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Characterization of polymorphic microsatellite loci in the neotropical plant-ant *Allomerus decemarticulatus* (Formicidae: Myrmicinae) and multiplexing with other microsatellites from the ant subfamily Myrmicinae

PIERRE-JEAN G. MALÉ[1, 2], ANNE LOISEAU[3], ARNAUD ESTOUP[3], ANGÉLIQUE QUILICHINI[4, 5] and JÉRÔME ORIVEL[1, 2, 4]

[1]Université de Toulouse, Laboratoire Evolution & Diversité Biologique (EDB), UMR 5174, 118 route de Narbonne, 31062 Toulouse cedex 9, France; e-mails: pjmale@cict.fr, orivel@cict.fr

[2]CNRS, EDB (Laboratoire Evolution et Diversité Biologique), F-31062 Toulouse, France

[3]INRA, UMR CBGP (INRA / IRD / Cirad / Montpellier SupAgro), Campus international de Baillarguet, CS 30016, F-34988 Montferrier-sur-Lez cedex, France; e-mails: loiseau@supagro.inra.fr, estoup@supagro.inra.fr

[4]CNRS, UMR Ecologie des Forêts de Guyane, Campus Agronomique, F-97379 Kourou cedex, France; e-mail: Angelique.Quilichini@ecofog.gf

[5]Jardin Botanique Henri Gaussen, 39 allées Jules Guesde, F-31062 Toulouse, France

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Abstract. Five polymorphic microsatellite loci of the arboreal ant *Allomerus decemarticulatus* (Myrmicinae) were isolated and characterized. The amplification and polymorphism of seven additional microsatellite loci, previously developed for the ant species *A. octoarticulatus* and *Wasmannia auropunctata*, were also tested and the amplification conditions necessary for genotyping the complete set of 12 multiplexed markers in *A. decemarticulatus* determined. The number of alleles per locus ranged from three to 15 and observed heterozygosity varied from 0.09 to 0.95. Cross-species amplification of these loci was also successfully achieved in additional species of the same ant subfamily, Myrmicinae. This set of microsatellite markers will be used in studies on the mating system and population genetic structure of Myrmicinae in general and *A. decemarticulatus* in particular.

INTRODUCTION

Allomerus decemarticulatus (Hymenoptera: Myrmicinae) is a neotropical obligate plant-ant. In French Guiana, it is strictly associated with the myrmecophytic understory treelet *Hirtella physophora* (Chrysobalanaceae) via a mutualistic relationship (Solano et al., 2003; Grangier et al., 2009). Its occurrence is thus restricted to areas where *H. physophora* is present. Populations of *H. physophora* are patchily distributed, even at small geographic scales (Solano et al., 2003), and the population genetic structure of *A. decemarticulatus* is expected to reflect that of its host. A set of polymorphic microsatellite loci for *A. decemarticulatus* was developed and characterized that can be multiplexed with microsatellite loci previously developed either in a congeneric species or a species from a different tribe of the same subfamily. This set of microsatellite markers will be used in studies on the mating system and population genetic structure of *A. decemarticulatus*.

MATERIAL AND METHODS

Total genomic DNA was extracted from four adult males using the DNeasy Blood & Tissue Kit (QIAGEN, Courtaboeuf, France). Extracts were mixed and concentrated using a SpeedVac Concentrator RC1010 (Jouan, Saint-Herblain, France) and re-suspended in ultrapure water to obtain a concentration of 65 ng/µL. *Rsa*I-digested DNA fragments from 600 to 1000 kb in size were extracted from the gel, after electrophoresis, using QIAquick Gel Extraction Kit (QIAGEN). These fragments were ligated to Rsa-21 and Rsa-25 linkers [Rsa-21 (5'–3') CTCTTGCTTACGCGTGGACTA; Rsa-25 (5'–3') phosphate-TAGTCCACGCGTAAGCAAGAG] for PCR amplification with Rsa-21 primers. The PCR products were purified with the QIAquick PCR Purification Kit (QIAGEN). Enrichment was conducted using Streptavidin MagneSphere® Paramagnetic Particles Kit (Promega, Carbonnières-les-Bains, France) and a mixture of the biotinylated oligonucleotide probes $(TC)_{10}$ and $(TG)_{10}$. Enriched DNA was then amplified using Rsa-21 primers, purified using the QIAquick PCR Purification Kit (QIAGEN) and ligated into pGEM-T vectors using the pGEM-T Easy Vector system II kit (Promega) following the manufacturer's protocol. A total of 1152 white transformant clones were PCR screened using $(TC)_{10}$ or $(TG)_{10}$ probes with T7 and SP6 primers. One hundred and fifty-two clones gave a positive signal and the inserted DNA fragment was subsequently sequenced. Thirty-six primer pairs were designed using the software Primer Designer v 2.0 (Scientific & Educational Software 1990, 91). PCR amplifications were carried out in a 15 µL final volume containing 1× QIAGEN Multiplex Master Mix, 0.5× Q-Solution, 2 µM of each primer, and 2 µL of genomic DNA. The thermal profile started with an initial denaturation at 95°C (15 min), followed by 35 cycles of denaturation at 94°C (30 s), annealing at 57°C (1 min 30 s) and extension at 72°C (1 min) and ended with a final extension at 60°C for 30 min. PCR products were sized using electrophoresis on a 1.5% agarose gel.

Loci for which amplifications produced only one DNA band corresponding to the predicted size of the PCR product were then amplified following the protocol described above but with forward primers fluorophore-labelled on the 5' end and non-labelled reverse primers. These loci were finally sized using an ABI 3730 sequencer (Applied Biosystems, Courtaboeuf, France) with the 500 LIZ™ GeneScan™ size standard and GENEMAPPER™ 4.0 software. Levels of polymorphism were estimated by genotyping one worker per colony from 68 colonies derived from two populations. DNA extractions were per-

TABLE 1. Primer sequences and polymorphism statistics for 12 microsatellite loci in two populations of *Allomerus decemarticulatus* [*Ta* – annealing temperature; *n* – number of colonies; *Na* – number of alleles studied; *Ho* – proportion of heterozygous individuals; *He* – expected heterozygosity ; * – significant deviation from Hardy-Weinberg equilibrium after correction for multiple tests (Sequential Bonferroni procedure; $P < 0.01$)].

Locus	Repeat motif (cloned allele)	Size range (bp)	5' dye	Petit Saut ($n = 46$)			Nouragues ($n = 22$)			*Ta* (°C)	Primer sequences (5'–3')	Multiplex markers set	Genbank accession numbers	Source
				Na	*Ho*	*He*	*Na*	*Ho*	*He*					
Adec-A21	$(AG)_{22}$	356–360	6FAM	3	0.54	0.45	3	0.68	0.52	63.8	TACCGGACCGAACTACTTGC ACTCACGCACTCGCCTCAT	2	GU982709	This study
Adec-A23	$(GA)_{30}$	289–295	6FAM	7	0.23	0.30	6	0.72	0.80	64.2	ACGAACGCTGCACTTCCA AGGAGGTGAGAGAGCTTGAGG	1	GU982710	This study
Adec-A41	$(AG)_{18}$	161–167	6FAM	3	0.65	0.90*	3	0.41	0.58	62.2	CGTATCCATAAGGCGCAATC GTCGTCAAGAAGCAGAGGCA	1	GU982711	This study
Adec-A49	$(AG)_{32}$	212–226	6FAM	8	0.73	0.64	6	0.81	0.74	62.1	CGTGCCATCGCGAGAA CCATTTCTGCCGAGGGT	1	GU982712	This study
Adec-A60	$(GA)_{38}$	271–283	NED	4	0.09	0.09	3	0.64	0.55	60.9	TCGCGCGGAAATCGT CATCTGTCGTGCACCGTAAT	2	GU982713	This study
Waur8Ω	$(GA)_{15}$	273–313	PET	7	0.76	0.82	12	0.82	0.89	57	GTAGTTGGCGAGACCGGATG CTCCAGCTGTGGTTCCGATG	1	FJ970016	Molecular Ecology Resources Primer Development Consortium et al., 2009
Waur872	$(GA)_{12}AA(GA)_{23}$	357–415	VIC	11	0.55	0.80*	8	0.20	0.87*	57	CGTGCTATCCTCGACGAAGT GCCGACAGTACAACAAACAGC	2	FJ970023	Molecular Ecology Resources Primer Development Consortium et al., 2009
Waur813	$(GA)_{17}$	106–145	NED	15	0.76	0.85	10	0.95	0.89	57	GTCCAAGGATGAATGTATTCAC GCCGCTAGAAGAAGAATGAC	2	FJ970021	Molecular Ecology Resources Primer Development Consortium et al., 2009
Waur730	(CT)18	182–209	VIC	7	0.39	0.42	9	0.80	0.87	57	GAATGAGCGAAGCGTGTTAC GAGATTCTCGACGCGTATGA	1	AY779642	Fournier et al., 2005
Waur225	$(CA)_{10}$	240–290	VIC	5	0.20	0.34*	8	0.67	0.75	63	GTGCGCAGACATAGATAAGG TGAATAGCTGCGACTCTACG	2	AY779634	Fournier et al., 2005
Ad166	$(TG)_{11}$	161–176	NED	3	0.32	0.51*	11	0.77	0.85	53.5	GGTCCTTTGAGCAACTTAGC CTGATCGCAATAGAGCAATG	1	DQ139976	Debout et al., 2006
Ad040	$(TG)_{16}(CG)_5(CA)_3$	301–331	VIC	9	0.39	0.85*	14	0.75	0.88	50.4	GAAAGACAGATCGCTTCATC GCCGATATTACTTCATTCAG	2	DQ139967	Debout et al., 2006

formed using 10% Chelex (BioRad, Marnes-la-Coquette, France) solution. Level of polymorphism and deviation from Hardy-Weinberg equilibrium were estimated for each locus using GENEPOP 4.0 (Raymond & Rousset, 1995; Rousset, 2008). Correction for multiple tests was performed when appropriate (Sequential Bonferroni procedure).

RESULTS AND DISCUSSION

Fourteen PCR products gave discrete bands of the predicted size on agarose gels. All of these loci were unambiguously sized using the sequencer. Among them, five loci were polymorphic. Individual loci had 3 to 8 alleles and observed heterozygosity ranged between 0.09 and 0.95 (Table 1). Five loci departed significantly from Hardy-Weinberg equilibrium ($P < 0.01$, Table 1). It is likely this can be accounted for by a high level of inbreeding in populations of *A. decemarticulatus* due to the clumped distribution of *H. physophora*.

Sequences of the five microsatellite loci are deposited in the GenBank database (see Table 1 for accession numbers). All loci were successfully cross-amplified in at least one species of three in the same genus (*A. octoarticulatus*, *A. septemarticulatus*, and *A. dentatus*), one species of the same tribe (*Monomorium subopacum*), and one species in a different tribe but the same subfamily (*Wasmannia auropunctata*) (Table 2).

To increase the number of loci available for mating system and population genetic studies in *A. decemarticulatus*, the co-amplification of the five above markers plus seven microsatellite loci previously developed for two ant species from the same subfamily (two loci from *A. octoarticulatus* and five from *W. auropunctata*; Fournier et al., 2005; Debout et al., 2006; Molecular Ecology Resources Primer Development Consortium

TABLE 2. Cross-species PCR tests for five *Allomerus decemarticulatus* microsatellite loci in five ant species (*A. octoarticulatus*, *A. septemarticulatus*, *A. dentatus*, *Monomorium subopacum*, and *Wasmannia auropunctata*). The number of alleles and their size range (in parentheses) are based on genotyping *n* individuals of each species. Amplification failure is indicated by a dash.

Locus	*A. octoarticulatus* (*n* = 18)	*A. septemarticulatus* (*n* = 3)	*A. dentatus* (*n* = 1)	*M. subopacum* (*n* = 1)	*W. auropunctata* (*n* = 5)
Adec-A21	5 (351–360)	3 (350–358)	1 (358)	1 (358)	3 (356–360)
Adec-A23	6 (275–290)	3 (268–290)	2 (300–304)	1 (289)	3 (291–307
Adec-A41	44 (161–171)	4 (161–169)	2 (165–175)	2 (161–165)	4 (162–184)
Adec-A49	5 (212-226)	2 (214–222)	2 (214–228)	–	–
Adec-A60	6 (214–226)	4 (271–279)	2 (272–284)	2 (272–284)	4 (273–279)

et al., 2009) was optimized. These seven markers were chosen based on their putative polymorphism in *A. decemarticulatus*. Forward primers were fluorescently labelled according to expected size ranges in order to allow typing of all 12 loci in a single electrophoresis run. Two sets of six primer pairs were each designed according to annealing temperature using Multiplex Manager 1.1 (Holleley & Geerts, 2009). This complete set of markers will be used in studies on the dispersal and mating biology of an ecologically and evolutionarily important ant-plant mutualism.

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