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TECHNICAL NOTE

Nine novel microsatellite markers for the army ant *Simopelta pergandei* (subfamily Ponerinae)

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Abstract *Simopelta* (subfamily Ponerinae) army ants are specialized predators of other ants in New World tropical forests. Although they show a striking convergence in overall life-history with the well known army ants of the subfamilies Aenictinae, Dorylinae, and Ecitoninae, the genus has been little studied. We developed and characterized nine novel microsatellite loci for *S. pergandei* with 2–8 observed alleles (mean: 5.2) and expected heterozygosities between 0.16 and 0.87 (mean: 0.68). Three of these loci reliably cross-amplified in a second species, *S. pentadentata*, with 4–8 alleles (mean: 8.0) and expected heterozygosities between 0.32 and 0.85 (mean: 0.65). These genetic markers will be useful in studying the sociobiology and molecular ecology of *Simopelta* army ants and in elucidating convergent evolutionary trajectories that have culminated in the army ant lifestyle.

Keywords Formicidae · Genetic marker · Microsatellite · Population genetics · Sociobiology

The New World army ant genus *Simopelta* (subfamily Ponerinae) presently comprises 21 species in two species groups (Mackay and Mackay 2008). Although *Simopelta* is phylogenetically only distantly related to the well known army ants in the subfamilies Aenictinae, Dorylinae, and Ecitoninae, they show a striking convergence in overall life-history (Gotwald and Brown 1966; Brady 2003; Mackay and Mackay 2008): (1) Queens are highly specialized egg-layers and permanently wingless, which strongly suggests that they mate inside the nest and that colonies reproduce by fission; (2) *Simopelta* colonies are specialized predators of other ants; (3) although colonies are rarely encountered in the field, several observations suggest that they are nomadic. This combination of traits makes *Simopelta* an optimal system to study the evolution of army ant mating systems, colony pedigree structure, and social conflicts using a comparative approach. While the major groups of army ants studied so far have the highest queen-mating frequencies among the ants, it is still unclear how this idiosyncratic mating system relates to the army ant lifestyle in evolutionary terms (Kronauer et al. 2007).

Furthermore, because army ant queens, unlike the queens of most ants, do not disperse on the wing, populations are expected to be particularly sensitive to habitat fragmentation, which makes them interesting model systems for conservation genetics (Berghoff et al. 2008). To address these issues, we developed nine novel microsatellite loci for the species *Simopelta pergandei* (*curvata* species group) (Table 1). We also tested these loci for cross-amplification in a second species, *S. pentadentata* (*williamsi* species group) (Table 2).

Samples of *S. pergandei* were collected in Henri Pittier National Park, Venezuela, and samples of *S. pentadentata* were collected in and around the Monteverde Cloud Forest area, Costa Rica. All samples were collected directly into 96% EtOH. Genomic DNA for microsatellite isolation was extracted from entire homogenized *S. pergandei* workers

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Table 1 Characterization of nine novel microsatellite loci in *S. pergandei*

Locus	Primer sequence (5′–3′)	Repeat motif	N_A	Size range (bp)	H_O	H_E	GenBank acc. no.
SpgA12	F CAAGACGTGCAGATTTCCAA	$(GA)_{17}$	6	238–266	0.867	0.748	HM595028
	R CCATTGATGACGTTGTTTCG						
SpgA37	F CGAACGGACGAATTATCGAG	$(TG)_4CG(TG)_9N_{18}(CT)_8$	4	152–160	0.852	0.704	HM595029
	R TGGCAAGGTAAGTGGATGAA						
SpgA40	F AGTTAGGGCGCGGCTATAAT	$(TC)_{11}$	5	174–186	0.500	0.553	HM595030
	R TGCAACGTTGAGAAGCGAAT						
SpgB6	F GCCAATAGTGCGCGAATAG	$(CT)_7CC(CT)_8$	7	191–234	0.750	0.827	HM595031
	R TCCTTGATAGCTCGGGGTAA						
SpgB11	F AAACGGGTCATTAGGTCACG	$(GT)_{12}$	2	148–149	0.133	0.157	HM595032
	R GCGCGAGATTGCAACTTATT						
SpgB20	F TAGGATCGACGTTCGTGAGA	$(TA)_7(TG)_9$	5	197–219	0.597	0.701	HM595033
	R AAATGAGTGGCGTCTTCACC						
SpgB27	F GGGTCCATAAGTCGAACTGA	$(GA)_{24}$	8	198–218	1.000	0.874	HM595034
	R AAGAAAGGGGTTCCAACGTC						
SpgB28	F TGGTTCGCTCTTCTTGTCCT	$(TC)_{13}N_{12}(TC)_8GC(TC)_6$	5	174–234	0.633	0.826	HM595035
	R GTTGCTAATTCACCGGTCGT						
SpgB33	F TGTGACGGTCAAGGATGGTA	$(TC)_{15}$	5	158–176	0.782	0.740	HM595036
	R TTGCGCGTTTTCATATTCAG						

Primer names and sequences, the number of observed alleles (N_A) and the corresponding size range of the amplified fragment, observed (H_O) and expected (H_E) heterozygosities, as well as GenBank accession numbers are given for each locus

Table 2 Cross-amplification of a subset of *S. pergandei* microsatellite loci in *S. pentadentata*

Locus	N_A	Size range (bp)	H_O	H_E
SpgA37	7	247–275	0.653	0.783
SpgB20	4	198–204	0.192	0.323
SpgB27	13	194–218	0.682	0.845

Column labels are as in Table 1

using the QIAGEN DNeasy Blood & Tissue kit. Microsatellite loci were isolated according to an enrichment protocol described in detail in Fischer and Bachmann (1998) and Rütten et al. (2001). Briefly, 2–3 μg of genomic DNA was digested with restriction enzyme *HinfI* and fragments were ligated to an adapter. In two separate reactions, biotin-labelled $(CA)_{10}$ and $(CT)_{10}$ probes were hybridized to the fragmented DNA and bound to streptavidin-coated magnetic beads (Dynal). A magnet was then used to enrich for poly CA and poly CT containing fragments. The enriched DNA was PCR amplified using adapter specific PCR primers and PCR products were ligated into pCR 2.1-TOPO vectors (Invitrogen™) and cloned into OneShot Chemically Competent *Escherichia coli* cells (Invitrogen™). We sequenced 70 clones from each of the two libraries using standard M13 primers. 16 and 23 inserts from the $(CA)_{10}$ and $(CT)_{10}$ enriched libraries contained microsatellite motifs. For a subset of these sequences, we designed PCR primers on the flanking regions of microsatellite repeats using the software Primer3Plus (Untergasser et al. 2007) (Table 1). Standard fluorescent tags were used to label the forward primers.

Genomic DNA for microsatellite genotyping for both species was extracted by freezing entire ants in liquid nitrogen, homogenizing the tissue with a pistil, and heating to 96°C for 15 min in 200 μl of 5% Chelex 100 (Bio-Rad). After centrifugation, 0.5 μl of the DNA containing supernatant was used as DNA template in PCR. The PCR cocktail had a total volume of 10 μl and contained 0.5 U QIAGEN *Taq* DNA Polymerase, 1× buffer, 2 mM $MgCl_2$, 0.25 mM of each dNTP, and 0.25 μM of each primer. PCR reactions were performed on a DNA Engine Dyad® Peltier Thermal Cycler (Bio-Rad) with an initial denaturation step of 3 min at 95°C, followed by 45 cycles of 30 s at 95°C, 30 s at 55°C, 30 s at 72°C and a final extension step of 15 min at 72°C. PCR products were run on a 3730xl DNA Analyzer (Applied Biosystems) and allele sizing was performed with Peak Scanner™ Software v1.0 (Applied Biosystems).

We genotyped a total of 68 *S. pergandei* individuals from four colonies for all nine markers. Three markers also consistently amplified and were polymorphic in *S. pentadentata* (Table 2). For this species we genotyped 216 individuals from 18 colonies. Observed heterozygosities for both species were calculated directly from these

genotypes in FSTAT version 2.9.3.2 (Goudet 1995) (Tables 1, 2). Individuals in social insect colonies are related, which can bias estimates of allele frequencies in the population if several individuals from the same colony are included in the dataset. We therefore used a second dataset in which we substituted replicate worker samples from the same colony with the deduced (mother) queen and (father) male genotypes from that colony (see Kronauer et al. 2006 for details) to calculate expected heterozygosities in FSTAT (Tables 1, 2). Tests of Hardy–Weinberg equilibrium and linkage disequilibrium were performed on datasets consisting of one diploid individual per colony with the software GENEPOP on the web, version 4.0 (Raymond and Rousset 1995). No significant evidence for linkage disequilibrium or deviations from Hardy–Weinberg equilibrium was found.

The microsatellite markers reported here will be useful in studying the sociobiology and population genetics of species in the army ant genus *Simopelta*, which will contribute to our general understanding of social evolution in complex insect societies.

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