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PRIMER NOTE

Five polymorphic microsatellite markers for the study of *Cardiocondyla elegans* (Hymenoptera: Myrmicinae)

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Abstract

We describe primer sequences for five microsatellite markers in *Cardiocondyla elegans*, an ant species with ergatoid males. Polymorphism of these loci was investigated using 236 individuals from 22 colonies from four locations. The microsatellites are dinucleotide repeats with four to 16 alleles, and the observed heterozygosity ranges from 0.244 to 0.720. We characterized these markers for the study of the population as well as the social structure of colonies.

Keywords: *Cardiocondyla elegans*, dinucleotide repeats, ergatoid ant males, microsatellites

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Cardiocondyla elegans Emery, 1869, is a small myrmicine ant (*c*. 2.3 mm long) that lives in sandy and moist soil. Bernard (1968) reported that *C. elegans* is a Mediterranean ant that builds nests on river banks. He accurately described workers and winged females, but mistook the males for queens of a new socially parasitic ant, *Xenometra gallica*. This error occurred because the orange to yellow males of *C. elegans* are ergatoid (wingless), unlike males of other ant species, and differ greatly in colour from the completely black workers and the winged queens. Baroni Urbani (1973), corrected this mistake by redescribing '*X. gallica*' queens as *C. elegans* males. Because the males are ergatoid, there are no nuptial flights and copulations occur inside the nests. Another peculiarity of *C. elegans* is that many (*c*. 10) ergatoid males can live peacefully in the same nest as opposed to other species of the same genus, e.g. *Cardiocondyla minutior* or *Cardiocondyla mauritanica* (Heinze 1997).

To gain a better understanding of the genetic structure of populations and colonies of this species, we developed specific microsatellite markers. DNA was extracted from pooled 140 *C. elegans* workers from two colonies, one from Montlouis-sur-Loire (Indre et Loire, France) and one from Carennac (Lot, France), using the DNEasy® Tissue Kit (QIAGEN). Extracted DNA was sent to the Savannah River Ecology Laboratory, Aiken, SC, USA for microsatellite isolation. Details of the protocol are available from Travis C. Glenn (*glenn@srel.edu*). Briefly, as outlined by Hauswaldt & Glenn (2003), the DNA was digested with *Rsa*I, ligated to SuperSNX linkers, hybridized to biotinylated microsatellite oligonucleotides and captured on Dynabeads (Dynal Biotech). Captured DNA was recovered by polymerase chain reactions (PCR) with SuperSNX-f (5′-GTTTAAGGCCTAGCTAGCAGAATC-3′) and cloned using the TOPO TA Cloning System 2.1 (Invitrogen). White colonies were amplified using M13 forward and reverse primers. PCR products of 500–1000 bp were sequenced using BigDye version 2.0 (Applied Biosystems). Sequences were assembled and edited in SEQUENCHER version 4.1.2 (Gene Codes Corporation) and exported to EPHEMERIS version 1.0 to automatically search sequences for microsatellite repeats. Twenty primer pairs were designed, but only five of them showed variability. This was investigated by genotyping 10 individuals from nine *C. elegans* colonies from three different locations for each of the primer pairs according to the protocol described in Dronnet *et al*. (2004). All of the five variable markers are 'AG' or 'AC' dinucleotide repeats (Table 1).

For a more detailed study of these five variable microsatellites, DNA of 236 workers from 22 colonies from four different locations (Montlouis-sur-Loire and Candes

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Table 1 Characterization of primer sequence of five variable microsatellite loci in *Cardiocondyla elegans*

Locus	Primer sequence (5′–3′)	Repeat sequence	Size range (bp)	T_a (°C)	Label of *F*-primer	*N*	No. of alleles	H_O	H_E	GenBank Accession no.
CE2–3A	F: CCGTCTTTTCCACTCAC R: GGAATCGTCGAGAGAGA	$(AG)_{19}$	103–129	60	TET	214	13	0.558	0.753	AY911417
CE2–4A	F: TGCGAGTGGATGTATGA R: CCCACCTTACAGCAATATC	$(AG)_{20}$	178–192	60	FAM	155	7	0.690	0.807	AY911418
CE2–5D	F: AGACGTAAGGTTTGAAGAGA R: ACAACTATGCCAAATTAAGTAT	$(AC)_{15}$	204–210	60	HEX	215	4	0.244	0.580	AY911419
CE2–12D	F: TCCGCTAAATTATCATGG R: TCGAGTGCATAAAGGAATA	$(AG)_{10}$	124–148	60	FAM	182	9	0.379	0.770	AY911420
CE2–4E	F: ATACAAAAGAATATGAAGTAATACA R: GTGTGCTTATGTATCTGGTAT	$(AC)_{25}$	139–175	60	HEX	148	16	0.720	0.847	AY911421

T_a, annealing temperature; *N*, number of individuals; no. of alleles, observed number of alleles; H_O, observed heterozygosity; and H_E, expected heterozygosity.

Saint Martin, Indre et Loire; Carennac, Lot; Chemilly, Allier), was extracted using a Puregene® DNA isolation kit (Gentra Systems), according to Foitzik & Herbers (2001). Standard PCRs (Biometra T1 thermocycler, Whatman) were carried out in a final 20-μL reaction volume containing 1 to 50 ng DNA, 1× buffer, 2 mM $MgCl_2$, 200 μM per dNTP (MBI Fermentas), 0.6 μM unlabelled reverse primer, 0.6 μM labelled (TET, FAM and HEX dyes) forward primer, 0.5 U *Taq* DNA polymerase (Qbiogene). Because locus *CE2-4E* is very sensitive to contamination, we used only half of the volume of DNA solution. PCR was performed using the following program: initial denaturation step at 94 °C (3 min), followed by 40 cycles at 94 °C (45 s), 60 °C (45 s) and 72 °C (45 s), with a final extension step at 72 °C (7 min). PCR products were visualized on an ABI PRISM 310 genetic analyser (PE Biosystems) and allele size was analysed by using a GeneScan-500 size standard (TAMRA) and GENESCAN 3.1 software (PE Biosystems).

The five polymorphic loci showed four to 16 alleles per locus and the observed heterozygosity ranged from 0.244 to 0.720. For each locus, observed heterozygosity was always significantly smaller than the expected heterozygosity ($P < 0.001$, GENEPOP web version 3.4 option: Hardy–Weinberg test). Linkage disequilibrium test from GENEPOP web version 3.4 indicates a nonrandom relation of these loci from each other ($P < 0.001$ for each pair of loci). This low heterozygosity and linkage between loci may in part result from allele frequencies differing between collecting sites, but is also consistent with the fact that ergatoid males are able to breed with their sisters inside the nest (Schrempf *et al*. 2005). These first variable microsatellites in *C. elegans* will be useful in studying social structure of the colonies as well as relationships between nests or populations.

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