Molecular Ecology Notes (2002) **2**, 404–407 doi: 10.1046/j.1471-8278 .2002.00254.x

PRIMER NOTE

PCR primers for polymorphic microsatellite loci in the facultatively polygynous plant-ant *Petalomyrmex phylax* (Formicidae)

A. DALECKY,* G. DEBOUT,* G. MONDOR,† J.-Y. RASPLUS† and A. ESTOUP†
CEFE–CNRS, 1919 route de Mende, 34293 Montpellier Cedex 5, France, †INRA–CBGP, campus international de Baillarguet, 34988 Montferrier/Lez Cedex, France

Abstract

Fourteen microsatellite loci were isolated and their level of polymorphism characterized in two populations of the facultatively polygynous plant-ant *Petalomyrmex phylax* (Formicinae). High levels of within-population variation were observed at most loci, with number of alleles ranging from two to 15, and heterozygosity from 0.050 to 0.925. Cross-species amplification of these loci was also tested in four plant-ant species belonging to three other genera, *Aphomomyrmex*, *Cladomyrma* (both Formicinae) and *Cataulacus* (Myrmicinae).

Keywords: Formicidae, microsatellites, Myrmelachistini, plant-ant relationships, *Petalomyrmex*, social insect

Received 27 February 2002; revision received 9 May 2002; accepted 9 May 2002

Communal breeding in eusocial species, particularly the occurrence of multiple reproductive individuals in the same colony, provides opportunities to study conflicts within societies (e.g. reproductive and sex allocation, reproductive skew; Bourke & Franks 1995; Crozier & Pamilo 1996). *Petalomyrmex phylax* is an ant associated with the myrmecophytic tree *Leonardoxa africana africana* (Leguminosae: Caesalpinioideae) in rain forests of Cameroon (McKey 1984, 2000; Gaume *et al*. 1997). In this species, some populations contain only single-queened colonies, whereas in other populations supernumerary queens are accepted (Gaume 1997). We isolated microsatellite loci in *P. phylax* in order to study the genetic structure of populations and colonies in this species, as well as to test predictions based on kin selection in relation to the monogynous/polygynous status of colonies.

Microsatellites were isolated following Estoup *et al*. (1993) and Estoup & Cornuet (1994) (detailed protocol available at http://www.inapg.inra.fr/dsa/microsat/microsat.htm). Briefly, total genomic DNA was extracted from a pool of 240 individuals (larvae and pupae) using the DNeasy™ kit (Qiagen). A partial genomic library was then constructed by ligating 400–900 bp Sau3A-digested DNA (50 ng) into a pUC-18 phagemic vector (Amersham) and transforming Epicurian coli Supercompetent Cells (Stratagene). A total of 1763 transformant (white) clones was transferred on Hybon-N nylon membranes (Amersham), and hybridized at 48 °C with a mixture of oligonucleotide probes $(TC)_{10}$, $(TG)_{10}$, $(CAC)_5CA$, $CT(CCT)_5$, $CT(ATCT)_6$, $(TGTA)_6TG$ labelled with the DIG oligonucleotide tailing kit (Boehringer). Of these clones, 102 (5.8%) gave a positive signal revealed by the DIG detection kit (Boehringer), and the insert was sequenced for 58 of these positive clones. From a total of 21 primer pairs designed using OLIGO 3.3 (Rychlik & Rhoads 1989), 14 pairs gave satisfactory amplification patterns (i.e. polymerase chain reaction (PCR) products of the predicted size, and stutter bands of low intensity). The primer sequences of the 14 loci are given in Table 1.

PCR amplifications were carried out in 10-μL final volume, which contained 2-μL of extracted DNA, 0.48–0.60-μL of 25 mM $MgCl_2$ (see Table 1), 0.3-μL of 2.5 mM dNTP, 1-μL of buffer 10× (750 mM Tris-HCl pH 8.8; 200 mM $(NH_4)_2SO_4$, 0.1% (v/v) Tween 20), 0.5 units of *Taq* DNA polymerase (Eurogentec Red GoldStar™), 0.022 pmol of reverse primer labelled with [γ^{33}P]-dATP, and 0.4 pmol of unlabelled forward primer. PCRs were performed in

Correspondence: A. Dalecky. Fax: 33 4 67 41 21 38; E-mail: ambroise.dalecky@cefe.cnrs-mop.fr

Table 1 Primer sequences, PCR conditions and polymorphism statistics for 14 microsatellite loci in two populations of *Petalomyrmex phylax*. Repeat motif is listed 5′–3′ with respect to the forward primer (F)

Locus	Primer sequences (5′–3′)	Repeat motif in library	Size (bp)	T_a (°C) [$MgCl_2$] (mM)	Size range (bp)	Ebodjé			Bondé		
						n	N_a	H_O/H_E	*n*	N_a	H_O/H_E
Pet3	F: TTGTCTTTCGCAAACATTTC R: ATAAACAAATGAAACGAAAAGTC	$(GT)_{10}TT(GT)_3$	289	53 [1.2]	283–299	17	5	0.529/0.453	19	6	0.737/0.659
Pet4	F: AAGATGTGCAGAGGGTGTGTAC R: GGATACCGCACATTGTTCTG	$(GT)_{17}AC(GT)_2GC(GT)_3$	126	55 [1.2]	92–138	18	8	0.611/0.725	19	10	0.684/0.835
Pet16b	F: CGTCACCCGTGTGTTCG R: GTTCCGTTCTCCACATTCAG	$(AG)_{12}G(AG)_8$	207	55 [1.2]	207–241	14	5	0.500/0.529	20	9	0.600/0.776
Pet29	F: TATAGGTCGGCAAAAAGAATG R: CTGTCGGAGAGTGAAAATCG	$(GA)_{12}$	106	55 [1.2]	94–118	18	6	0.632/0.555	20	4	0.800/0.706
Pet30b	F: CGTTAGGTAACCACATTAGG R: AAGTGCCGTAATAAAGGG	$(TC)_2TA(TC)_2TTT(TC)_{13}T(TC)_2$	152	50 [1.2]	142–178	18	13	1.000/0.925	20	10	0.950/0.885
Pet32	F: GCGTTATCTATAAACCATCTCCG R: TCCTCACCCGCACTATTTTG	$(TC)_{12}CC(TC)_7$	204	58 [1.2]	204–228	19	8	0.789/0.853	20	10	0.750/0.812
Pet36a	F: TCTTCCCTGTAACACCGCAC R: GCTTTTTCACCCGACATACG	$(TC)_{17}GTG(GAC)_2G(TC)_8$	132	58 [1.2]	94–160	20	15	0.850/0.883	20	13	1.000/0.868
Pet37	F: ATTTGCTGCGTTTCCAG R: AGCGGCGGTGTAGTATG	$(TG)_{12}$	240	50 [1.2]	239–279	17	8	0.765/0.815	19	11	0.684/0.814
Pet41	F: ATAATGAGAAACACCTTGGC R: CGGTATTGTGGCGTAGG	$(TC)_{20}$	223	50 [1.2]	205–253	19	9	0.737/0.848	20	15	0.900/0.886
Pet44	F: GCCCGGAGGTTGACAATATAC R: GTCTCTGGTTTCGCAATAAAATG	$(GA)_{14}$	102	53 [1.5]	93–102	20	2	0.050/0.050	20	5	0.500/0.612
Pet81	F: GCTCTCGCCTCACGCAG R: CGAGAGTGAGTTGGAATCAGG	$(CA)_{14}$	208	55 [1.2]	199–216	18	4	0.444/0.449	20	8	0.750/0.792
Pet83	F: GGCAAACAACCACCCTCC R: AGAAACGAGGCGAATGGC	$(TG)_{11}$	309	55 [1.2]	305–313	19	4	0.684/0.602	20	4	0.400/0.599
Pet90a	F: TTGCGACACCAGAAGGG R: CGACTTGCGAGAGACCG	$(GAG)_8$	125	55 [0.8]	116–128	19	2	0.158/0.149	20	4	0.400/0.556
Pet97	F: ATGCGTTTGAATGTAGATGG R: TTAATGGTGCGACGATACTC	$(TC)_{14}$	142	53 [1.2]	116–150	19	5	0.632/0.599	19	5	0.895/0.761

'Size' refers to the length of the cloned allele. T_a: lower annealing temperature.
[$MgCl_2$]: concentration of $MgCl_2$ for PCR reactions.
n: number of individuals analysed.
N_a: number of allele size variants observed.
H_O: observed proportion of heterozygous individuals.
H_E: heterozygosity (i.e. gene diversity; Nei 1987).
GenBank accession nos of primer sequences AF478112–AF478125.

Table 2 Cross-species amplification results for 14 microsatellites isolated in *P. phylax*. *n* is the number of individuals analysed (one worker per colony). Number of allele size variants between brackets

Species	*n*	Pet3	Pet4	Pet16b	Pet29	Pet30b	Pet32	Pet36a	Pet37	Pet41	Pet44	Pet81	Pet83	Pet90a	Pet97
Aphomomyrmex afer	7	[2] (243–245)	—	[4] (183–205)	[6] (96–140)	—	—	—	—	[8] (205–259)	[3] (92–96)	[1] (196)	[1] (311)	—	[5] (110–136)
Cladomyrma maschwitzi	3	—	—	—	—	—	[2] (176–203)	—	—	—	—	[1] (188)	—	—	[1] (154)
Cladomyrma yongi	2	—	—	—	—	—	[2] (179–181)	—	—	—	—	[1] (164)	—	—	[1] (154)
Cataulacus mckeyi	5	—	—	—	—	—	[2] (202–204)	—	—	—	—	[1] (224)	—	—	—

Allele size range between parentheses. –: no PCR amplification observed. Heterozygosities were not calculated as most individuals were collected from different populations.

a PTC-100™ thermal cycler using the following cycling conditions: initial denaturation at 94 °C (1 min); five 'touchdown' cycles (10 cycles for locus Pet4): 92 °C (20 s), 1 °C drop per cycle to a final annealing temperature of T_a + 1 °C (30 s) (Table 1), 72 °C (1 min); followed by a further 25 cycles of 92 °C (20 s), T_a (30 s), 72 °C (1 min); and a final elongation at 72 °C (10 min). PCR products were separated by electrophoresis in a 5% denaturing polyacrylamide gel, and visualized by autoradiography using the cloned allele as size reference and H_2O instead of DNA extract as negative control.

Microsatellite loci were genotyped for a total of 40 diploid individuals sampled in two populations located in Ebodjé (2°33′58.4″ N, 9°50′37.1″ E) and Bondé (3°13′18.6″ N, 10°15′00.6″E), Cameroon (20 individuals per population, one worker per colony). All 14 loci were polymorphic, with an observed number of alleles per population ranging between two and 15, and heterozygosities between 0.050 and 0.925 (Table 1). No significant deviation from Hardy–Weinberg equilibrium and linkage equilibrium between loci was observed in both population samples when exact tests (GENEPOP 3.3 package; Raymond & Rousset 1995) and correction for multiple tests (sequential Bonferroni procedure) were performed.

Cross-species amplifications were tested on workers from four plant-ant species belonging to three other genera: *Aphomomyrmex afer*, *Cladomyrma maschwitzi*, *C. yongi* and *Cataulacus mckeyi* (Table 2). PCR conditions were identical to those used in *P. phylax*. In agreement with phylogenetic relationships (Chenuil & McKey 1996), the closely related species *A. afer* amplified successfully for eight of the 14 microsatellites and showed polymorphism (more than one allele size variant) at six of them, while more distantly related species could be amplified at only two or three loci.

Acknowledgements

We thank the INRA–CBGP lab for welcoming us and for help during library construction, and the CEFE–CNRS laboratory during genotyping. We thank J. Moog for providing *Cladomyrma*, and L. Gaume and D. McKey for providing *Aphomomyrmex* samples. We thank F. Kjellberg, D. McKey and K. Livingstone for critical reading of the manuscript. This work was supported by a grant from the Institut Français de la Biodiversité.

References

Bourke AFG, Franks NR (1995) *Social Evolution in Ants*. Princeton University Press, Princeton.

Chenuil A, McKey DB (1996) Molecular phylogenetic study of a myrmecophyte symbiosis: did *Leonardoxa*/ant associations diversify via cospeciation? *Molecular Phylogenetics and Evolution*, **6**, 270–286.

Crozier RH, Pamilo P (1996) *Evolution of Social Insect Colonies: Sex Allocation and Kin Selection*. Oxford University Press, Oxford.

Estoup A, Cornuet JM (1994) Utilisation de sondes oligonucléotides marquées à la digoxigénine pour la recherche de microsatellites. *Journal des Biotechnologies, Boehringer Mannheim*, **10**, 5–7.

Estoup A, Solignac M, Harry M, Cornuet JM (1993) Characterization of $(GT)_n$ and $(CT)_n$ microsatellites in two insect species: *Apis mellifera* and *Bombus terrestris*. *Nucleic Acids Research*, **21**, 1427–1431.

Gaume L (1997) *Mutualisme, parasitisme, et évolution des symbioses plantes-fourmis: le cas de* Leonardoxa *(Légumineuse) et de ses fourmis associées*. PhD Thesis, Ecole Nationale Supérieure d'Agronomie de Montpellier.

Gaume L, McKey DB, Anstett MC (1997) Benefits conferred by 'timid' ants: active anti-herbivore protection of the rainforest tree *Leonardoxa africana* by the minute ant *Petalomyrmex phylax*. *Oecologia*, **112**, 209–216.

McKey DB (1984) Interaction of the ant-plant *Leonardoxa africana* (Caesalpiniaceae) with its obligate inhabitants in a rainforest in Cameroon. *Biotropica*, **16**, 81–99.

McKey DB (2000) *Leonardoxa africana* (Leguminosae: Caesalpinioideae): a complex of mostly allopatric subspecies. *Adansonia, Sér. 3*, **22**, 71–109.

Nei M (1987) *Molecular Evolutionary Genetics*, 2nd edn, p. 512. Columbia University Press, New York.

Raymond M, Rousset F (1995) GENEPOP Version 1.2: population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Rychlik W, Rhoads RE (1989) A computer program for choosing optimal oligonucleotides for filter hybridization, sequencing and in vitro amplification of DNA. *Nucleic Acids Research*, **17**, 8543–8551.