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* (Tsuj & 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
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 Sau3A and a fraction ranging from 400 to 800 base
pairs was isolated after electrophoresis in low melting aga-
nose 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

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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 MgCl₂) 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 (Hₑ) 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 GENEPOL 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

<table>
<thead>
<tr>
<th>Locus</th>
<th>AN</th>
<th>Repeat motif</th>
<th>Size (bp)</th>
<th>Nₐ</th>
<th>Hₒ</th>
<th>Hₑ</th>
<th>Freq (A)</th>
<th>Primers (5′–3′)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ccur11</td>
<td>AY590645</td>
<td>[GA]₁₅</td>
<td>239–279</td>
<td>10</td>
<td>0.851</td>
<td>0.827</td>
<td>0.237</td>
<td>F: GATTTGCTCTGCAGTGATAC*&lt;br&gt;R: GCTGCAAGATCTGCACAG</td>
</tr>
<tr>
<td>Ccur26</td>
<td>AY590646</td>
<td>[GA]₁₄</td>
<td>102–114</td>
<td>5</td>
<td>0.781</td>
<td>0.780</td>
<td>0.333</td>
<td>F: TGGCAACTTGCTGTCTGATG*&lt;br&gt;R: ATGCGCCGATATCTCTTCTC</td>
</tr>
<tr>
<td>Ccur46</td>
<td>AY590647</td>
<td>[TC]₁₇</td>
<td>151–181</td>
<td>10</td>
<td>0.750</td>
<td>0.733</td>
<td>0.474</td>
<td>F: TGCACACTCTCCGATAGAG*&lt;br&gt;R: CACCAACAGATGGATGCAC</td>
</tr>
<tr>
<td>Ccur58</td>
<td>AY590651</td>
<td>[TG]₁₅</td>
<td>134–182</td>
<td>9</td>
<td>0.833</td>
<td>0.810</td>
<td>0.316</td>
<td>F: TGATGTTGTGTTGCGAGTGG*&lt;br&gt;R: TGTCCTCTGCTGGATGATCG</td>
</tr>
<tr>
<td>Ccur63b</td>
<td>AY590654</td>
<td>[GA]₁₉</td>
<td>180–214</td>
<td>9</td>
<td>0.787</td>
<td>0.765</td>
<td>0.421</td>
<td>F: GGTATTTAATGTCTGCGAGAG*&lt;br&gt;R: GTACCTACCTGCGGATGC</td>
</tr>
<tr>
<td>Ccur76</td>
<td>AY590656</td>
<td>[TG]₁₉</td>
<td>190–212</td>
<td>9</td>
<td>0.799</td>
<td>0.800</td>
<td>0.389</td>
<td>F: TGGCTCCGCGTTGAATGAG*&lt;br&gt;R: TGCTGCTGAGAAATGAGG</td>
</tr>
<tr>
<td>Ccur89</td>
<td>AY590658</td>
<td>[AG]₂₂</td>
<td>126–144</td>
<td>9</td>
<td>0.729</td>
<td>0.780</td>
<td>0.500</td>
<td>F: CTGAGAATTCCTCGCGACTATAG*&lt;br&gt;R: GNAGCAAG GGCGAGAGG</td>
</tr>
<tr>
<td>Ccur99</td>
<td>AY590662</td>
<td>[CA]₆ᵬ</td>
<td>101–137</td>
<td>10</td>
<td>0.840</td>
<td>0.840</td>
<td>0.306</td>
<td>F: GCGGAAATGCTAGCCTGAC*&lt;br&gt;R: GGTATAGGCGAGAAGCGAC</td>
</tr>
</tbody>
</table>

*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)

<table>
<thead>
<tr>
<th>Locus</th>
<th>C. fortis (3)</th>
<th>C. velox (2)</th>
<th>C. mauritanicus (5)</th>
<th>C. bicolor (6)</th>
<th>C. nodus (3)</th>
<th>C. cursor (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ccur11</td>
<td>Tunisia</td>
<td>Spain</td>
<td>Tunisia</td>
<td>Tunisia</td>
<td>Greece</td>
<td>Mongolia</td>
</tr>
<tr>
<td></td>
<td>243 (1)</td>
<td>254–257 (2)</td>
<td>242–250 (3)</td>
<td>258–267 (3)</td>
<td>—</td>
<td>239–243 (2)</td>
</tr>
<tr>
<td>Ccur26</td>
<td>106–126 (2)</td>
<td>104–121 (2)</td>
<td>100–106 (4)</td>
<td>109–119 (3)</td>
<td>94 (1)</td>
<td>94–112 (2)</td>
</tr>
<tr>
<td>Ccur46</td>
<td>137 (1)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>137 (1)</td>
</tr>
<tr>
<td>Ccur58</td>
<td>138 (1)</td>
<td>175–193 (2)</td>
<td>138–201 (4)</td>
<td>154 (1)</td>
<td>—</td>
<td>145–154 (2)</td>
</tr>
<tr>
<td>Ccur63b</td>
<td>171 (1)</td>
<td>164–185 (2)</td>
<td>171–185 (6)</td>
<td>171 (1)</td>
<td>—</td>
<td>179–192 (2)</td>
</tr>
<tr>
<td>Ccur76</td>
<td>—</td>
<td>176–184 (3)</td>
<td>184–194 (4)</td>
<td>—</td>
<td>194–196 (2)</td>
<td>196–206 (3)</td>
</tr>
<tr>
<td>Ccur89</td>
<td>118–124 (2)</td>
<td>113–121 (2)</td>
<td>112–114 (2)</td>
<td>118–123 (3)</td>
<td>118 (1)</td>
<td>112–118 (2)</td>
</tr>
<tr>
<td>Ccur99</td>
<td>—</td>
<td>122–132 (2)</td>
<td>90–94 (2)</td>
<td>100–117 (2)</td>
<td>102 (1)</td>
<td>103–117 (2)</td>
</tr>
</tbody>
</table>
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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