Polymerase chain reaction primers for polymorphic microsatellite loci in the ant *Pseudomyrmex gracilis* (Formicidae: Pseudomyrmecinae)

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**Abstract**

The elongate twig ant *Pseudomyrmex gracilis* is widely distributed in the New World ranging from southern USA to Brazil and northern Argentina. To investigate its within-colony relatedness, we developed primers for nine polymorphic microsatellite loci. High levels of within-population variation were observed, with number of alleles ranging from two to 13, and heterozygosity from 0.28 to 0.90. The loci showed no deviations from Hardy–Weinberg equilibrium and no linkage disequilibrium.

**Keywords**: Hymenoptera, microsatellites, *Pseudomyrmex*, social insects

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Within-colony conflicts have been studied in few ant subfamilies (mainly Ponerinae, Myrmicinae, Formicinae). Nevertheless, those studies form the empirical basis for current established knowledge about kin conflicts in ants (see Ratnieks et al. 2006). To broaden this basis, it is necessary to examine species from other subfamilies. Therefore, we developed microsatellite markers for *Pseudomyrmex gracilis* (*Pseudomyrmecinae; common name ‘elongate twig ant’), a widespread neotropical ant species. This taxon is not closely related to the subfamilies mentioned above (Moreau et al. 2006) and thus well suited for providing new empirical data on kin conflicts in ants.

Specimens were collected from dead twigs in Southern Brazil and from swollen-thorn acacias in Southern Mexico in 2007, and stored in 96% ethanol. DNA was extracted from 36 workers (six colonies) following a modified cetyltrimethyl ammonium bromide (CTAB) protocol (Sambrook & Russell 2001) and pooled. Genomic DNA was restricted with Tsp 509 I (New England Biolabs) and ligated using two adaptors (MWG Biotech; Tsp AD short and Tsp AD long, Tenzer et al. 1999). After purification (Ultrafree-4 spinning columns, Millipore), fragments were amplified [32 polymerase chain reactions (PCR), 25 μL each] containing 0.5 μL restricted and ligated product, 1.25 U *Taq* DNA polymerase (MBI Fermentas), 1 μM *Taq* buffer (containing 100 mM Tris-Cl pH 8.8, 500 mM KCl, 0.8% Noidet P40; MBI Fermentas), 1.5 mM MgCl₂ (Fermentas), 250 μM each dNTP (Fermentas). Thermal cycling was performed in a T-Gradient Thermocycler (Whatman-Biometra): 20 cycles of 93 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min, preceded by 72 °C for 5 min to synthesize the nick between the linker and the genomic DNA and a final elongation at 72 °C for 10 min.

To enrich repeat motifs, (GA)₁₃ biotinylated probes were linked to streptavidin-coated magnetic beads (Dynabeads® M-280 streptavidin; Dynal) and probes were subsequently hybridized to the DNA. Hybridization and washing was carried out following Tenzer et al. (1999). Enriched DNA was recovered from the beads and amplified again using the same settings as before but without the initial extension step. The PCR was directly performed with 1 μL of bead solution as template.
Subsequently, PCR fragments were cloned using the TOPO-TA Cloning kit (Invitrogen) following the manufacturer’s protocol. White colonies (N = 159) were identified and dot blotted on nylon membranes (Hybond™-N+ Amersham). These clones then were probed with (GA)\(_{13}\) oligonucleotide labelled with fluorescein (MWG Biotech) and detected based on Fisher’s exact test as implemented in GENEPOP (Raymond & Rousset 1995). Thus, we developed nine primer pairs with nine microsatellite loci for *P. gracilis* (Mexican population). Forward primers (the first primers given) were 5’-fluorescent labelled in PCRs. The repeat motif is given for the cloned allele.

Table 1. Primer sequences and characteristics of nine microsatellite loci for *P. gracilis* (Mexican population). Forward primers (the first primers given) were 5’-fluorescent labelled in PCRs. The repeat motif is given for the cloned allele.

<table>
<thead>
<tr>
<th>Locus</th>
<th>GB accession</th>
<th>Primer sequence</th>
<th>Repeat motif</th>
<th>(T_a) (°C)</th>
<th>Size range (bp)</th>
<th>N</th>
<th>A</th>
<th>(H_e)</th>
<th>(H_o)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V1A01</td>
<td>FJ463656</td>
<td>5’ TET-CGCTCCTCCTCAGATAGTGA GCCAGGAAAGTTTGGCATC</td>
<td>(GA)(_{12})(GA)(_2)(GA)(_3)GA</td>
<td>55.0</td>
<td>141–160</td>
<td>32</td>
<td>8</td>
<td>0.77</td>
<td>0.78</td>
</tr>
<tr>
<td>V1B08</td>
<td>FJ463657</td>
<td>5’ 6-FAM-ATCCCCACCTCTCTTATTTA ACGAAATAATAGGAGGGA</td>
<td>(GA)(_2)(GA)(_3)</td>
<td>52.5</td>
<td>91–109</td>
<td>32</td>
<td>10</td>
<td>0.80</td>
<td>0.69</td>
</tr>
<tr>
<td>V1B09</td>
<td>FJ463658</td>
<td>5’ HEX-CCTCTCTTACCTTTTGTA AATCCCTGAGGATTTCTACG</td>
<td>(GA)(_2)(GA)(_3)GA</td>
<td>52.5</td>
<td>115–137</td>
<td>32</td>
<td>7</td>
<td>0.62</td>
<td>0.53</td>
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<tr>
<td>V1B10</td>
<td>FJ463659</td>
<td>5’ HEX-CAATTCCTTTCCTTATTTA AATACCTTGGACACACG</td>
<td>(GA)(_2)(GA)(_3)GA</td>
<td>47.5</td>
<td>129–164</td>
<td>32</td>
<td>4</td>
<td>0.69</td>
<td>0.72</td>
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<tr>
<td>V1D07</td>
<td>FJ463660</td>
<td>5’ TET-CTCTCATCCTTCCCTGAGTATC</td>
<td>AGR(GA)(_2)(GA)(_3)</td>
<td>50.0</td>
<td>111–113</td>
<td>32</td>
<td>2</td>
<td>0.25</td>
<td>0.28</td>
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<tr>
<td>V1D10</td>
<td>FJ463661</td>
<td>5’ HEX-GTACAGGGGTTTTGCTG</td>
<td>AAGR(GA)(_2)(GA)(_3)</td>
<td>55.0</td>
<td>77–142</td>
<td>32</td>
<td>20</td>
<td>0.92</td>
<td>0.88</td>
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<tr>
<td>V1E10</td>
<td>FJ463662</td>
<td>5’ 6-FAM-TCTAGAAAATACCTCCGAT</td>
<td>AATAGCCCTGACAGCGACCTAC</td>
<td>(GA)(_2)(GA)(_3)</td>
<td>52.5</td>
<td>104–174</td>
<td>32</td>
<td>13</td>
<td>0.87</td>
</tr>
<tr>
<td>V1F03</td>
<td>FJ463663</td>
<td>5’ HEX-ATATAGGAAATGGCTGAGC</td>
<td>AATTACCGTACAGCGCA</td>
<td>(GA)(_2)(GA)(_3)</td>
<td>55.0</td>
<td>77–102</td>
<td>32</td>
<td>5</td>
<td>0.77</td>
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<tr>
<td>V2A01</td>
<td>FJ463664</td>
<td>5’ 6-FAM-CTTTCTTCCAGGAAATACACGC</td>
<td>TTAATATCAAGGGGTTTGG</td>
<td>(GA)(_2)(GA)(_3)</td>
<td>55.0</td>
<td>86–147</td>
<td>32</td>
<td>13</td>
<td>0.74</td>
</tr>
</tbody>
</table>

GB, GenBank; \(T_a\), annealing temperature; N, number of genotypes that amplified from 32 individuals screened; A, observed number of alleles; \(H_e\), expected heterozygosity; \(H_o\), observed heterozygosity.
Acknowledgements

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References


Supporting Information

Additional supporting information may be found in the online version of this article:

**Table S1** Primer sequences and characteristics of seven additional microsatellite loci for *Pseudomyrmex gracilis*. Forward primers (the first primers given) were 5'-fluorescent labelled in PCRs. The repeat motif is given for the cloned allele. GB, GenBank; T_a, annealing temperature; N, number individuals that amplified from 12/7 individuals (Mexican/Brazilian population, respectively)/number of colonies; A, observed number of alleles; H_E, expected heterozygosity; H_O, observed heterozygosity. Observed and expected heterozygosities, and exact Hardy–Weinberg probability test using the Markov chain method with default parameters were calculated using the GENEPOP software (Raymond & Rousset 1995). No significant deviation (P < 0.001) between expected and observed heterozygosities based on Hardy–Weinberg probability tests was observed. We tested for null alleles using Micro-Checker (Shipley 2003) and found no evidence for null alleles. No linkage disequilibrium between any of the loci was detected based on Fisher’s exact test as implemented in the online version of the GENEPOP software (Raymond & Rousset 1995).

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Highly polymorphic microsatellite markers for *Radix balthica* (Linnaeus 1758)

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Abstract

We present data for eight polymorphic microsatellite markers isolated from a microsatellite-enriched DNA library for the freshwater snail *Radix balthica*. Three of them were specific for *R. balthica* while five also amplified polymorphic products in two congeneric species. Test application on populations from all over the species range has shown that these loci are highly informative for analysing population structure and estimating migration rates. Observed deviations from Hardy–Weinberg equilibrium are attributed to a mixed mating system.

Keywords: freshwater snail, genetic markers, microsatellites, *Radix balthica*

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