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PRIMER NOTE

Characterization and PCR multiplexing of polymorphic microsatellite loci for the invasive ant *Wasmannia auropunctata*

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Abstract

Highly polymorphic genetic markers provide a useful tool for estimating genetic parameters in studies of the evolution of sociality in insects. We isolated and characterized 12 polymorphic microsatellite loci in the invasive ant, *Wasmannia auropunctata*, and described experimental conditions for PCR (polymerase chain reaction) multiplexing and simultaneously genotyping these loci in two sets of five and seven markers. The number of alleles per locus ranged from two to 14 and the observed heterozygosity ranged from 0.233 to 0.967. Moreover, results of cross-species amplification tests are reported in three other species of *Wasmannia* and in two species of the genus *Allomerus*.

Keywords: ants, Blepharidattini, invasion, microsatellites, Myrmicinae, pest, *Wasmannia*

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Introduction of nonindigenous species is an increasing problem. Ants do not derogate from this rule. At least 147 out of 11 816 indexed ant species (http://antbase.org/ — 5 January 2005) have been observed outside their native range, their introduction being often human-mediated (McGlynn 1999). Although most of these ant species are observed in a transitory way, about 26 species are able to colonize new territories in a definitive way (McGlynn 1999). Biological invasions by ants represent a severe threat to biodiversity as well as to agriculture and other human interests, and five particularly threatening ant species are registered in the list of '100 of the World's Worst Invasive Alien Species' (Lowe *et al*. 2001). Previous works on invasive species, mainly on the fire ant, *Solenopsis invicta*, and the Argentine ant, *Linepithema humile*, have shown that their widespread success is largely because of low levels of intraspecific aggression and territorial behaviours (Hölldobler & Wilson 1990; Passera 1994), and to a concomitant reduction of the genetic diversity (reviewed in Holway *et al*. 2002). The little fire ant *Wasmannia auropunctata* is one of these particularly threatening ant species. Native from Neotropical forests of South America where it does not normally dominate the biological community, *W. auropunctata* is now widespread throughout the tropical zones of the world where it shows extreme population explosions (Wetterer & Porter 2003). Here, we report the isolation and characterization of 12 polymorphic microsatellite markers for the invasive ant *W. auropunctata*. These markers will be useful in investigating the population genetic structure and mating system both in the native and introduced ranges of this polygynous (i.e. colonies containing several queens) species.

A partial DNA library of 1768 recombinant clones was obtained from *W. auropunctata* individuals originating from New Caledonia, and was screened using a nonradioactive method (detailed protocols available at http://www.inapg.inra.fr/dsa/microsat/microsat.htm, Estoup *et al*. 1998). One

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Table 1 Microsatellite loci developed for the ant *Wasmannia auropunctata*. Cloned allele size is based on New Caledonian individuals. The observed size range, number of alleles (N_a), frequency of the most common allele (f), and estimates of observed (H_O) and expected (H_E) heterozygosities were estimated from 30 workers collected in French Guiana (FG) and 30 workers collected in New Caledonia (NC). T_a annealing temperature

Locus	Core repeat (cloned allele)	Size (bp)	Size range (bp)	N_a		f		H_O		H_E		T_a	Primers (5′–3′)	GenBank Accession no.
				FG	NC	FG	NC	FG	NC	FG	NC	(°C)		
PCR multiplex set 1														
Waur-1gam	$(AG)_{19}$	300	276–326	8	3	0.367	0.767	0.767	0.467	0.813	0.389	57	F: Fam-TGCTCTCAGTGTTCCGACAG R: CCCTAAAGAACATGAATATGACG	AY779632
Waur-2164	$(AG)_{40}$	199	276–328	12	5	0.567	0.517	0.233	0.933	0.668	0.672	57	F: Ned-GCCCGTTATTATACAGCTGG R: CTACTCGCAGCACCTCCATA	AY779633
Waur-3176	$(GA)_{17}$	235	222–246	9	2	0.383	0.750	0.867	0.467	0.800	0.381	57	F: Fam-AAGGCCGTCCTGTGCGTTA R: GAACAGGTCCTCGCCAGTCT	AY779636
Waur-418	$(GA)_{18}$	331	100–144	12	3	0.433	0.483	0.667	0.967	0.773	0.644	57	F: Fam-GCATCAATTTTCTCCCGAACC R: CGCAATGTGCAATGTCGTGT	AY779637
Waur-566	$(CT)_{14}$	261	257–279	10	2	0.233	0.550	0.900	0.900	0.879	0.503	57	F: Hex-GTTCAACGAGGATGGTTACG R: GCTTAATCGCACGTGAACTG	AY779639
Waur-716	$(GA)_{18}$	178	180–210	10	4	0.417	0.750	0.633	0.500	0.772	0.415	57	F: Ned-TCTGACTGGTCGAATTCCTG R: GACTGCACGAATGTGACGTA	AY779641
Waur-730	$(CT)_{18}$	181	156–200	13	3	0.283	0.600	1	0.800	0.864	0.549	57	F: Fam-GAATGAGCGAAGCGTGTTAC R: GAGATTCTCGACGCGTATGA	AY779642
PCR multiplex set 2														
Waur-1166	$(GA)_{22}$	101	91–119	13	2	0.233	0.817	0.867	0.300	0.880	0.305	63	F: Hex-GAACCAGAAGCGAGAACG R: AGCTGCATGAACTCCAAG	AY779631
Waur-225	$(CA)_{10}$	223	223–233	4	2	0.683	0.650	0.533	0.700	0.503	0.463	63	F: Ned-GTGCGCAGACATAGATAAGG R: TGAATAGCTGCGACTCTACG	AY779634
Waur-275	$(CT)_{14}$	113	103–131	9	3	0.433	0.550	0.300	0.900	0.713	0.595	63	F:Ned-AGAACTTTGATGCTCCACTTTC R: CTTTGAACTTCCTGGTTAATGC	AY779635
Waur-521	$(GA)_{15}$	200	200–216	—	3	—	0.583	—	0.833	—	0.581	63	F: Hex-AGATATGAGCGGAACCAGTC R: TCAGCCTCCTGGGTAGC	AY779638
Waur-680	$(AG)_{22}$	167	157–191	14	3	0.350	0.5	0.767	0.967	0.840	0.525	63	F: Hex-GCACAGTGCCGACTTGTTGC R: CGCACGTCTGGCACTCAATC	AY779640

hundred and forty of the 1768 recombinant clones showed a strong hybridization signal with $(GT)_{10}$ and $(CT)_{10}$ probes. Among them, 78 inserts were sequenced by the private company Genome Express, using BigDye Terminator Cycle sequencing and an ABI 3730XL automated sequencer (Applied Biosystems). Sixty-seven of the 78 sequenced inserts contained microsatellites and 24 were tested for amplification using fluorescent polymerase chain reaction and (PCR) RAND a MegaBace DNA sequencer (Amersham Biosciences). DNA extraction was performed following a standard CTAB-based protocol (Doyle & Doyle 1987). Briefly, individual ants were ground in CTAB solution and incubated for two hours at 55 °C, DNA was purified through chloroform/isoamyl alcohol extraction and isopropanol precipitation. PCR amplifications were performed with an MJ Research PTC-200 thermocycler and consisted of an initial denaturing step of 15 min at 94 °C; 35 cycles of 30 s at 94 °C, annealing for 90 s, and extension at 72 °C for 60 s; and a final extension at 60 °C for 30 min. Twelve polymorphic loci with nonambiguous allelic pattern (i.e. with a low number of stutter bands) were selected for further population studies. Primer sequences and PCR conditions are given for each selected locus in Table 1. The sequences of the 12 microsatellite loci have been deposited in the GenBank database (Accession nos: AY779631 to AY779642). According to annealing temperatures, two sets of five and seven loci were coamplified using Multilocus Amplification Kit (QIAGEN) in a 10-μL volume containing 1 × QIAGEN Multiplex Master Mix, 2 μM of each primer and 2 μL of genomic DNA (= 1 μg DNA/reaction). The forward primer of each pair of microsatellite DNA primers was labelled with a fluorescent dye (FAM, HEX or NED) in order to allow detecting alleles at all 12 loci in a single electrophoresis run (see Table 1).

The level of polymorphism and allelic distribution of the 12 microsatellite loci were estimated using GENEPOP 3.4 (Raymond & Rousset 1995) by typing 30 workers collected in five nests (six workers per nest) in a population from New Caledonia (i.e. introduced populations) and 30 workers collected in a same way in French Guiana (i.e. native area). The number of alleles per locus and the expected heterozygosities ranged between two and 14, and 0.305 and 0.880, respectively (Table 1). One locus (Waur-521) showed clear allelic pattern only for the New Caledonian samples. Differences between observed and expected heterozygosities are a result of relatedness of individuals within nests.

We examined the conservation of the primer sequences and the level of polymorphism of the selected loci in five other ant species of the subfamily Myrmicinae. Three of these species belong to the tribe Blepharidattini genus *Wasmannia* (*Wasmannia iheringi*, *Wasmannia rochai* and *Wasmannia scrobifera*) and two species to the tribe Solenopsidini genus *Allomerus* (*Allomerus octoarticulatus* and *Allomerus decemarticulatus*). For each species, extraction and PCR amplification were performed following the procedure described above. Results are reported in Table 2. Eight loci successfully cross-amplified in at least one species. Six loci cross-amplified in all *Wasmannia* species tested. However, only two, five and five loci were polymorphic in *W. iheringi*, *W. rochai* and *W. scrobifera*, respectively. A similar rate of cross-amplification success was obtained in the two species of the genus *Allomerus*.

Our results show that the microsatellite loci isolated from *W. auropunctata* will be useful for population genetic studies in both native and introduced populations of this species and, to a less extent, in some other ant species of the subfamily Myrmicinae. These markers are expected to provide important insight into the evolution of colony and population genetic structures which follows the introduction of ant species in new environments.

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Table 2 Cross-species PCR tests for 12 *W. auropunctata* microsatellite loci in five ant species of the subfamily Myrmicinae. The number of alleles and the allelic size range are based on *N* workers. Amplification failure is indicated by a dash

	Waur-1gam	Waur-1166	Waur-2164	Waur-225	Waur-275	Waur-3176	Waur-418	Waur-521	Waur-566	Waur-680	Waur-716	Waur-730
Tribe Blepharidattini, genus *Wasmannia*												
W. iheringi	1	1	—	3	—	2	1	—	1	1	—	—
(*N* = 3)	290	91		221–225		248–250	126		263	143		
W. rochai	2	3	—	2	—	1	1	—	2	3	—	—
(*N* = 3)	284–290	95–135		223–225		126	118		259–263	135–177		
W. scrobifera	2	2	—	3	—	3	1	—	3	—	—	—
(*N* = 2)	288–294	97–135		223–233		220–226	110		263–267			
Tribe Solenopsidini, genus *Allomerus*												
A. octoarticulatus	3	3	—	—	—	1	1	—	1	1	—	1
(*N* = 8)	286–296	101–111				228	114		252	143		143
A. decemarticulatus	2	—	—	4	—	1	1	—	2	1	—	3
(*N* = 8)	296–298			223–265		224	112		263–265	147		135–177

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