## **PRIMER NOTE Characterization of microsatellite loci for the Argentine ant**, *Linepithema humile*, and their potential for analysis **of colony structure in invading Hawaiian populations**

KRISTA K. INGRAM\* and STEPHEN R. PALUMBI 16 Divinity Ave Harvard University, Cambridge, MA 02138, USA

## Abstract

We developed primers for five polymorphic microsatellite loci to analyse the genetic structure of colonies in an invading Argentine ant population located in Haleakala National Park on the island of Maui, Hawaii. Microsatellite loci were isolated using both a polymerase chain reaction-based and a cloning-based method. With a range of 3–18 alleles and expected levels of heterozygosity of 0.46-0.77, these loci provide useful markers for the detection of colony and population structure in new or expanding populations of this species.

*Keywords*: Argentine ant, colony structure, *Linepithema humile*, microsatellite, population structure, social insects

Received 22 August 2001; revision accepted 22 October 2001

The Argentine ant, *Linepithema humile*, is a highly polygynous, unicolonial species that has successfully invaded a diversity of habitats worldwide (Newell & Barber 1913; Haskins & Haskins 1965; Cole *et al.* 1992; Human & Gordon 1996; Holway 1998; Kennedy 1998; P. Krushelnycky *et al.*, unpublished results). Although there is much evidence of the detrimental ecological impact of Argentine ant invasions, little is known about the biology and nest ecology of this species during an invasion.

Argentine ants were introduced into the Hawaiian Islands in 1967 (Huddleston & Fluker 1968). The spread of a population of these ants in Haleakala National Park on the island of Maui provides a unique opportunity to study the changes in colony structure of a unicolonial population during an invasion. We developed five polymorphic microsatellite loci to study colony structure in this population. These loci were isolated from genomic DNA using two different methods. Initially, we employed a polymerase chain reaction (PCR)-based method using di- and trinucleotide repeat primers (CA)<sub>10</sub> and (AAG)<sub>8</sub> to probe a limiting dilution series of a target genomic library as described in Grist *et al.* (1993). Our library was made using Pbluescript KS(+) vector (Stratagene) and diluted to 1:100 000. Initial insert

stocks were amplified using M13 forward and reverse primers at an annealing temperature ( $T_a$ ) of 60 °C. The PCR method identified 11 trinucleotide repeat sequences and four dinucleotide repeat sequences. We designed primers for five trinucleotide and three dinucleotide primers, which were subsequently tested for polymorphism in this population. Two of these loci were polymorphic, with one being highly polymorphic with 18 alleles in this population.

We also used a size-selected partial genomic library and a standard cloning method to characterize additional loci (Glenn 1996). Genomic DNA was digested with *Dpn*II (New England Biolabs) and cloned into pBluescript KS (+) vector (Stratagene) that had been cut with *Bam*HI (GibcoBRL). Colonies were grown on plates, lifted onto membranes and hybridized with (CA)<sub>10</sub> and (ATT)<sub>8</sub> probes that were labelled with Digoxigenin-11-dUTP/dATP (Boehringer Mannheim) at 60 °C and 50 °C, respectively. This method yielded 12 microsatellite sequences (nine di- and three trinucleotide repeat regions) and primers were designed for three polymorphic dinucleotide repeat regions.

Alcohol-preserved adults and pupae were soaked in distilled water for 15–30 min, pulverized, and boiled in 100  $\mu$ L of a 10% Chelex (Bio-Rad) solution for 15 min After boiling, the extraction solutions were centrifuged for 1 min and supernatant was removed. One microliter (approximately 10 ng) of each sample was used in a 12.5  $\mu$ L PCR reaction containing 1 unit of AmpliTaq (Applied Biosystems/Perkin-Elmer), 80  $\mu$ M dNTPs, 1  $\mu$ M of each primer,

Correspondence: Krista K. Ingram. \*Present address: 371 Serra Mall, Stanford University, Stanford, CA 94305, USA. Fax: 650-723-6132; E-mail: kingram@ants.stanford.edu

Table 1	Microsatellite loci	designed for A	argentine ants,	, Linepithema	humile. T <sub>a</sub> ,	annealing t	emperature;	H <sub>O</sub> , observe	d heterozy	gosity;
$H_{\rm E}$ , expe	ected heterozygosit	у								

Locus	Method	Primer sequences	$T_{a}$ (°C)	Repeat motif*	Allele size (bp)†	No. of alleles	H <sub>O</sub>	$H_{\rm E}$	GenBank Accession no.
Lihu-H	PCR	F: TCGTTGAACGCATGCGG R: CCAGAGACTCTTNGCGCATGC	55	(AAT) <sub>16</sub>	351–384	18	0.83	0.77	AF388657
Lihu-L	PCR	F: ccgtgtgcacatgtaggcac R: cgagccatgaggtccttatcc	55	$(GT)_{10}^{-}(GT)_{12}^{-}$	142–158	6	0.46	0.60	AF409065
Lihu-N	Cloning	F: gatgattctacgtgtagcg R: caaacggtacacataacgc	53	$(GT)_7(CT)_8$	116–124	3	0.54	0.46	AF409063
Lihu-O	Cloning	F: cgcgggcatggattggag R: cgcctctctcgcgaggc	53	(CA) <sub>14</sub>	120–132	4	0.45	0.53	AF388658
Lihu-P	Cloning	F: GTTATAATCATGTCACGTGG R: GCAGGCCAGCTCTAGAAC	50	(AC) <sub>12</sub>	172–190	5	0.69	0.63	AF409064

\*Sequenced allele; trange of allele sizes detected at each locus.

and a buffer consisting of 10 mM Tric-Hcl, 50 mM KCl, 1.5 mM MgCls, 0.01% Gelatin, NP-40, and Triton X100. All PCR reactions were performed on a Perkin-Elmer thermocycler with the following cycle parameters: 30 cycles of denaturation at 95 °C (30 s),  $T_a$  °C (40 s), elongation at 75 °C (45 s). The annealing temperatures vary among the five loci (Table 1). PCR products were run on 5% Sequagel (National Diagnostics) acrylamide gels using fluorescent-labelled forward primers (Sigma-Genosys) and the gels were analysed using GENESCAN 3.1.2 software (Applied Biosystems/Perkin Elmer).

In total, 27 novel microsatellite repeat sequences were identified for the Argentine ant. Primers were developed for 11 of these sequences. Five of these 11 loci were polymorphic in the Haleakala population (n = 396 individual ants) with the number of alleles ranging from 3 to 18 alleles and levels of expected heterozygosity per locus ranging from 0.46 to 0.77. The primers and characteristics of these five loci in the Haleakala population are given in Table 1. Although microsatellite polymorphism has been found to be relatively low in other introduced populations of Argentine ants (Krieger & Keller 1999), the variability of these new microsatellite markers in the Haleakala population provides a valuable molecular tool to study the fine genetic structure of new or rapidly expanding populations of this species.

## Acknowledgements

We would like to thank Chris Dick for assistance during the development of the microsatellites and Lloyd Loope, Art Medeiros, Paul Krushelnycky and Ellen VanGelder of the Biological Research Division of the Haleakala National Park for assistance in Hawaii.

## References

- Cole FR, Medeiros AC, Loope LL, Zuehlke WW (1992) Effects of the Argentine ant on arthropod fauna of Hawaiian high-elevation shrubland. *Ecology*, **73**, 1313–1322.
- Glenn T (1996) *Microsatellite Manual*. Unpublished manuscript available from the author or via ftp://ftp.onxy.si.edu/protocols/msatmanV#.rtf.
- Grist SA, Firgaira FA, Morley AA (1993) Nucleotide repeat polymorphisms isolated by the polymerase chain reaction. *Biotechniques*, **15**, 304–309.
- Haskins CP, Haskins EF (1965) *Pheidole megacephala* and *Iridomyrmex humilis*. Bermuda-equilibrium or slow replacement. *Ecology*, **46**, 736–740.
- Holway D (1998) Effects of Argentine ant invasions on grounddwelling arthropods in northern California riparian woodlands. *Oecologia*, **116**, 252–258.
- Huddleston EW, Fluker S (1968) Distribution of ant species of Hawaii. *Proceedings of the Hawaiian Entomological Society*, **20**, 45–69.
- Human KG, Gordon DM (1996) Exploitation and interference competition between the invasive Argentine ant, *Linepithema humile*, and native ant species. *Oecologia*, **105**, 405–412.
- Kennedy TA (1998) Patterns of an invasion by Argentine ants (*Linepithema humile*) in a riparian corridor and its effects on ant diversity. *American Midland Naturalist*, **140**, 343–350.
- Krieger MJB, Keller L (1999) Low polymorphism at nineteen microsatellite loci in a French population of Argentine ants (*Linepithema humile*). *Molecular Ecology*, 8, 1078–1079.
- Newell W, Barber TC (1913) *The Argentine Ant*. Bureau of Entomology Bulletin. United States Department of Agriculture, Washington, D. C.