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Haskins & Haskins 1965; Cole *et al.* 1992; Human & Gordon 1996; Holway 1998; Kennedy 1998; P. Krushelnysky *et al.*, unpublished results).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)*₁₀ and *(AAG)*₈ to probe a limiting dilution series of a target genomic library as described in Grist *et al.* (1993). Our library was made using Pluescript KS (+) vector (Stratagene) that had been cut with BamHI (GibcoBRL). Colonies were grown on plates, lifted onto membranes and hybridized with *(CA)*₁₀ and *(ATT)*₈ 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 µL 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 µL PCR reaction containing 1 unit of AmpliTaq (Applied Biosystems/Perkin-Elmer), 80 µm dNTPs, 1 µm of each primer,
and a buffer consisting of 10 mm Tric-Hcl, 50 mm KCl, 1.5 mm MgCl2, 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), T5 °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 Krushelnycy and Ellen VanGelder of the Biological Research Division of the Haleakala National Park for assistance in Hawaii.

**References**


