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PERMANENT GENETIC RESOURCES

Isolation and characterization of microsatellite loci from the invasive ant *Pheidole megacephala*

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Abstract

We report the characterization of eight microsatellite markers in the big-headed ant *Pheidole megacephala*, a pest ant registered in the list of '100 of the world's worst invasive alien species'. An enrichment protocol was used to isolate microsatellite loci, and polymorphism was explored with 36 individuals collected in an invasive population from Australia and 20 individuals collected in a population from the native mainland location in South Africa. These primers showed a number of alleles per locus ranging from two to 10, and expected heterozygosities ranging from 0.083 to 0.826. Moreover, results of cross-species amplification are reported in five other *Pheidole* species and in seven other ants of the subfamily Myrmicinae.

Keywords: big-headed ant, colony structure, invasive species, microsatellite, *Pheidole megacephala*, population structure

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Ants are popular organisms to address a variety of questions in ethology, genetics, ecology, evolution or conservation biology. Among the family Formicidae, *Pheidole* is one of the most speciose and widespread genera: 898 described species are distributed over the eight zoogeographical regions where ant fauna are found (Neotropical, Nearctic, Palearctic, Afrotropical, Malagasy, Oriental, Indo-Australian and Australasian) (Hölldobler & Wilson 1990; Bolton 1995). In the New World, species of the genus *Pheidole* are the most abundant and diverse ants, and range from the northern USA to Argentina (Wilson 2003). *Pheidole* species have been studied for various aspects of their biology, including the ecological correlates of their temporal and physical castes and the associated division of labour (e.g. *P. dentata*, Calabi & Traniello 1987; *P. morrisi*, Brown & Traniello 1998); their highly biased colony sex ratio (*P. desertorum*, Helms 1999; *P. pallidula*, Fournier *et al*. 2003); their foraging strategies and territorial behaviour (e.g. *P. pallidula*, Passera *et al*. 1996; *P. xerophylla* (previously *P. tucsonica*) and *P. gilvescens*, Langen *et al*. 2000; Tripet *et al*. 2006); and their impact on biodiversity (e.g. *P. megacephala*, Passera 1994 and references therein; Wetterer 2007; *P. fervens* and *P. moerens*, Garrison 1996).

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The big-headed ant *P. megacephala* is well known as a household and agricultural pest, and its negative ecological impact on biodiversity may be greater than any other invasive ant (Wetterer 2007). The species is nominated as one of the 100 of the world's worst invaders by the IUCN Species Survival Commission Invasive Species Specialist Group (Lowe *et al*. 2001). However, despite its ecological and economical impacts, no genetic studies have been conducted to decipher the evolutionary processes associated with the invasion of the big-headed ant. Here, we describe the development of eight microsatellite loci for this invasive species and their application to individuals from Africa, the supposed native range of the species (Wilson & Taylor 1967), and Australia.

Samples were collected in March 2005 from four Australian populations and stored in ethanol at –80 °C. Total genomic DNA was isolated using a standard phenol–chloroform extraction protocol (Sambrook & Russell 2001). An enriched library was made by ecogenics GmbH from size-selected genomic DNA ligated into SAULA/SAULB-linker (Armour *et al*. 1994) and enriched by magnetic bead selection with biotin-labelled $(CT)_{13}$, $(GT)_{13}$ $(GTAT)_7$ and $(GATA)_7$ oligonucleotide repeats (Gautschi *et al*. 2000a, b). Of 374 recombinant colonies screened, 163 gave a positive signal after hybridization. Plasmids from 64 positive clones were sequenced and primers were designed for 16 microsatellite inserts.

Table 1 Microsatellite loci developed for the ant *Pheidole megacephala*. The observed size range (in base pairs), the number of alleles (N_a), the frequency of the most common allele (f), and the estimates of observed (H_O) and expected (H_E) heterozygosities are based on 36 individuals collected in a single population from Australia and 20 individuals collected in a South African population. The forward (F) primers were labelled with fluorescent dyes (either FAM, HEX or NED) for detection *Tann* is the annealing temperature

			Australia					South Africa					
Locus	Core repeat (cloned allele)	Primer sequence (5′–3′)	Size	N_A	f	H_O	H_E	Size	N_A	f	H_O	H_E	GenBank Accession no.
PCR multiplex set 1 T_{ann} = 60 °C													
Pmeg-06	$(GA)_{15}$	F: *FAM*-GTTTGAAAATGCGAGGAAGG	98–114	4	0.583	0.611	0.532	102–143	10	0.300	0.900	0.826	EF503571
		R: TTCGGTATGTCTGCAAATCC											
Pmeg-09	$(CATA)_{18}(CATG)(CATA)_2$	F: *FAM*-TCACGCAAGATTAGAGTGATTTC	191–223	3	0.517	0.371	0.415	196–228	7	0.275	0.700	0.800	EF503572
		R: TCTTACGTGTATGCGTATGTAAGG											
Pmeg-12	$(CT)_{29}$	F: *HEX*-CGAAAGAAAATCGGTAGCTTTG	152–186	4	0.733	0.694	0.583	127–178	8	0.475	0.550	0.733	EF503573
		R: AGGTAAGATTGCCGCAGTTG											
Pmeg-14	$(GTAT)_{13}$	F: *NED*-TTTAATCAAAGTTGTAACTTAATGTCG	121–138	3	0.917	0.086	0.083	92–142	8	0.350	0.350	0.790	EF503574
		R: AAAGTTGGCAAATAAATATATACACG											
PCR multiplex set 2 T_{ann} = 56 °C													
Pmeg-07	$(CT)_4TT(CT)_{20}$	F: *HEX*-TTGGATTTTCCTTCCCCTTC	103–115	3	0.817	0.306	0.323	119–167	10	0.342	0.842	0.810	EF503575
		R: ACGCCCAACGAATAACACAC											
Pmeg-10	$(GA)_{29}$	F: *FAM*-GGTCTCCCTTGAAAGACAAAG	152–161	4	0.817	0.500	0.539	119–125	4	0.450	0.650	0.651	EF503576
		R: GTTTCCCGGCAATATAAAGG											
Pmeg-11	$(CA)_{13}$	F: *NED*-TCAACATCGCTTTCATACCG	148–164	4	0.567	0.472	0.497	152–166	6	0.375	0.650	0.703	EF503577
		R: GAACGCGTGAATGAATAATTG											
Pmeg-15	$(GA)_{21}TA(GA)_3$	F: *NED*-GCATAGAAAGACGAGGAGAGG	92–94	2	0.867	0.333	0.346	75–106	4	0.591	0.364	0.583	EF503578
		R: TTTTTGCTTTCCTCCATTCC											

The allelic distribution and the level of polymorphism measured as the expected (H_E) and observed (H_O) heterozygosities, were estimated using GENALEX 6 (Peakall & Smouse 2006). Test for linkage disequilibrium (LD) and exact tests for Hardy–Weinberg equilibrium (HWE) for each locus and location were conducted with the program GENEPOP version 3.4 (Raymond & Rousset 1995). The presence of null alleles was estimated using MICRO-CHECKER (Van Oosterhout *et al*. 2004). Microsatellite loci were tested on 36 workers collected in one Australian population (Howard Springs Natural Park, Northern Territory) and 20 workers from one South African population (Skukuza). Multiplex polymerase chain reactions (PCR) amplifications were optimized and performed in a 10 μL reaction volume containing 2 μL of genomic DNA (about 4–8 ng of DNA), 5 μL of 2× HotStar *Taq* Master Mix (QIAGEN), 0.3 μM of forward and reverse primers each and double- distilled water. PCRs were carried out with an MJ Research PTC-200 thermocycler. After an initial denaturing step of 15 min at 95 °C, the PCR consisted in 35 cycles of 30 s at 94 °C, 90 s at the annealing temperature (see Table 1), and 60 s at 72 °C, followed by a final extension step of 30 min at 60 °C. Microsatellite loci were analysed on an ABI 3100 automated sequencer (Applied Biosystems); the lengths of PCR products were determined using GENEMAPPER software (Applied Biosystems) and used to construct a multilocus genotype for each individual.

Eight polymorphic loci with unambiguous allelic pattern were selected for further population studies. Primer sequences and PCR conditions are given for each selected locus in Table 1. The sequences of the eight loci have been deposited in the GenBank database (Accession nos EF503571–EF503578). In the Australian sample, the number of alleles per polymorphic locus and the expected heterozygosities ranged from two to four, and from 0.083 to 0.583, respectively. Loci developed here were more polymorphic for the native South African population, with the number of alleles ranging from four to 10 and levels of expected heterozygosity per locus varying between 0.583 and 0.826 (Table 1). Such a discrepancy in allelic frequency between native and introduced populations has also been reported in other ant species (Holway *et al*. 2002; Fournier *et al*. 2005). No significant deviation from Hardy–Weinberg expectation over all loci was found for the Australian population (all $P > 0.342$), except locus *Pmeg-12* ($P < 0.001$) showing a significant heterozygote deficit. Similarly, in the South African population, all loci were at HWE (all $P > 0.069$), except loci *Pmeg-12* and *Pmeg-14* characterized by a significant heterozygote deficit ($P < 0.001$ for both loci). Such a deficiency in heterozygotes may result from the presence of null alleles. This seems to be the case for the two loci *Pmeg-12* and *Pmeg-14* from the South African population (with a respective frequency of 0.13 and 0.27) but not for *Pmeg-12* from the Australian population. High relatedness among individuals within nests can also result in departures from HWE; however, we would expect this to be evident across more, if not all, loci. Despite significant heterozygote deficit

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

	Pmeg-06	*Pmeg-09*	*Pmeg-12*	*Pmeg-14*	*Pmeg-07*	*Pmeg-10*	*Pmeg-11*	*Pmeg-15*
Tribe Pheidolini, genus *Pheidole*								
P. dentata	–	–	–	–	–	2 133–174	3 176–204	–
P. gilvescens	–	–	–	–	–	2 125–127	1 160	–
P. longipes	–	–	–	–	–	–	1 152	–
P. rhea	–	–	–	–	–	1 127	3 193–199	–
P. xerophylla	–	–	–	–	–	4 133–178	1 160	–
Tribe Pheidolini, genus *Aphaenogaster*								
A. gibbosa	–	–	–	–	–	–	–	–
A. senilis	–	–	–	–	–	–	–	–
Tribe Pheidolini, genus *Messor*								
M. barbarus	–	–	–	–	–	–	–	–
M. capitata	–	–	–	–	–	–	–	–
M. sancta	–	–	–	–	–	–	–	–
Tribe Pheidologetonini, genus *Pheidologeton*								
P. affinis	–	–	–	–	–	–	–	–
P. silensis	–	–	–	–	–	–	–	–

at one and two loci, both Australian and African populations were at HWE. No evidence for LD was detected both in Australian and South African samples (all $P > 0.075$).

In addition, we performed cross-species amplifications of the selected loci on 11 ant species of the subfamily Myrmicinae. Five species belong to the genus *Pheidole* (*P. dentata*, *P. gilvescens*, *P. longipes*, *P. rhea* and *P. xerophylla*), five species belong to a different genus of the same tribe Pheidolini (*Aphaenogaster gibbosa*, *A. senilis*, *Messor barbarus*, *M. capitata*, *M. sancta*), and two species are of the tribe Pheidologetonini (*Pheidologeton affinis*, *P. silensis*). Extractions and amplifications were performed on four individuals of each species plus two positive and one negative controls (DNA of *P. megacephala* workers and distilled water, respectively), following the procedure described above. As shown in Table 2, two loci (*Pmeg-10* and *Pmeg-11*) amplified for each species of *Pheidole* and six loci did not amplify with any of the species sampled. Individuals of *Aphaenogaster senilis*, *A. gibbosa*, *Messor barbarus*, *M. capitata*, *M. sancta*, *Pheidologeton affinis* and *P. silensis* never amplified with any of the loci tested.

In spite of relatively low allelic variation, the microsatellite loci characterized in this study will provide useful information for studies of population genetic structure in both invasive and native populations of the ant *P. megacephala*, and will help to clarify the origin of this pest species. In the African population, the presence of null alleles at two loci (*Pmeg-12* and *Pmeg-14*) should be considered and corrected for allele frequency errors by using different procedures (e.g. Van Oosterhout *et al*. 2004, 2006; Chapuis & Estoup 2007). Thus, data from these microsatellite loci will contribute to providing insight into the evolution of colony and population genetic structure following the introduction of ant species in a new environment.

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