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MICROSATELLITE LETTERS

Development of twenty-one polymorphic microsatellite markers for the fungus-growing ant, *Mycocepurus goeldii* (Formicidae: Attini), using Illumina paired-end genomic sequencing

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Abstract Obligate social parasites, or inquilines, exploit the colonies of free-living social species and evolved at least 80 times in ants alone. Most species of the highly specialized inquiline social parasites are rare, only known from one or very few, geographically isolated populations, and the sexual offspring of most inquiline species mates inside the maternal colony. Therefore, inquiline populations are believed to be small and genetically homogeneous due to inbreeding. To comparatively study the genetic diversity of the socially parasitic fungus-growing ant, *Mycocepurus castrator,* and its only known host species, *Mycocepurus goeldii*, and to infer the parasite's conservation status, we developed 21 microsatellite markers for the host species, *M. goeldii*, and evaluated whether these markers cross-amplify in the social parasite, *M. castrator*. We isolated and characterized a total of 21 microsatellite loci for *M. goeldii.* The loci were screened for 24 individuals from geographically distant and genetically divergent populations in Brazil. The number of alleles per locus ranged from 18 to 4, the observed heterozygosity ranged from 0.25 to 0.636, and the probability of identity values ranged from 0.011 to 0.146. Preliminary analyses show that these markers cross amplify in the closely related social parasite species *M. castrator*. These newly developed loci provide tools for studying the genetic diversity and the evolution of social parasitism in the *Mycocepurus* host–parasite system.

Keywords Polygyny · Polyandry · Social parasitism · Inquilinism · PAL_FINDER · SSR

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Microsatellite letter

Socially parasitic ants exploit their host species to maximize their own fitness and evolved at least 250 times in ants alone, one-third of which ($n > 80$) are workerless inquiline parasites (Buschinger 2009). Although diverse in number of species, social parasites are rarely encountered (Hölldobler and Wilson 1990). Host specificity and restricted geographic distribution contribute to their rareness. Some parasites are likely inbred because their sexual offspring mate inside the maternal nest. Due to inbreeding and small population sizes it is believed that many parasite species are genetically homogeneous and potentially endangered, which is reflected by the fact that 89 % ($n = 133$) of the 149 ant species listed on the Red List of Threatened Species are social parasites (IUCN 2013), even

Table 1 Details for twenty-one polymorphic microsatellite loci characterized for *Mycocepurus goeldii*

Locus	Primer sequence 5′–>3′	Repeat motif	Size (bp)	N	*k*	H_o	H_e	PI
Mygo4	F[a]: TCTAATCGGGACGCACGC	TCG	230–302	22	18	0.636	0.924	0.011
	R: AATTGCGCTGAAAGCATCG							
Mygo7[b]	F[a]: GTTGGGCTTGCAGGTTCG	AACG	308–320	20	4	0.250	0.690	0.146
	R: ACGATGTATCCAGTTGGTCCC							
Mygo9[b]	F[a]: AATAGATGACGATAAAGACGCACG	TGCG	106–158	24	13	0.583	0.877	0.026
	R: AGAATAATGCATTGGAATAATATAGAGGG							
Mygo12	F[a]: CCTAAAGGGAATCACGAGCG	TTC	324–357	22	11	0.591	0.834	0.044
	R: AGTCGAGGCTCGCAATAACG							
Mygo13[b]	F[a]: GTCACAATGTGCATAAATCTCTCC	ATT	165–282	23	17	0.522	0.916	0.014
	R: AGTCGTGGCGATAAGCTCG							
Mygo14[b]	F[a]: GCTCCCGTTCGTTGTTGC	AACG	190–306	23	14	0.478	0.867	0.031
	R: ATACGACTTCATTTCCGCTGG							
Mygo15[b]	F[a]: ACTCTCCCAACACACACACG	TGCG	179–261	23	15	0.435	0.832	0.041
	R: ATCCGGATGCTCATTATTGC							
Mygo16[b]	F[a]: CGGATCGTCATCAGAGTCCC	AAC	155–188	24	7	0.500	0.788	0.077
	R: ATGAGTCGGCGAGGATGG							
Mygo17[b]	F[a]: ACAAATAGCCGGTGCAAAGC	TCG	154–184	24	8	0.542	0.822	0.055
	R: ATGCGAGTGCGTTAGAGTGC							
Mygo21	F[a]: AAGTTCTCTCGTCGGGTCTCC	TCG	282–327	20	10	0.550	0.849	0.041
	R: CGAGTCCGATAAGACGAGGG							
Mygo22[b]	F[a]: ACGAATGACTTCGACAATTAGAGG	TGC	227–269	21	9	0.429	0.789	0.067
	R: CGCATGCTTAAATTCACTCGG							
Mygo23	F[a]: GTAGCGACGAGGACGACG	ATC	307–352	23	12	0.652	0.867	0.030
	R: CGGTCAATTCCGATCTCTACC							
Mygo28[b]	F[a]: GCTCGATTGCATCAGACACC	TCG	190–259	24	16	0.458	0.902	0.018
	R: GACGAGCGAAACCTCTGACG							
Mygo29[b]	F[a]: CGGTCTTTCCACCTTGTGC	TTC	274–382	22	13	0.455	0.873	0.029
	R: GACGAGTGAGATAAGAAGAAGACTACG							
Mygo30[b]	F[a]: CAGGTCCGAACAAACAAGAAGG	TTC	311–341	30	9	0.609	0.824	0.049
	R:GCGGGAGAGTGGGAGAGG							
Mygo32[b]	F[a]: ATATCATGCACGTTGCTCGC	TGCG	124–160	22	8	0.364	0.788	0.070
	R: GTCGACGTATCGGAGGGC							
Mygo33[b]	F[a]: GAATCGCCTCGTGTGTGC	TGCG	247–271	23	7	0.348	0.802	0.075
	R: GTGCAAGCTAATTATCCGCC							
Mygo34[b]	F[a]:CGGGATCGGCTTTATCAGG	TTGG	173–201	24	8	0.542	0.786	0.075
	R:GTTGCACAGCACGAGAGACG							
Mygo35[b]	F[a]:ATCGAATCCGCGCTCTACG	TCG	168–264	23	13	0.478	0.884	0.024
	R: GTTTCTCCGTGCTGGAGTCG							
Mygo44	F[a]: TTTCCGACTACTTCCCTCGC	TCG	152–191	24	12	0.542	0.870	0.030
	R: TGGAGGATAAAGGGTGCTGC							
Mygo48[b]	F[a]: AAATGTATAAGGCTCTTAACGACACG	ATT	208–241	23	9	0.348	0.853	0.038
	R: TTTGACACAGAAAGTCCCGC							

The size indicates the range of observed alleles in base pairs and includes the length of the CAG tag; number of individuals genotyped is N; k is number of alleles observed; Ho and He are observed and expected heterozygosity, respectively; PI is the probability of identity for each locus, and TD refers to the touchdown protocol used for PCR (see text)

[a] Indicates CAG tag (5′-CAGTCGGGCGTCATCA-3′) label

[b] Indicates significant deviations from Hardy–Weinberg expectations after Bonferroni corrections

though parasite taxa comprise only 2 % of the total number of ant species worldwide.

Recently, we discovered the inquiline *Mycocepurus castrator*, a social parasite of the geographically widespread fungus-growing ant *Mycocepurus goeldii* (Rabeling and Bacci 2010). To study the genetic diversity of this host parasite pair, infer the conservation status of the social parasite, and investigate the population genetic conditions associated with the evolution of social parasitism, we developed 21 polymorphic microsatellite markers for *M. goeldii*. Genomic DNA used to isolate the microsatellite loci was extracted from a single individual, utilizing a QIAamp DNA Micro Kit (QIAGEN). An Illumina paired-end shotgun library was prepared by shearing 1 μg of DNA using a Covaris S220 and following the standard protocol of the Illumina TruSeq DNA Library Kit and using a multiplex identifier adaptor index. Illumina sequencing was conducted on the HiSeq with 100 bp paired-end reads. Five million of the resulting reads were analyzed with the program *PAL_FINDER_v0.02.03* (Castoe et al. 2012) to extract those reads that contained di-, tri-, tetra-, penta-, and hexanucleotide microsatellites. Once positive reads were identified in *PAL_FINDER_v0.02.03,* they were batched to a local installation of the program Primer3 (version 2.0.0) for primer design. To prevent issues with copy number of the primer sequence in the genome, loci for which the primer sequences only occurred once or twice in the 5 million reads were selected. Forty-eight loci of the 5,338 that met this criterion were chosen. One primer from each pair was modified on the 5′ end with an engineered sequence (CAG tag 5′-CAGTCGGGCGTCATCA-3′) to enable the use of a third primer in the PCR (identical to the CAG tag) that was fluorescently labeled. The sequence GTTT was added to primers without the universal CAG tag addition.

Forty-eight primer pairs were tested for amplification and polymorphism using DNA obtained from eight individuals. PCR amplifications were performed in a 12.5 μL volume (10 mM Tris pH 8.4, 50 mM KCl, 25.0 μg/ml BSA, 0.4 μM unlabeled primer, 0.04 μM tag labeled primer, 0.36 μM universal dye-labeled primer, 3.0 mM $MgCl_2$, 0.8 mM dNTPs, 0.5 units AmpliTaq Gold® Polymerase (Applied Biosystems), and 20 ng DNA template) using an Applied Biosystems GeneAmp 9700. Touchdown thermal cycling programs encompassed a 10 °C span of annealing temperatures ranging between 65 and 55 °C. Touchdown cycling parameters consisted of an initial denaturation step of 5 min at 95 °C followed by 20 cycles of 95 °C for 30 s, highest annealing temperature (decreased 0.5 °C per cycle) for 30 s, and 72 °C for 30 s; and 20 cycles of 95 °C for 30 s, lowest annealing temperature for 30 s, and 72 °C for 30 s, and a final extension at 72 °C for 5 min. PCR products were run on an ABI-3130xl sequencer and sized with Naurox size standard, except for the unlabeled primers started with GTTT. Results were analyzed using GeneMapper version 3.7 (Applied Biosystems). Twenty-one of the tested primer pairs amplified high quality PCR product that exhibited polymorphism.

We assessed the variability of the 21 polymorphic loci in 24 specimens from Brazil (Table 1). We estimated the number of alleles per locus (*k*), observed and expected heterozygosity (H_o and H_e), and probability of identity (PI) using GenAlEx v6.4. Tests for deviations from Hardy–Weinberg equilibrium (HWE) and for linkage disequilibrium were conducted using GENEPOP v4.0. After Bonferroni correction for multiple comparisons 16 loci showed significant deviations from expectations under HWE, possibly because the random mating condition of the HW principle was violated for some population, possibly because some *M. goeldii* populations are not freely interbreeding due to geographic and genetic distance from each other.

These newly developed loci will be used to (1) examine the genetic diversity of the parasitic *M. castrator* and its host *M. goeldii* and (2) study the population genetic conditions associated with the evolution of social parasitism in fungus-growing ants. Preliminary analyses indicate that these microsatellite markers cross amplify in *M. castrator*, which will allow us to assess the genetic diversity in this rare and potentially endangered species.

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