Development of single sequence repeat markers for the ant Aphaenogaster senilis and cross-species amplification in A. iberica, A. gibbosa, A. subterranea and Messor maroccanus

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Abstract Eight polymorphic microsatellite loci were isolated and characterized for *Aphaenogaster senilis*, a common ant species distributed in the Western Mediterranean. Characterization of 15 individuals form southern Spain showed moderate to high allelic diversity ranging from 2 to 9 alleles per locus. Cross-species tests on 10 individuals of *A. iberica*, *A. gibbosa*, *A. subterranea* and *Messor maroccanus* revealed successful amplification for most loci. This set of markers can be useful for population genetic studies and might even prove useful in other phylogenetically close species of the subfamily Myrmicinae.

The ant *Aphaenogaster senilis* is a common species distributed in the Western Mediterranean basin from South France to Morocco (Cagniant et al. 1991). Strictly monogynous colonies (one single queen per colony) are formed by fission whereby adult colonies split in two fragments (Boulay et al. 2007). The old queen heads one fragment while the other contains a newly mated queen, daughter of the old queen. Such a reproductive replacement in the colony is expected to temporarily reduce the average relatedness between nestmates during the first step of colony formation (André et al. 2001). Because young queens dispersal occurs by foot, colony fission may also generate population viscosity and local resource competition (Pearcy and Aron 2006), that is the competition of related individuals for common resources.

Although *A. senilis* is a suitable model for studying the evolution of colony fission, genetic information is lacking. Here, we isolated and characterized polymorphic microsatellite markers for this species with the aim to investigate the underlying colony—and population level—mechanisms of fission. Furthermore, we report their variability on three congener species (*A. iberica*, *A. gibbosa* and *A. subterranea*) and one sympatric species of a related genus (*Messor maroccanus*).

Workers of *A. senilis* and *M. maroccanus* were collected at the Doñana National Park (South East Spain). Workers of *A. iberica* and *A. gibbosa* were collected near the village of Cazalla de la Sierra (South East Spain). Workers of *A. subterranea* were collected near the city of Tours (Centre of France). Microsatellite markers were identified for *A. senilis* through the development of an enriched genomic library as described by Glenn et al. (2000). DNA extractions were performed from brain tissue and approximately 10 µg of high molecular weight DNA was isolated by phenol-chloroform extraction (Sambrook et al. 1989). Simultaneous restriction-ligation of genomic DNA was carried out using *Rsa*I restriction enzyme and double stranded linker-adapted primers according to Hamilton et al. (1999). Ligated DNA was enriched with a biotin-labelled probe mixture consisting of $(GT)_{10}$ and $(CT)_{10}$ at 10 µM each. DNA fragments with repetitive sequences were then selectively captured by

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streptavidin-coated Dynabeads (Oxoid) and separated by a magnetic field. Enriched DNA was eluted in 200 µl dH_2O from the bead probes and concentrated by vacuum centrifugation to a final concentration of ~100 ng/µl. DNA was then reamplified by polymerase chain reaction (PCR), purified and ligated into a cloning vector using pGEM-T Easy Vector II (Promega). A total of 48 positive clones were screened and checked for inserts using ABI PRISM BigDye Terminator Cycle kit (Applied Biosystems) and resolved on an ABI 3100 Genetic Analyzer (Applied Biosystems). Primer pairs for eight potentially usable microsatellite loci were designed using the software package OLIGO 6.4. Polymorphism was tested by PCR reactions performed in 20 µl total volume, which include 50 ng of DNA, 2 mM of $MgCl_2$, 0.25 µM of each primer, 200 µM dNTP's, 1X reaction buffer [75 mM Tris–Hcl, 20 mM $(NH_4)_2SO_4$] and 0.5 units *Taq* polymerase (BIOTAQ). Reaction conditions were as follows: an initial denaturation step of 5 min at 95°C, 16 cycles consisting of 30 s at 92°C, 30 s at 66°C annealing temperature decreasing one degree/cycle, 30 s at 72°C followed by an additional 30 cycles consisting of 30 s at 92°C, 30 s at 50°C annealing temperature, 30 s at 72°C and a final elongation step of 10 min at 72°C. Microsatellite variability was assessed in 15 individuals from the different colonies. All were genotyped by assessing allele size on an ABI 3100 Genetic Analyzer (Applied Biosystems). Allele scoring was carried out using GENEMAPPER software version 3.5 (Applied Biosystems). Expected and observed values for heterozygosity were determined using ARLEQUIN V.2.0 (Schneider et al. 2000). The number of

Table 1 Characterization of eight *Aphaenogaster senilis* microsatellite loci ($N = 15$)

Accession no.	Locus	Repeat motif	Primer sequence (5′ → 3′)	Allele no.	Allele size (bp)	H_O	H_E	F_{IS}
EU491492	Asen12	$(TG)_{36}$	F: TGGTAGTTACATGAATGAAAGTCG	4	99–111	0.983	0.698	−0.435
			R: ATGGAGGGTGAAGGCAAC					
EU491493	Asen15	$(TG)_4TA(TG)_{13}$	F: TTTTTATTTCCGCCCTGTAG	2	102–104	0.5188	0.482	−0.075
			R: ATATTTCCCGCGCAATAATC					
EU491494	Asen58	$(CA)_{15}$	F: TCCGAAAGAATATCACTCTATTATTG	3	253–261	0.8656	0.605	−0.435
			R: GAAATTTGCTGCTCAATGTG					
EU491495	Asen83	$(GA)_{26}$	F: TCTGTCTCGTAAGTGGCTTTG	8	314–382	0.783	0.849	0.113
			R: CAGTTGGTAACTTTTCCAAAATC					
EU491496	Asen94	$(GA)_{17}G(GA)_8$	F: GCTATCTCTGCACCGAACTAC	9	165–194	0.791	0.853	0.089
			R: CTTTTCGTCGGCTAATTTTC					
EU491497	Asen155	$(CA)_9$	F: AACGACGGATGTGCTTTTTC	5	198–208	0.679	0.722	0.108
			R: TATAACGGTGAGCGTCATGG					
EU491498	Asen178	$(TC)_4CCC(TC)_{17}$	F: TCGGAGCCTTAAGTCAACAG	7	267–289	0.8	0.894	0.089
			R: GACGCACGTCATACACTAGG					
EU491499	Asen181	$(CT)_{22}$	F: TTTCCAAGCGGTTAAATTGC	8	168–188	0.952	0.893	−0.118
			R: ATCGCTCAAAATCTGCCTTG					

Note: H_O = observed heterozygosity; H_E = expected heterozygosity under Hardy–Weinberg equilibrium; F_{IS} = inbreeding coefficient

Table 2 Cross-species amplification of eight microsatellite loci from *A. senilis* in *A. iberica*, *A. gibbosa*, *A. subterranea* and *M. maroccanus*

Locus	*A. iberica* ($N = 10$)		*A. gibbosa* ($N = 10$)		*A. subterranea* ($N = 10$)		*M. maroccanus* ($N = 10$)	
	Allele no.	Range	Allele no.	Range	Allele no.	Range	Allele no.	Range
Asen12	4	99–109	4	95–119	7	93–117	4	93–99
Asen15	3	94–104	2	94–96	2	94–104	1	104
Asen58	na		na		na		na	
Asen83	na		3	300–310	na		na	
Asen94	3	141–151	3	153–155	2	147–153	2	133–135
Asen155	4	210–158	4	210–258	1	254	3	200–204
Asen178	2	261–265	2	265–273	1	261	na	
Asen181	na		na		na		na	

Note: na = no amplification

alleles per locus, allele size range as well as deviations from Hardy–Weinberg expectations and linkage disequilibrium between pairs of loci were estimated using FSTAT V.2.9 (Goudet 1995). All loci were polymorphic; the total number of alleles per locus and heterozygosity estimates are listed in Table 1. We found no evidence of linkage disequilibrium or deviations from Hardy–Weinberg expectations at any locus.

Polymorphism was examined in *A. iberica*, *A. gibbosa*, *A. subterranea* and *M. maroccanus* using the same conditions detailed for *A. senilis*. Four loci amplified in all species with the number of alleles ranging from 1 to 7 depending on the locus and the species (Table 2). The lowest success of amplification was encountered in *M. maroccanus*. This is consistent with the taxonomical relationship between the species. This set of markers can be useful for the evaluation of colony genetic composition, genetic diversity, demographic processes such as the monitoring of colony dispersal and might even prove useful in other phylogenetically close species of the subfamily Myrmicinae.

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