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PRIMER NOTE

Isolation and characterization of microsatellite loci from Galápagos lava lizards (*Microlophus* spp.)

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Abstract

We describe the isolation of microsatellite loci from Galápagos lava lizards (*Microlophus* spp.) using an enriched genomic library. Twelve loci that are polymorphic among six populations from two species are described. Characterization of these loci in 20 individuals within one population (Isla Plaza Sur) showed seven to be polymorphic with 3–11 alleles. Heterozygosities within this population were high (0.32–0.90) and did not deviate from Hardy–Weinberg expectations. We suggest that these markers will be useful in studies of population differentiation within and among islands across the Galápagos archipelago.

Keywords: enriched library, Galápagos Islands, microsatellites, population structure, Tropiduridae

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Studies based on protein electrophoresis and mitochondrial DNA suggest that Galápagos lava lizards (*Microlophus* spp.) include seven or eight species that have evolved from two colonizations of the Galápagos Islands from the western coast of South America (Wright 1983; Heise 1998). While these studies have also aided our understanding of *Microlophus* evolution across the major islands of the archipelago, they do not have sufficient resolution to analyse the population differentiation that occurs among or within islets. Importantly, there is significant variation in morphology, ornamentation, and ecological performance among populations at this smaller scale (Snell *et al*. 1988; Miles *et al*. 2001; Stone *et al*. 2002). Neutral genetic markers with high variability will be useful in testing hypotheses concerning the origin and maintenance of this phenotypic variation.

To this end we have developed a series of polymorphic microsatellites to screen genetic variation within and among populations of lava lizards. We prepared a genomic library enriched for microsatellites using subtractive hybridization (following Hamilton *et al*. 1999). Genomic DNA of 10 individuals (combined total 11.25 μg), representing five islands [*M. albemarlensis*: Isla Santa Cruz (n = 3), Isla Plaza Norte (n = 3), Isla Plaza Sur (n = 2), Roca Bainbridge no. 6 (n = 1); *M. bivittatus*: Isla Champion (n = 1); island names follow Snell *et al*. (1996)], was mixed and digested with restriction enzymes *Nhe*I, *Hae*III and *Rsa*I. Linkers were ligated to restriction fragments before their hybridization to 3′ biotinylated probes overnight at 60 °C and 45 °C for CA and GATA, respectively. Enrichment for GATA was performed twice to increase the proportion of clones containing these repeats. Microsatellite-enriched DNA was ligated into pBluescript® II SK(+) (Stratagene) prior to transformation into *Escherichia coli* competent cells (ElectroMAX™ DH10B, Life Technologies) using electroporation.

Plasmid DNA was isolated from individual colonies by boiling for 10 min in 100 μL TE buffer (10 mM Tris–HCl, pH 8.0; 0.1 mM ethylenediaminetetraacetic acid, pH 8.0) and used as a template in a polymerase chain reaction (PCR)-based procedure for microsatellite screening (see Lunt *et al*. 1999). Briefly, two PCR reactions (25 μL) for each clone were carried out: one with the repeat sequence used as a primer in addition to two universal primers (M13 forward and reverse), and another with the universal primers only to serve as a control. Clones containing a microsatellite were identified when the former showed two bands while the control showed one. Control PCR products from positive clones were purified (Geneclean® III, Anachem) prior to sequencing (ABI PRISM Big Dye™

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Table 1 Microsatellite loci for *Microlophus* lizards from the Galápagos Islands, Ecuador. Size range and number of alleles for all individuals across six islands (n = 32). Number of alleles in parentheses and heterozygosities are for Isla Plaza Sur only (n = 20)

Locus	GenBank Accession number	Repeat motif	Primer sequence 5′–3′	[$MgCl_2$] (mM)	Size range (bp)	No. of alleles	H_E	H_O
Mic1	AF463628	$(AC)_{13}$	F: HEX-TGCTAAGCATGAGCTACACC R: CCAACTAGCTGGCAC	1.5	136–146	6 (3)	0.52	0.59
Mic2	AF463629	$(TAGA)_2TAGC(TAGA)TGA(TA)_2(TAGA)_8$	F: FAM-CAGTGGAACACACAGTATCC R: ACCTTAGGAATGACAGAAGG	2.5	186–426	24 (11)	0.86	0.90
Mic3	AF463630	$(TAGA)_8$	F: TET-GTGAGAACTGAAACTGAAAGC R: TTTGTCTACAAACAAAACTGC	2.5	177–193	6 (3)	0.58	0.58
Mic4	AF463631	$(GATA)_6$	F: FAM-TGTTCATTTCATCATCAAGC R: CTTCACAAACATTGCAACC	2.5	212–452	9 (5)	0.75	0.75
Mic5	AF463632	$(CA)_{14}$	F: FAM-AGGAACATTCTGCACTAAGG R: TTCCACTCTGCTCTACATCC	2.5	194–216	7 (4)	0.61	0.63
Mic6	AF463633	$(AC)_4TC(AC)_3TC(AC)_3$	F: HEX-ATTCTCCCATAAAATCTGC R: CAAGGAGCTCTTAGAAAAACC	4.0	158–172	5 (3)	0.65	0.53
Mic7	AF463634	$(CA)_5TA(CA)_{14}$	F: TET-TTTATACACTATTTACAACCAAAGG R: TTCTTGATCTTCCCAGTAGG	2.5	155–171	3 (1)	—	—
Mic8	AF463635	$(CA)_3TA(CA)_2AACACG(CA)_8$	F: HEX-TTCATGTAACCCTAATGATCC R: TGCTTTCCTCACACATGC	2.5	105–121	5 (3)	0.28	0.32
Mic9	AF463636	$(CA)_{13}$	F: FAM-ATTCTTGTGCTGCTTACAGC R: TGTCCTAGCAGAGGTCTCAT	1.5	215–230	4 (1)	—	—
Mic10	AF463637	$(CA)_9$	F: TET-ATAGTGGGATTTTCTCATGG R: CTTGATGGAGCTTTATTTCC	2.5	193–197	3 (1)	—	—
Mic11	AF463638	$(CA)_{17}$	F: TET-TGTGTTGATGGGGATACAG R: GCTTTTCCCAGAAGAACC	2.5	104–115	3 (1)	—	—
Mic12	AF463639	$(CA)_{11}$	F: TET-AGTACAATCGTTTAACTCTCTCC R: CCTCAGTTTCTGTACGATGG	2.5	193–211	3 (1)	—	—

Terminator Cycle Sequencing Ready Reaction Kit version 2.0) on an ABI 377 DNA sequencer (PE Biosystems). Inserts containing at least six repeats were selected for the design of primers (Primer3, Whitehead Institute for Biomedical Research).

Genomic DNA was extracted using a Qiagen QIAamp® DNA Mini Kit. We amplified microsatellite loci using 'touchdown' PCR [11 μL reaction: 20 mM Tris–HCl, pH 8.4, 50 mM KCl, 1.5–4.0 mM $MgCl_2$, 100 μM each dNTP, 0.24 μM each primer, 0.75 units *Taq* polymerase (Gibco), and 2.5 ng of template; cycles comprising 94 °C for 5 min, then 15 cycles of 94 °C for 20 s, 60 °C with a 1 °C decrease per cycle for 30 s and 72 °C for 30 s, followed by 20 cycles of 94 °C for 20 s, 45 °C for 30 s and 72 °C for 30 s, then finally one cycle of 72 °C for 7 min]. Amplifications were performed with fluorescently labelled primers using a GeneAmp® PCR System 9700 (Perkin Elmer). PCR products were visualized on an ABI 373 DNA sequencer (Perkin Elmer) using Genescan-500 TAMRA for sizing.

We genotyped 32 individuals originating from six islands in the archipelago [*M. albemarlensis*: Isla Plaza Sur (n = 20), Isla Plaza Norte (n = 3), Isla Santa Cruz (n = 6), Roca Bainbridge no. 6 (n = 1), Islote Marielas Sur (n = 1); *M. bivittatus*: Isla Champion (n = 1)]. Twelve loci amplified reliably and were polymorphic among populations (Table 1). Seven of 12 loci were polymorphic within Plaza Sur (3–11 alleles) and heterozygosities ranged from 0.32 to 0.90. No locus deviated from Hardy–Weinberg expectations ($P > 0.1$; using GENEPOP on the Web, at http://wbiomed.curtin.edu.au/genepop/index.html). Our results suggest that the loci presented here will be useful for studies of population structure within and among islands in the Galápagos archipelago.

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Female Galápagos lava lizard (*Microlophus albemarlensis*) from Isla Santa Cruz in the Galápagos Islands, Ecuador. Lava lizards exhibit marked phenotypic variation among populations isolated on the islands and islets of the archipelago. Photo by Heidi M. Snell.
