Molecular Ecology Notes (2001) 1, 126–127

PRIMER NOTE

Identification of polymorphic microsatellite loci in the queenless, ponerine ant *Diacamma ceylonense*

ASHOK GOPINATH,* RAGHAVENDRA GADAGKAR† and M. R. S. RAO*
*Department of Biochemistry, Indian Institute of Science, Bangalore 560 012, India, †Centre for Ecological Sciences, Indian Institute of Science, Bangalore 560 012, India.

Abstract

***Diacamma ceylonense* is a queenless, ponerine ant whose colonies are headed by a single, mated, egg-laying worker referred to as the gamergate. Thus, new colonies are a result of dispersal by wingless gamergates. This is expected to influence patterns of colony dispersal and spatial distribution of genetic variablity. In order to facilitate the study of population genetic structure we have identified six unique, polymorphic, microsatellite loci. We have used fluorescence tagged primers to detect polymorphism at these loci.**

Keywords: *Diacamma*, fluorescent primers, microsatellites, Ponerine ants, social insects

Received 12 October 2000; revision received 14 December 2000; accepted 18 January 2001

Queenless, ponerine ants provide interesting model systems for studies of intracolony genetic relatedness as well as population genetic structure. Because colonies of such ants are headed by mated, egg-laying workers (gamergates), rather than by morphologically differentiated queens (Peeters 1991), gamergate turnover and consequent effects on intracolony relatedness are of great interest. In the absence of winged female alates, which are seen in queen-right species, new colonies can only be produced by fission, by walking (not flying) individuals. The consequence of such a mode of dispersal on the population genetic structure (in relation to physical distance) is expected to be different from that seen in queen-right species. Microsatellite markers are increasingly being used as a powerful tool to study intracolony genetic relatedness and population genetic structure of social insects, particularly ants (Chapuisat 1996; Herbers & Mouser 1998). Doums (1999) has recently reported eight polymorphic microsatellite markers for studying the queenless, ponerine ant *Diacamma cyaneiventre* using the more conventional radioactive detection assay for polymorphism. We have used a fluorescence (nonradioactive) detection assay to show polymorphism at six other microsatellite loci in the closely related *Diacamma ceylonense*.

Genomic DNA was isolated by grinding the ants in CTAB lysis buffer (1% CTAB, 0.75 M NaCl, 50 mM Tris-HCl pH 8.0, 10 mM EDTA, Proteinase K 100 μg/mL) for 1–2 min and subjected to phenol:chloroform extractions (Sambrook *et al.* 1989). DNA was handled gently by using cut tips, so as not to shear the DNA. A small insert genomic library was constructed by digesting the genomic DNA with *Sau*3AI (New England Biolabs) restriction enzyme. The products below 3 kb were cloned into pUC18 vector (Pharmacia Amersham *Bam*HI, BAP vector) at the *Bam*HI site. Four thousand recombinant clones were obtained.

The library was screened for the presence of $(CA)_n$ and $(AG)_n$ containing microsatellite loci using γ^{32}P end-labelled $(TG)_{15}$ (Life Technologies) and $(CT)_{15}$ (Bangalore Genei Ltd) synthetic oligonucleotides. A total of 28 repeat-containing clones were identified. Of these, 13 unique repeat-containing clones were identified by subjecting all the clones to sequencing, using the asymmetric PCR cycle sequencing protocol (ABI Prism) and detection by ABI Prism 377 Automated Sequencer.

Primers were synthesized to the flanking regions of these repeat sequences. Polymerase Chain Reaction (PCR) was used to amplify each repeat containing locus in the genomic DNA of the individual ants. Twenty-five μL reaction mixes were set up, using 200 μM dNTPs (final concentration), 1× *Taq* Buffer (Bangalore Genei) containing 10 mM Tris-Cl, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, and 0.5 U of *Taq* DNA Polymerase (Bangalore Genei). Three out of 13 primers failed to amplify any product, two showed multiple bands, two were monomorphic, and six were polymorphic. The forward and reverse primer sequences, repeat lengths,

Correspondence: M. R. S. Rao. Fax: 91(80) 3600118; E-mail: mrsrao@biochem.iisc.ernet.in

Table 1 Characteristics of microsatellite loci from *Diacamma ceylonense*. The number of alleles observed (N_a), observed heterozygosity (H_O), expected heterozygosity (H_E), inbreeding coefficient (F_{IS}) and *P*-value for assessing deviation from Hardy–Weinberg equilibrium, were calculated from a sample of unrelated workers (one worker per colony) using GENEPOP (Raymond & Rousset 1995). The samples were collected from IISc, Bangalore (13°00′ N, 77°32′ E), in Karnataka State (India). N_i is the number of individuals analysed and T_a the annealing temperature

Locus	GenBank Accession No.	Repeat array	Primer Sequences (5′ to 3′)	T_a (°C)	N_a	N_i	Size Range (bp)	H_O	H_E	F_{IS}	*P*
DCI-12	AY007982	$(CT)_{12}$	F: ATCTCGCGCGCAGCCTTCTTTC	55	3	24	139–143	0.23	0.25	−0.09	1.0
			R: AATGCACGCCGCATCCTGGGC								
DCI-34	AY007985	$(CT)_{28}$	F: GCGGCTACGTATGGAAATGCG	57	11	26	151–179	0.88	0.87	−0.02	0.03
			R: TCGACGACCGAAGCAGCTGA								
DCI-56	AY007986	$(CT)_{11}$	F: GAGTAGACGCGGGTCAAGAA	55	5	26	171–181	0.62	0.66	+0.06	0.09
			R: CACCGCGAAGAGCGTCGTTG								
DCI-78	AY007987	$(CT)_{12}$	F: GCCGTCACGGCGGAGCATTT	55	3	16	198–202	0.19	0.23	+0.20	0.19
			R: GTTGTCTCGAACCTTGGGAA								
DCI-1516	AY007983	$(CT)_{12}$	F: CGAACCCGAAGGCCGACGAG	55	4	20	104–110	0.20	0.43	+0.54	0.003
			R: AAGCGACGACGTTTCACCGC								
DCI-2122	AY007984	$(CT)_5/(AG)_7$	F: TCGATCGCGTGTCCGAGTCC	55	6	26	137–147	0.50	0.43	−0.17	1.0
			R: GAACGAAAGAGCTCGGGGAA								

annealing temperature and other details of the six polymorphic loci are given in Table 1.

Nonradioactive (fluorescent) PCR was set up in two ways: (i) in the case of loci DCI-34, DCI-56, DCI-78 and DCI-2122 the primers were commercially synthesized with HEX, TET, 6-Fam, and TET derivatives of fluorescein (Perkin Elmer), respectively. Five pmoles each of the fluorescently labelled and unlabelled forward primer and 10 pmoles of the unlabelled reverse primer were added to each reaction tube. (ii) In the case of primer pair DCI-1516 and DCI-12, 400 nm of fluorescent dUTP analogues (Perkin Elmer) and 200 μM nonfluorescent dNTPs were added to each reaction tube. Ten pmoles each, of unlabelled forward and reverse primer were added to the reaction mix, respectively. The products were loaded on a 6% denaturing sequencing gel, along with a standard size marker, TAMRA 500, in each lane and the different sized bands were detected (ABI Prism 377 Automated sequencer) and interpreted using GENESCAN software (ABI Prism) and Genotyper (ABI Prism). Four out of the six polymorphic loci showed no significant deviation from Hardy–Weinberg equilibrium, one showed significant deviation and one was borderline (Table 1). We suspect that one or more of the loci deviate from Hardy–Weinberg equilibrium because null alleles may be present at these loci.

The usage of fluorescently tagged primers in such studies has some distinct advantages. Calibration of allele sizes is more accurate because each lane has a standard molecular weight marker to compare with, eliminating size calibration problems due to improper gel runs (eg. gel smiling). PCR products obtained with different fluorescence tags for different loci can be loaded in the same lane making this a high throughput technique. Finally, and just as importantly, exposure to harmful radioactivity can be avoided.

Acknowledgements

We thank M. Kumaran, S. Karthik, and T. Varghese for their enthusiastic participation during ant collections, and J. Robert for constructive criticisms. All sequencing reactions and genotype analysis were conducted using the Department of Biotechnology supported Automated DNA Sequencing facility, IISc. We also thank IFCPAR and DST for partial support. Ashok Gopinath is a Senior Research Fellow of Council of Scientific and Industrial Research (CSIR).

References

Chapuisat M (1996) Characterization of microsatellite loci in *Formica lugubris* B and their variability in other species. *Molecular Ecology*, **5**, 599–601.

Doums C (1999) Characterization of microsatellite loci in the queenless Ponerine ant *Diacamma cyaneiventre*. *Molecular Ecology*, **8**, 1957–1969.

Herbers JM & Mouser RL (1998) Microsatellite DNA markers reveal details of social structure in forest ants. *Molecular Ecology*, **7**, 299–306.

Peeters C (1991) The occurrence of sexual reproduction among ant workers. *Biological Journal of the Linnean Society*, **44**, 141–152.

Raymond M & Rousset F (1995) GENEPOP (Version 3.2) population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Sambrook J, Fritsch E, Maniatis T (1989) *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor Laboratory Press, New York.