 20 years (Gordon & Kulig 1996). To study the colony and population genetic structure of *P. barbatus*, we developed primers for 10 polymorphic microsatellite loci.

All ant samples were collected from the study population mentioned above, which is located in southeastern Arizona, USA. Samples were frozen while in the field, and later stored at −80 °C. Microsatellites were isolated from genomic DNA following the protocols described by Toonen (1997), with some modifications. Genomic DNA was digested with *Sau*3A1 (Sigma). Fragments between 300 and 800 base pairs in length were purified, and ligated into pBluescript II SK(+) vectors (Stratagene) that had been cut with *Bam*HI (GibcoBRL). XL1-Blue *Escherichia coli* cells (Stratagene) were made competent following the protocol described by Hillis *et al*. (1996). Competent cells were transformed with plasmids, and grown on nutrient agar containing ampicillin, X-gal and IPTG for blue/white selection of positive clones. Sterile toothpicks were used to transfer positive clones to Bio-Block 96-place Tube Arrays (Rainin) containing 0.5 mL nutrient broth, and were grown overnight at 37 °C. A multichannel pipette was used to transfer 1 μL of each culture to nitrocellulose membranes, which were incubated overnight on nutrient agar plates at 37 °C. After fixing the DNA to the membranes, the membranes were probed with $(AC)_{15}$, $(AG)_{15}$, $(AAC)_{10}$, $(AAG)_{10}$, $(AAT)_{10}$ and $(ACC)_{10}$ oligonucleotides that were end-labelled with [γ^{33}P]dATP. A total of 40 positive clones were identified. Plasmid DNA was isolated from the clones, and insert DNA was sequenced on a GeneAmp 9700 thermocycler (Perkin-Elmer/Applied Biosystems) using M13 (–20)/reverse primers (Stratagene) and ABI Prism Big-Dye Terminators (Applied Biosystems). Primers were developed for 13 of the 40 sequenced clones.

To test each primer pair, the initial polymerase chain reactions (PCRs) were carried out on a RoboCycler Gradient Temperature Cycler (Stratagene). DNA was extracted from ant heads by crushing the sample using a Teflon pestle, adding 150 μL of 10% Chelex (Bio-Rad) solution, and incubating at 95 °C for 20 min. The samples were centrifuged for 2 min, and the supernatant containing the DNA was removed. PCRs were carried out in a final volume of

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Table 1 Characteristics of microsatellite loci designed for the red harvester ant, *Pogonomyrmex barbatus*

Locus	Primer sequence (5′ to 3′)	T_a (°C)	Repeat motif	N_a	Size range (bp)	N_i	N_c	H_E	H_O	GenBank accession no.
Pb 1	F: CTGGAGGCAAAGAGTCACATC R: CTTCTTGAATCGGTCGCTACG	57	$(CA)_{13}(CG)_{12}(CA)_{11}$	39	228–286	209	18	95	91	AF481933
Pb 2	F: GTCTCATCATCTCCCTCGTTG R: CGGTAATCGGCAGCAAGTG	57	$(GA)_{13}$14 bp$(GA)_{12}$	26	288–361	176	15	90	93	AF481934
Pb 3	F: GCGACGACCAAGATGCTTC R: GAGGATGAGCCACGGTGAGAC	57	$(GA)_{11}GG(GA)_{16}$	33	289–328	240	18	94	95	AF481935
Pb 4	F: GTGGGACAACCTGTCAGAACG R: CCTCGGGAATGCCTATATGAG	57	$(GT)_{24}$	34	218–275	199	18	90	79	AF481936
Pb 5	F: CGCCGATGTCGCTATACC R: CTCAGAAGACGCAGGAACG	57	$(CTTT)_4TT(CT)_{16}$	20	221–242	233	18	81	60	AF481937
Pb 6	F: GCTGACGACGACTCAAATC R: GATCTTATCACCACTGCTCAT	57	$(CA)_4A(CA)_{33}$	28	127–177	244	18	91	95	AF481938
Pb 7	F: CACAAGAATCAGCGACGAC R: GCCAATAACACAGCCGTG	57	$(GT)_{12}$	19	141–185	134	12	81	95	AF481939
Pb 8	F: GAGATGGGCAAGGAACAGGAC R: GGAAGAATCTGCGGAGTGC	57	$(CT)_4TT(CT)_{17}$	17	268–297	158	12	82	100	AF481940
Pb 9	F: GTCGTGAAATAATAATCAGTACG R: GAACACAATAGAAATCCAGC	50	$(CA)_{21}AA(CG)_9$	25	320–358	154	15	92	100	AF481941
Pb 10	F: GCTGCTCTCGTAACTAAGTCG R: CCGTACTTTACCGTGCTGG	57	$(CAA)_{10}$	3	272–278	246	19	58	91	AF481942

T_a, annealing temperature; N_a, number of alleles; N_i, number of individuals genotyped; N_c, number of colonies from which the genotyped individuals were collected; H_E, expected heterozygosity; H_O, observed heterozygosity.

15 μL containing 2 μL genomic DNA, 0.5 U *Taq* DNA Polymerase (Qiagen), 0.8 mM dNTPs, 1× Q-solution (Qiagen), 1× PCR buffer (Qiagen), and 0.5 μM of each primer. The PCR cycle consisted of 95 °C for 5 min, followed by 35 cycles of 95 °C for 45 s, 50–61 °C gradient for 45 s, 72 °C for 45 s, followed by 72 °C for 7 min.

To check for polymorphism, PCR products were internally labelled by adding 0.2 μM fluorescent [R110]dCTPs (Applied Biosystems) to the PCRs described above. PCR cycling profiles (same as above) were carried out on a GeneAmp 9700 thermal cycler (Perkin-Elmer/Applied Biosystems). PCR products were run on 5% Sequagel (National Diagnostics) acrylamide gels on an ABI 377 automated sequencer. Gels were analysed using GENESCAN 3.1.2. software (Perkin-Elmer/Applied Biosystems).

Of the 13 primer pairs tested, 10 amplified scorable, polymorphic loci. The reverse primer for each of these loci was fluorescently labelled with either TET or 6-FAM (Operon), and was used in 15 μL reactions containing 2 μL of each sample, 0.5 U *Taq* DNA Polymerase (Qiagen), 0.8 mM dNTPs, 1× Q-solution (Qiagen), 1× PCR buffer (Qiagen), and 0.5 μM of each primer. PCRs were carried out using the PCR profile described above, with 25 cycles, and locus-specific annealing temperatures. The primer sequences, annealing temperatures, and characteristics of all 10 microsatellite loci are summarized in Table 1.

Given the large number of alleles and high levels of observed heterozygosity found at most of the 10 loci, the microsatellites developed for *P. barbatus* promise to be useful tools in the study of intra- and intercolony relationships.

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