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PRIMER NOTE

Eight highly polymorphic microsatellite markers for the army ant *Eciton burchellii*

A. JAY DENNY, NIGEL R. FRANKS and KEITH J. EDWARDS
School of Biological Sciences, University of Bristol, Woodlands Road, Bristol, BS8 1UG, UK

Abstract

The army ant *Eciton burchellii* is a nomadic predator in the rain forests of Central and South America. Detailed work has documented many aspects of this species' ecology, behaviour and life history. However, a detailed investigation into within colony relatedness structure requires the development of genetic tools. Here we present eight microsatellite markers with between nine and 25 alleles. For each loci there is close agreement between observed and expected heterozygosity.

Keywords: Army ant, *Eciton*, hymenoptera, microsatellite

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A population of the army ant *Eciton burchellii* from Barro Colorado Island, Panama, has been studied intensively for 70 years (Rettenmeyer 1963; Franks 1985; Boswell *et al.* 1998). These behavioural and ecological studies have led to a good understanding of the colony life cycle. Colonies have a single queen and may have up to half a million workers. They follow a regular 35-day activity cycle during which a new cohort of workers is raised. If colonies are sufficiently large at the start of the annual dry season they may produce a reproductive brood of about 4000 males and a small number of new queens (Franks 1985; Franks & Hölldobler 1987). When the males and new queens emerge from their pupal cases the old parental colony reproduces by binary fission, splitting into two daughter colonies. This occurs in approximately one third of the 50 colonies on Barro Colorado Island each year (Franks 1985).

Despite this detailed information, certain life history characteristics of *Eciton burchellii* remain unknown. One important consideration in social hymenoptera is the paternity level within colonies (Bourke & Franks 1995). Multiple mating of colony queens leads to variable levels of relatedness between colony members (Pamilo 1991), allowing the predictions of kin selection theory (Hamilton 1964) to be rigorously tested. In addition, the mean mating frequency of queens within a population is required for the calculation of the effective population size, a measure that has implications for conservation practice (Chapman & Bourke 2001). The development of highly variable microsatellite markers has allowed detailed analysis of parentage and relatedness of individuals within colonies of some ant species (e.g. Boomsma & Ratnieks 1996; Hammond *et al.* 2001). However, no such tools have as yet been developed for army ants. Here we address this shortfall and present eight microsatellite markers for the army ant *Eciton burchellii*.

We extracted total genomic DNA from 250 mg of worker ants (20 individuals from two colonies) using a phenol/chloroform protocol (Batley *et al.* 1998). One microgram of this DNA was digested with 2 units of *Rsa*I in a volume of 30 μL for 2 h at 37 °C. To this we added half a microlitre of 10 mM ATP, 1 unit of T_4 DNA ligase and 50 ng of *Mlu*I adaptor (consisting of a 21-mer: 5 CTCTTGCTTACGCGTGGACTA3 and a phosphorylated 25-mer: 5 pTAGTCCACGCGTAAGCAAGAGCACA3) before incubation at 37 °C for 3 h. The ligated DNA was then denatured by boiling for 5 min. The library was enriched for microsatellites following the protocol of Edwards *et al.* (1996) using a Hybond N+ filter bound with either a $(CA)_{20}$ or $(CT)_{20}$ oligonucleotide. Plasmids from individual colonies were prepared using a Qiagen R.E.A.L. kit and sequenced using a DYEnamic ET dye terminator cycle sequencing kit and M13 forward primer on a MegaBACE 1000 capillary sequencer (Amersham Pharmacia Biotech Ltd). No CA dinucleotide repeat sequences were found from 288 sequenced clones. However, 288 sequenced clones enriched for CT microsatellites yielded a return of 35%. Primer sequences were determined for 10 loci using the software PRIMER 3 (Rozen & Skatetsky 2000). These were selected because of

Correspondence: Jay Denny. E-mail: jay.denny@bristol.ac.uk

Table 1 Primer sequences and characteristics of eight microsatellite loci in *Eciton burchellii*. Sequences are registered with the GenBank database under the Accession Numbers AY499659 to AY499666. T_a: annealing temperature; N_a: number of alleles observed; H_O: observed heterozygosity; H_E: expected heterozygosity

Locus	Primer sequences (5 –3)	T_a (°C)	Repeat motif	Clone Allele	Allele size range (bp)	N_a	H_O	H_E
Eb04	TCGGAGTTTAATAACGGAACG GAATCGATCGCCTCGAAGTA	55	$(CT)_{34}$	128	110–151	16	0.85	0.84
Eb10	TTGTATTGAACGACCACACGA CGCAGTCTCCGGTGTAGAAC	55	$(CT)_{14}CG(CT)_{11}TT(CT)_{11}$	191	191–228	11	0.75	0.69
Eb14	GCAGTGCTGATGCAAACG GAGGATGGATCGTCCGATAA	55	$(CT)_{31}$	192	178–219	19	0.89	0.90
Eb21	TGGCCGTGTTTCCACTTT TGATAGGGAGATGGGGACAG	55	$(CT)_{26}$	158	150–195	16	0.96	0.89
Eb24	GACAAAGACGCGAGATGCTA AGGGACACTCGTTGTTCGTC	55	$(CT)_{27}CG(CT)_{11}$	186	143–276	25	0.95	0.93
Eb25	GCTGCAGACGGAAGAGGTTA CTCAAGTGTCCTTCGCGACT	55	$(CT)_{21}$	156	156–191	15	0.85	0.86
Eb42	AGCAGGGTATCATGGCGTAG TGTGACGATCGCCTAACATT	55	$(CT)_5CC(CT)_{11}$	108	106–129	9	0.83	0.82
Eb51	AATCGCAGGGAAAGTAACGA GGTCCCTTAGGTGTGACTCG	55	$(CT)_{14}T_4(CT)_{27}$	152	129–176	19	0.89	0.90

a long, relatively clean (few extraneous bases) repeat motif. Nine loci were found to be polymorphic. However, one of these showed evidence of null alleles and was rejected.

We tested the markers on 160 worker ants from five colonies. DNA was extracted from individuals by phenol/chloroform extraction. One microlitre of template DNA (approx. 25 ng), 7.5 pmol of forward and reverse primer, 200 μM of each dNTP (Promega), 2.5 μL of 10x reaction buffer (Qiagen) and 1 unit of Hotstart *Taq* DNA polymerase (Qiagen) were used in a 25 μL volume polymerase chain reaction (PCR). Amplifications were performed in a GeneAmp PCR system 9700 with the following thermal profile. Thirty-five cycles of 94 °C, 30 s, 55 °C 30 s, 72 °C 60 s with a final extension at 72 °C for 10 min. Each forward primer was 5 end labelled with a fluorescent dye, either 6-FAM, HEX, or TET, and visualized against ET-Rox 400 bp size standard using the MegaBACE 1000. Fragment sizing and allele calling were performed with the MegaBACE-associated software, GENETIC PROFILER. Allele calling was rechecked by eye. Allelic diversity, and average observed and expected heterozygosity were calculated using GENEPOP (Raymond & Rousset 1995). The number of alleles ranged from 9 (*Eb42*) to 25 (*Eb24*) and for all loci the observed heterozygosity agreed closely with the expected heterozygosity (Table 1). *Eb21* was the only locus to deviate significantly from Hardy-Weinburg expectations $\chi^2_1 = 6.79$, $P < 0.01$), although, because of the high proportion of heterozygotes at this locus this was not due to the presence of null alleles. DNA from 48 workers was re-amplified and samples genotyped at all eight loci. In all cases the initial and repeat worker genotype were in agreement.

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