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TECHNICAL NOTE

Polymorphic microsatellite loci for the ant-garden ant, *Crematogaster levior* (Forel)

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Abstract Throughout Amazonia, the ant *Crematogaster levior* is known for its participation in a complex ant-garden mutualism with the ant *Camponotus femoratus* and several species of epiphytic plants for which it plays an important role in seed viability. We isolated nine polymorphic microsatellite loci for *C. levior* from a genomic library enriched for di-, tri-, and tetra-nucleotide repeats. Two to 14 alleles were detected per locus, with levels of observed heterozygosity ranging from 0.103 to 0.785.

Keywords *Crematogaster* · Formicidae · Di-nucleotide microsatellite · Tri-nucleotide microsatellite · Tetra-nucleotide microsatellite

Ants in the genus *Crematogaster* are common and dominant members of Neotropical forest fauna (Longino 2003). Among the most abundant *Crematogaster* species in the Amazon is *Crematogaster levior*, an ant outstanding for its participation in a complex ant-garden mutualism with the ant *Camponotus femoratus* and several species of epiphytic plants, for which the ants play an important role in seed dispersal and viability (Davidson 1988; Longino 2003; Orivel et al. 1997; Vantaux et al. 2006; Wilson 1987). Ant-gardens are arboreal carton nests built primarily by *C. femoratus,* which also collects seeds of specific epiphytes and embeds them in the nests, where both ant species cultivate and defend the growing plants (Davidson 1988; Vantaux et al 2006). The gardens of *C. femoratus* and *C. levior* occur in aggregations of up to 30 nests, each of which can contain multiple dealate queens of both species (Davidson 1988). These two species represent the best-described example of parabiosis, the peaceful cohabitation of two or more ant species in the same nest (Davidson 1988; Orivel et al. 1997; Vantaux et al. 2006). This unusual phenomenon occurs in several species throughout the tropics, and defies the typical closure of insect societies toward heterospecifics or non-kin (Hölldobler and Wilson 1990). Even though their behavioral interactions are well characterized, the colony and population genetic structure of *C. levior* and other ant-garden species are undocumented, limiting their value as an important model for the understanding of parabiosis. The vast geographic range of this interaction suggests that population structuring is expected among populations and across geographical regions. Here, we present primer sequences, polymerase chain reaction (PCR) conditions, and initial characterization of the genetic variation for nine microsatellite markers.

Microsatellite isolation essentially followed the enriched protocol described by Dopman et al. (2004), with minor modifications, as described by Booth et al. (2008). Biotinylated dimer, trimer, and tetramer repeat motif probes employed in this method were described by Perera et al. (2007). A total of 40 clones containing inserts within the desired size range (200–600 bp) were sequenced using the M13 forward primer at the Genome Research Laboratory at North Carolina State University, NC. Twenty-five sequences contained tandem repeats considered likely to yield intraspecies polymorphism. Of these, 14 sequences contained sufficient flanking region for PCR primer design using the GENEFISHER software (Giegerich et al. 1996).

PCR reactions were carried out in 12 µl volumes, each containing 1× PCR buffer, 1.5–2.0 mM $MgCl_2$, 100 µM

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Table 1 Characteristics of nine microsatellite DNA loci developed for the ant *Crematogaster levior* and screened for a total of 28 specimens collected in Madre de Dios, Perú: locus designation (GenBank Accession nos: EU554543–EU554549; EU571470–EU571471), primer sequences, repeat motif, PCR conditions, sample size (N), number of alleles observed (N_A), average expected (H_E), and observed (H_O) heterozygosities, conformance to Hardy–Weinberg equilibrium (HW test) and range of PCR product sizes in (bp)

Locus	Primer sequences	Repeat motif	Annealing temperature (°C)	μM each primer	mM $MgCl_2$	No. of cycles	Individuals collected at one location						GenBank Accession nos
							N	N_A	H_E	H_O	HW test	Product size range (bp)	
Cl-4	F: GTTACACGATCACGCAA	$(GA)^{12}(GGA)^2$	45	1.5	2.0	35	28	5	0.788	0.521	ns	346–356	EU554543
	R: GAGACTGTTGTTGCTCA												
Cl-12	F: TCCCTTCCTCTTCTGAGA	$(CA)^3(CT)^{16}$	50	0.75	2.0	35	28	8	0.841	0.555	*	236–258	EU554544
	R: TCTTCTCGCGAGTCCGA												
Cl-22	F: CCGAGGGAATCGCGAA	$(GA)^2(GTGA)^4(GA)^3GAAA$ $(GA)^2(AT)^2GTCA\ (TG)^4$	52	0.5	1.75	35	28	2	0.099	0.103	ns	316–318	EU554545
	R: AGTGGATCGGGCAGGA												
Cl-23	F: AATCTGCTAGCGAGCAA	$(GA)^{40}$	52	0.5	1.75	35	28	13	0.921	0.720	*	241–287	EU571470
	R: GCCTAGGTAGTTCGTCGA												
Cl-24	F: ACTATCCTGTGGCTTGGA	$(GA)^3AA(GA)^{13}$	52	0.5	1.5	30	28	4	0.655	0.629	ns	162–170	EU554546
	R: GCCTAGGTAGTTCGTCGA												
Cl-26	F: TTCGACTGTACACAGGA	$(CT)^{14}TT(CT)^9$	55	3.5	1.5	30	28	11	0.845	0.785	*	206–230	EU554547
	R: CTGCTTTCATCGAGACA												
Cl-31	F: AGAATTACGCCCGGTGA	$(GA)^{22}$	52	0.6	1.5	30	28	14	0.909	0.689	*	290–362	EU554548
	R: TAGTGCCACTCCATCGTA												
Cl-34	F: CCTGACGGTATCGATCGA	$(GA)^{13}$	55	0.75	1.5	35	28	5	0.703	0.600	ns	278–288	EU554549
	R: TGGGTCGTGTGAATCTGA												
Cl-37	F: AGTTCCCGGTACGCTA	$(GA)^{13}$	52	0.5	1.75	35	28	5	0.715	0.519	ns	163–173	EU571471
	R: GTGTGAATCTGACGTGCA												

HW test, Hardy–Weinberg test (Raymond and Rousset 1995)—*significant probability test ($P < 0.05$)

dNTPs, ~50 ng DNA template, 0.3 U *Taq* DNA Polymerase (Bioline), and ddH_2O to 12 µl. Primer concentration varied between 0.5 and 3.5 pM with the forward primer of each end-labeled with a M13F-29 IRDye^{TM} tag (Li-Cor, Inc). Annealing temperatures ranged from 45°C to 55°C. PCR cycling conditions were comprised of an initial denaturation stage of 3 min at 95°C, followed by either 30 or 35 cycles each consisting of 30 s at 95°C, 30 s at the ideal temperature for each primer set, and 30 s at 72°C, with a subsequent terminal extension at 72°C for 3 min, carried out using ABI 2720 thermal cyclers (Applied Biosystems). Following PCR, 4 µl of stop solution (95% formamide, 20 mM EDTA, bromophenol blue) was added to each 12 µl reaction. Reactions were subsequently denatured at 90°C for 4 min, and 1 µl was loaded onto 25 cm 6% 1× TBE polyacrylamide gels, mounted on a Li-Cor 4300 automated DNA sequencer. Loci were sized using a 70–400 bp standard (Microstep-20a, Microzone^{TM}). Gels were run at a constant power of 40 W at 50°C for 2 h. Results were analyzed using GENEPROFILER^{TM} software (Scanalytics, Inc.).

Fourteen primer pairs produced unambiguous PCR products, with nine loci demonstrating allelic variation when screened across 28 individuals, representing two workers from each of 14 colonies collected at the Centro de Investigación y Capacitación Río Los Amigos, located in Madre de Dios, Perú. Approximate distance between sampled colonies ranged from 165 m to 5,858 m. Locus characteristics are provided in Table 1. Diversity indices were calculated using GENEPOP v3.3 (Raymond and Rousset 1995). Among the 14 colonies, two to 14 alleles were detected per locus. Observed heterozygosities ranged from 0.103 to 0.785. No evidence for linkage disequilibrium was detected among the 36 possible pairwise locus comparisons. Within this same sample location four loci showed deviations from Hardy–Weinberg equilibrium (see Table 1). Given the spatial scale over which colonies were collected, this is likely to be due to the admixture of two or more populations. Preliminary evidence suggests that these loci will prove useful in studies of phylogeography, colony structure, and mating system of the ant-garden ant *C. levior*.

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