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

DENIS FOURNIER,* JULIEN FOUCAUD,* ANNE LOISEAU,* SANDRINE CROS-ARTEIL,* HERVÉ JOURDAN,† JÉRÔME ORIVEL,‡ JULIEN LE BRETON,§ JEAN CHAZEAU,† ALAIN DEJEAN,‡ LAURENT KELLER§ and ARNAUD ESTOUP*

*Centre de Biologie et de Gestion des Populations, INRA, Campus International de Baillarguet CS 30016, 34988 Montferrier/Lez, France, †Laboratoire de Zoologie Appliquée, IRD, 98948 Nouméa, Nouvelle-Calédonie, ‡Laboratoire Evolution et Diversité Biologique, Université Paul Sabatier, Campus de Rangueil, 118 route de Narbonne, 31062 Toulouse, France, §Laboratory of Sub-Tropical Zoology, University of the Ryukyus, 903–0213 Nishihara, Okinawa, Japan §Department of Ecology and Evolution Bâtiment de Biologie, University of Louvanne, 1015 Louvanne, Switzerland

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

Received 20 October 2004; revision accepted 2 December 2004

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...
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

<table>
<thead>
<tr>
<th>Locus</th>
<th>Core repeat (cloned allele)</th>
<th>Size (bp)</th>
<th>Size range (bp)</th>
<th>$N_a$</th>
<th>$f$</th>
<th>$H_O$</th>
<th>$H_E$</th>
<th>$T_a$ (°C)</th>
<th>Primers (5’–3’)</th>
<th>GenBank Accession no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR multiplex set 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Waur-1gam | $(AG)_9$ | 300 | 276–326 | 8 | 3 | 0.367 | 0.767 | 0.467 | 0.813 | 0.389 | 57 | F: Fam-TGCTCTCAGTTGGTTCCAGACAG  
R: CCCCCAGACTGAGTTGACG |
| Waur-2164 | $(AG)_{40}$ | 199 | 276–328 | 12 | 5 | 0.567 | 0.517 | 0.233 | 0.333 | 0.668 | 0.672 | 57 | F: Ned-GCCCGTTATTATACAGCTGG  
R: CTACCTGACAGACCTGATT |
| Waur-3176 | $(GA)_{17}$ | 235 | 222–246 | 9 | 2 | 0.383 | 0.750 | 0.867 | 0.467 | 0.800 | 0.381 | 57 | F: Fam-AGACGCGCTCCCCATTA  
R: CTACCTGACAGACCTGATT |
| Waur-418 | $(GA)_{18}$ | 331 | 100–144 | 12 | 3 | 0.433 | 0.483 | 0.667 | 0.967 | 0.773 | 0.644 | 57 | F: Fam-GCATCATAATTTTCCTCCGAACCC  
R: GCCAAGTGCAGGTCAGG |
| Waur-566 | $(CT)_{14}$ | 261 | 257–279 | 10 | 2 | 0.233 | 0.550 | 0.900 | 0.900 | 0.879 | 0.503 | 57 | F: Hex-GTTCAACGAGTGGTGGATACG  
R: GCTTAATCGCACGTGAACT |
| Waur-716 | $(GA)_{18}$ | 178 | 180–210 | 10 | 2 | 0.177 | 0.750 | 0.633 | 0.500 | 0.772 | 0.415 | 57 | F: Ned-TCTGGACCTGGATGACTT |
| Waur-730 | $(CT)_{18}$ | 181 | 156–200 | 13 | 3 | 0.283 | 0.600 | 1.000 | 0.864 | 0.549 | 0.57 | 57 | F: Fam-GAATGAGCGAGCGCTGGTTAC  
R: GAGATATCTGACGACGATAG |
| 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-GAACAGAAGGAGGACGAG  
R: AGCTGACATCGACTGACAG |
| Waur-225 | $(CA)_{10}$ | 223 | 223–233 | 4 | 2 | 0.683 | 0.650 | 0.533 | 0.700 | 0.503 | 0.463 | 63 | F: Ned-GTGGCGAGCAGTGAATTAGGC  
R: TGAATGCTGACGACTCAAAG |
| Waur-275 | $(CT)_{14}$ | 113 | 103–131 | 9 | 3 | 0.433 | 0.550 | 0.300 | 0.900 | 0.713 | 0.595 | 63 | F: Ned-AGAATTTTGATGACTGCCACTTCTTC  
R: CTACCTGACAGACCTGATT |
| Waur-521 | $(GA)_{15}$ | 200 | 200–216 | 13 | 3 | 0.583 | 0.833 | 0.833 | 0.581 | 0.353 | 0.63 | 63 | F: Hex-AATATGACGACGACAGTTGC  
R: TCAGATCTGACGACGACAG |
| Waur-680 | $(AG)_{22}$ | 167 | 157–191 | 14 | 3 | 0.350 | 0.50 | 0.767 | 0.967 | 0.840 | 0.525 | 63 | F: Hex-GCAGATGAGCAGCGACTGGTGC  
R: CCCAGTCTGACGACCTTATT |

© 2005 Blackwell Publishing Ltd, Molecular Ecology Notes 5, 239–242
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 a standard 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 Multiplex 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 Solenop- sidini 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.

**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.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>W. iheringi</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td></td>
<td>2</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(N = 3)</td>
<td>290</td>
<td>91</td>
<td>221–225</td>
<td>248–250</td>
<td>126</td>
<td>263</td>
<td>143</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>W. rochai</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td></td>
<td>1</td>
<td>1</td>
<td></td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>W. scrobifera</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td></td>
<td>3</td>
<td>1</td>
<td></td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. octoarticulatus</td>
<td>3</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(N = 8)</td>
<td>286–296</td>
<td>101–111</td>
<td></td>
<td></td>
<td>228</td>
<td>114</td>
<td>252</td>
<td>143</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. decemarticulatus</td>
<td>2</td>
<td></td>
<td>4</td>
<td></td>
<td>1</td>
<td>1</td>
<td></td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(N = 8)</td>
<td>296–298</td>
<td>223–265</td>
<td></td>
<td></td>
<td>224</td>
<td>112</td>
<td>263–265</td>
<td>147</td>
<td></td>
<td></td>
<td>135–177</td>
</tr>
</tbody>
</table>

@ 2005 Blackwell Publishing Ltd, Molecular Ecology Notes, 5, 239–242
References


Estoup A, Gharbi K, San Cristobal M et al. (1998) Parentage assignment using microsatellites in turbot (Scophthalmus maximus) and rainbow trout (Oncorhynchus mykiss) hatchery populations. Canadian Journal of Fisheries and Aquatic Sciences, 57, 715–723.


