PRIMER NOTE

Characterization of nuclear DNA microsatellite markers in the ant *Cataglyphis cursor*

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Abstract

The ant *Cataglyphis cursor* is exceptional in that unmated workers are potentially able to lay both male and female eggs. We characterized eight pairs of primers for microsatellite loci, developed from genomic DNA for this species. Variability was tested with DNA from 19 workers and all eight loci were highly polymorphic, displaying 5–10 alleles and a high level of heterozygosity. Cross-species amplifications indicate that these microsatellites might be useful in genetic studies of other species belonging to the genus *Cataglyphis*.

Keywords: ant, Cataglyphis, cross-species amplifications, microsatellite, thelytoky

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Kin selection (Hamilton 1964) explains the evolution of a sterile worker caste among social insects in a context of natural selection. Altruistic individuals do not reproduce but still can transmit copies of their genes to the next generation by favouring reproduction of kin, such as parents and siblings. Ants are a well known example of societies where workers sacrifice their own reproduction to participate in cooperative tasks, such as foraging, nest building or rearing offspring nestmates (Hölldobler & Wilson 1990). In the ant *Cataglyphis cursor*, workers have kept functional ovaries and can lay eggs that will develop into males, by arrhenotokous parthenogenesis, but also into females, by thelytokous parthenogenesis (Cagniant 1979). Worker reproduction is rare in the Formicidae and thelyto-kous parthenogenesis has been shown in only four other species: *Pristomyrmex pungens* (Itow *et al.* 1984), *Cerapachys biroi* (Tsuji & Yamauchi 1995), *Platythyrea punctata* (Heinze & Hölldobler 1995) and *Messor capitatus* (Grasso *et al.* 2000). Despite its peculiar reproductive biology, population and colony genetic structure in *C. cursor* remains unknown and no genetic markers are available for the genus.

Here, we characterize eight highly polymorphic micro-satellite markers from *Cataglyphis cursor* nuclear DNA. We also report the results of cross-amplification of these primers on five other *Cataglyphis* species belonging to four

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different groups of the genus: *C. bicolor* (*bicolor* group), *C. fortis* (*fortis* group), *C. mauritanicus* and *C. velox* (*altisquamis* group) and *C. nodus* (*niger* group). In addition to these five species, we also tested these primers on workers belonging to a remote *C. cursor* population from Mongolia.

Genomic DNA was extracted from three cocoons of *C. cursor* collected at Port Leucate (France) using a high salt procedure (Sambrook *et al.* 1989). RNA strands were removed by incubating the sample for 2 h at 37 °C with 1% RNAase. Genomic DNA was then partially digested with the enzyme *Sau*3A and a fraction ranging from 400 to 800 base pairs was isolated after electrophoresis in low melting aga-rose gel (*NuSieve®*). The resulting fragments were puri-fied with GFX PCR DNA and Gel Band Purification Kit (Amersham Biosciences), ligated into a phagemid vector (*pbluescript®II SK*(+), Stratagene), and cloned in *Escherichia coli* XL-1 Blue competent cells (Stratagene). Synthetic oligo-nucleotides $(TC)_{10}$ and $(TG)_{10}$, labelled with the DIG system (Boehringer Mannheim) were used to screen about 3150 recombinant colonies. Out of the 73 positives clones that were successfully sequenced (single strand) by Genome Express (France), 65 contained repeated patterns. Twenty-seven sequences contained mononucleotide sequences: 8 polyA and 21 polyT. Thirty-eight sequences contained dinucleotide sequences. From these sequences, 20 pairs of primers were designed using the online software PRIMER 3 (Rozen & Skaletsky 2000; URL: http://www.molbiol.ox.ac. uk/cgi-bin/primer3_www.cgi/). After a prescreening using

Table 1 Characteristics of microsatellite loci in the ant *Cataglyphis cursor*. The size range, the number of alleles (N_a), the observed (H_O) and expected (H_E) heterozygosities and the frequency of the most common allele [Freq (A)] are based on 19 workers collected in St Hyppolite (France) in 2001

Locus	AN	Repeat motif	Size (bp)	N_a	H_O	H_E	Freq (A)	Primers (5′–3′)
Ccur11	AY590645	[GA]$_{15}$	239–279	10	0.851	0.827	0.237	F: GATTGGCTTGGCGGTATC*
								R: GGTCGCAAGAGTCACGAG
Ccur26	AY590646	[GA]$_{14}$	102–114	5	0.781	0.780	0.333	F: TGACTCGTGATCTGCTCGTG*
								R: ATGGGCGTGAATCTCTCTTTC
Ccur46	AY590647	[TC]$_{17}$	151–181	10	0.750	0.733	0.474	F: TGACAACTACCTGCAATAGACG*
								R: CCAACCAACGAGATTCGAC
Ccur58	AY590651	[TG]$_{15}$	134–182	9	0.833	0.810	0.316	F: TAGTTGGCGTGCTCGAATG*
								R: TGTGCCTGTCGTTGAATCTC
Ccur63b	AY590654	[GA]$_{19}$	180–214	9	0.787	0.765	0.421	F: GTGTATTAACTCTCCGAAGCAAG*
								R: GTACCTCACCTGGCATACCG
Ccur76	AY590656	[TG]$_{19}$	190–212	9	0.799	0.800	0.389	F: TGCCTCCCGTTGAGTATG
								R: TGCTTGGAAGGTAAATGAGC*
Ccur89	AY590658	[AG]$_{22}$	126–144	9	0.729	0.780	0.500	F: CTGAAGTCCTCCGACATATAGC
								R: GAACGAGAACCAGGCAAGG*
Ccur99	AY590662	[CA]$_8$	101–137	10	0.840	0.840	0.306	F: GCGGAACATTACACGCATAC*
								R: GTTGAATGACCAGTAACACACG

*Labelled primer.

Table 2 Cross-species amplifications of four polymorphic microsatellite loci. The localization of the sampling is indicated for each species, and the number of individuals (workers) screened is given in brackets. The size range of the amplification product is given for each locus together with the number of alleles found among the sample (in brackets)

Locus	C. fortis (3) Tunisia	C. velox (2) Spain	C. mauritanicus (5) Tunisia	C. bicolor (6) Tunisia	C. nodus (3) Greece	C. cursor (2) Mongolia
Ccur11	243 (1)	245–257 (2)	242–250 (3)	258–267 (3)	—	239–243 (2)
Ccur26	106–126 (2)	104–121 (2)	100–106 (4)	109–119 (3)	94 (1)	94–112 (2)
Ccur46	137 (1)	—	—	—	—	137 (1)
Ccur58	138 (1)	175–193 (2)	138–201 (4)	154 (1)	—	145–154 (2)
Ccur63b	171 (1)	164–185 (2)	171–185 (6)	171 (1)	—	179–192 (2)
Ccur76	—	176–184 (3)	184–194 (4)	—	194–198 (2)	196–206 (3)
Ccur89	118–124 (2)	113–121 (2)	112–114 (2)	118–123 (3)	118 (1)	112–118 (2)
Ccur99	—	122–132 (2)	90–94 (2)	100–117 (2)	102 (1)	103–117 (2)

radioactive dATP, eight markers out of the nine that successfully amplified were selected on the basis of the quality of amplification and potential variability. Their variability was then assessed further on a sample of 19 workers originating from 19 different colonies (one worker per colony) collected in a population close to St Hyppolite (France) in 2001.

Amplifications were carried out in a 10 µL volume containing about 20 ng of genomic DNA, 0.19 mM of each dNTP, 0.25 µM of each primer, 1X *Taq* Buffer (containing 15 mM MgCl$_2$) and 0.03 U *Taq* DNA polymerase (Qiagen). Amplifications were performed in a GeneAmp PCR system 2700 (Applied Biosystems) thermal cycler with the following parameters: after an initial denaturing step of 2 min at 94 °C, the PCR consisted of 10 cycles of 15 s at 94 °C, 15 s at 52 °C (the annealing temperature) and 30 s at 72 °C, followed by 20 cycles of 15 s at 89 °C (to preserve the fluorescent markers), 15 s at 52 °C and 30 s at 72 °C, with 10 more minutes at 72 °C for the final extension. Amplified

fluorescent fragments were visualized using the automated Applied Biosystems Prism 310 sequencer (Perkin-Elmer, USA). The size of the different alleles was determined using the ROX-400HD internal size standard and the GENESCAN version 3.2.1 analysis software (Applied Biosystems).

All eight loci were highly polymorphic (Table 1). The expected heterozygosity (H_E) ranged from 0.73 to 0.83 and we found five to 10 alleles within the study population. The probability of deviation from Hardy–Weinberg equilibrium or linkage disequilibrium between pair of loci were both calculated using GENEPOP version 3.4 (Raymond & Rousset 1995), and alpha was adjusted using sequential Bonferroni correction for multiple tests. No significant deviation from Hardy–Weinberg equilibrium were detected for any locus, and all pairwise tests of linkage disequilibrium were nonsignificant.

Results of the cross-species amplifications are given in Table 2. Seven of the eight loci amplified successfully on

most other *Cataglyphis* species. The locus Ccur46 successfully amplified only in samples of *C. fortis* and *C. cursor* from Mongolia. The occurrence of different alleles indicate that these primers are potentially useful for genetic studies in other species of this genus.

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