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TECHNICAL NOTE

Characterization of 8 polymorphic microsatellite loci in the neotropical ant-garden ant, *Camponotus femoratus* (Fabricius)

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Abstract *Camponotus femoratus* is an abundant and behaviorally dominant ant in lowland Amazonian rainforests, where this species participates in a complex and obligate seed-dispersal mutualism. *C. femoratus* typically cohabits with another ant species *Crematogaster levior* in an apparently amiable but poorly understood interaction. Despite these outstanding characteristics, the population genetics and dispersal patterns of *C. femoratus* are unknown. We isolated eight polymorphic microsatellite loci for *C. femoratus* from a genomic library enriched for di-, tri-, and tetra-nucleotide repeats. We detected 2 to 17 alleles per locus, with levels of observed heterozygosity ranging from 0.286 to 0.714.

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

Camponotus is one of the two largest genera of ants. It occupies every terrestrial habitat and is ecologically important throughout its cosmopolitan distribution (Hölldobler and Wilson 1990; Wilson 2003). One species of particular interest is *Camponotus femoratus*, an abundant and behaviorally dominant ant in lowland Amazonian rainforests that participates in a complex and obligate seed-dispersal mutualism (Davidson 1988; Wilson 1987). *C. femoratus* constructs arboreal carton nests in which it cultivates at least 10 species of epiphytic plants, including the protected cactus *Epiphyllum phyllanthus*, in so-called ant-gardens (AGs). The AG plant species rely upon *C. femoratus* for seed dispersal and growth substrate (Davidson 1988).

Despite its ecological dominance and its role in a complex and intimate seed dispersal mutualism, as well as its characteristic amiable cohabitation with another ant species *Crematogaster levior*, for which microsatellites were recently developed (Booth et al. 2008b), the population genetics of *C. femoratus* are unknown. Its nest-gardens occur in aggregations of two to 30 or more nests in territories that can occupy up to 39% of forest area (Davidson 1988). These appear to represent polygynous, polydomous colonies, but social structure has not been confirmed and dispersal dynamics are unknown. While microsatellite markers are reported for other members of the genus *Camponotus* (Gertsch et al. 1995; Crozier et al. 1999; Goodisman and Hahn 2005), these either did not amplify or were monomorphic in *C. femoratus*. Therefore we present eight polymorphic microsatellite markers developed for *C. femoratus* in order to clarify its population structure and better understand its role as an ecologically dominant ant and obligate seed disperser in the Amazon, an endangered ecosystem of global importance.

We isolated microsatellites according to the enriched protocol described by Dopman et al. (2004), with slight modifications, as described by Booth et al. (2008a). Biotinylated dimer, trimer and tetramer repeat motif probes employed in this method were described by Perera et al. (2007). We sequenced a total of 48 clones containing inserts within the desired size range of 200 to 600 bp using the M13 forward primer at the Genomic Sciences Laboratory at North Carolina State University, NC. Forty-four sequences contained tandem repeats likely to be polymorphic within *C. femoratus*; of these, 25 sequences included

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Table 1 Characteristics of eight microsatellite DNA loci developed for the ant-garden ant, *Camponotus femoratus* and screened for a total of 56 specimens collected in Madre de Dios, Perú: locus designation

Locus	Primer sequences	Repeat motif	Annealing temperature (°C)	μM each primer	mM $MgCl^2$	No. of cycles	Individuals collected at one location ($n = 56$)					GenBank accession no
							N_A	H_E	H_O	Size (bp)	HW test	
Cf-2	F: CAATTTACGACGCTCCA	$(CT)^{11}CC(CT)^{10}TC(CT)^{4}TT(CT)^{2}TT(CT)^{2}$	58	0.75	1.5	35	6	0.595	0.518	216–238	*	FJ041324
	R: TTCTCGAGGACTTTCCA											
Cf-3	F: GTCATCGCGAATTCATG	$(CT)^{19}CC(TC)^{3}(CT)^{3}CC(CT)^{2}TT(TC)^{3}$	58	0.6	1.5	35	10	0.875	0.714	192–214	ns	FJ041325
	R: CGATCGAACTTGACAAG											
Cf-7	F: GCGTGCAAAGTTCATCGA	$(GA)^{4}AATAGAGG(GA)^{2}AC(GA)^{7}$	50	4	1.75	35	3	0.531	0.375	148–152	ns	FJ041326
	R: CTCGTTTGCCCTTTCGAA											
Cf-10	F: AGATCGTGTGGATGGAA	$(CT)^{19}CGCAGA(TA)^{4}$	52	1.25	1.5	35	16	0.858	0.714	192–228	*	FJ041327
	R: CCGTCACTATACGTGGAA											
Cf-13	F: GCGCGTGCAAACGCAA	$(CT)^{4}TT(CT)^{8}TT(CT)^{3}TT(CT)^{9}$	60	0.4	1.5	35	5	0.730	0.464	226–252	ns	FJ041328
	R: ATGGGACGCGACGAGGA											
Cf-35	F: ACTCAGTAAGCACGAGA	$(GA)^{8}$	55	0.5	1.5	30	2	0.378	0.286	240–242	ns	FJ041329
	R: GTATGTCCAATCGCAGA											
Cf-37	F: ATTGCGTGGTTCGCAGGA	$(GA)^{23}$	55	1	2	35	17	0.920	0.661	165–207	*	FJ041330
	R: ATCTTGTTGCCCGCGCAA											
Cf-38	F: GATCTCGGCGAGATCAAG	$(TCCC)^{3}TC(CT)^{9}$	50	0.75	1.75	35	2	0.446	0.339	237–239	ns	FJ041331
	R: ACATGATCGACGGACTCT											

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

(GenBank Accession nos: FJ041324 -FJ041331), primer sequences, repeat motif, PCR conditions, 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)

sufficient flanking region for PCR primer design using the GENEFISHER software (Giegerich et al. 1996).

PCR were 12 µl each, including 1× PCR buffer, 1.5–2.0 mM $MgCl_2$, 100 µM dNTPs, ~50 ng DNA template, 0.3 U *Taq* DNA Polymerase (Bioline), and ddH_2O to 12 µl. Primer concentration varied between 0.4 and 4 pM with the forward primer of each end-labeled with a M13-F-29 IRDye™ tag (Li-Cor, Inc). PCR cycling was carried out with an ABI 2720 thermal cycler (Applied Biosystems) programmed for a 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 annealing temperature for each primer set (50–60°C), and 30 s at 72°C, with a subsequent terminal extension at 72°C for 3 min. Following PCR, each reaction was stopped with 4 µl of stop solution (95% formamide, 20 mM EDTA, bromophenol blue). Reactions were subsequently denatured at 90°C for 4 min, and 1 µl was loaded onto 25 cm polyacrylamide gels (25 cm 6% 1× TBE), mounted on a Li-Cor 4300 automated DNA sequencer. Loci were sized using a 70–400 bp standard (Microstep-20a, Microzone™). Gels were run at a constant power of 40 W at 50°C for 2 h. Results were analyzed using GENEPROFILER™ software (Scanalytics, Inc.).

Twenty primer pairs yielded unambiguous PCR products. Eight of these loci demonstrated allelic variation among 56 individuals, representing four workers from each of 14 colonies collected at the Centro de Investigación y Capacitación Río Los Amigos in Madre de Dios, Perú. Approximate distance between sampled colonies ranged from 165 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, 2–17 alleles were detected per locus. Observed heterozygosities ranged from 0.286 to 0.714. We detected no evidence for linkage disequilibrium among the 28 possible pairwise locus comparisons. Within this same sample location three 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 admixture of two or more populations. We anticipate that these loci will be valuable in further studies to clarify the population genetic structure of the neotropical ant-garden ant *C. femoratus*.

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