PRIMER NOTE

Polymorphic microsatellite markers for the Florida scrub lizard (*Sceloporus woodi*)

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Abstract

The Florida scrub lizard (*Sceloporus woodi*) is one of a suite of species restricted to Florida scrub, a threatened ecosystem. We characterized eight microsatellite loci from scrub lizards based on screening of 75–91 individuals per locus. Polymorphism was high (8–20 alleles per locus). Observed and expected heterozygosities ranged from 0.32–0.83 and 0.77–0.91, respectively. These markers will be useful for population-level analyses and can contribute to a genetic foundation for conservation strategies for this endemic species.

Keywords: Florida scrub lizard, microsatellite, *Sceloporus woodi*

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The Florida scrub lizard is endemic to scrub habitats found on relic Pliocene and Pleistocene sand ridges in the centre of the Florida peninsula and in strands along more recent coastal sand dunes. Within these ridges, scrub is naturally fragmented into an archipelago of habitat islands surrounded by more mesic environments unsuitable for obligate scrub organisms. In recent decades, conversion of scrub to citrus groves and urbanization have reduced the number and size of scrub patches and increased patch isolation; as a result, the endemic flora and fauna of Florida scrub are severely threatened (McCoy & Mushinsky 1992). An analysis of mitochondrial DNA cytochrome *b* revealed deep genetic separations among scrub lizard populations on disjunct sand ridges (Clark *et al*. 1999). We developed microsatellite markers to examine finer-scale genetic structure of lizard populations across habitat islands within ridges and, subsequently, to inform conservation strategies such as reintroduction, translocation and reserve design (United States Fish and Wildlife Service 1991; Branch *et al*. 2003).

DNA isolations were performed using the DNeasy Tissue Kit (Qiagen, Inc.) following the protocol for animal tissue, and an enriched genomic DNA library was prepared from one individual (Kandpal *et al*. 1994; Fleischer & Loew 1996). Genomic DNA was digested with *Sau*3AI restriction enzyme. Fragments within 400–1500 base pairs were size selected by excision from a low-melt agarose gel. DNA fragments were ligated to *Sau*3AI linkers, and excess linkers were removed using an Ultrafree-MC Centrifugal Filter Unit (Millipore). Linker-ligated fragments were then amplified by polymerase chain reaction (PCR).

A library enriched for $(CA)_n$ and $(GT)_n$ repeats was created by initially hybridizing the DNA fragments to a biotinylated nucleic acid probe, followed by capture of probe–target fragments using VECTREX Avidin D (Vector Laboratories, Inc.). Probe–target fragments were amplified by PCR, ligated to a TOPO TA pCR 2.1 vector (Invitrogen Corp.), and transformed into One Shot *Escherichia coli* cells (Invitrogen Corp.). Colonies were screened on nitrocellulose transfer membranes (Osmonics Laboratory Products) and hybridized with a chemiluminescent $(CA)_n$ probe (Lifecodes, Inc.). Detection of colonies containing $(CA)_n$ repeats was accomplished using Lumi–Phos 480 (Lifecodes, Inc.). Colonies containing repeats were grown overnight in LidBac Safe–Lock tubes (Eppendorf), purified using a QIAprep Spin Miniprep Kit (Qiagen, Inc.), and then sequenced on an ABI 377 automated sequencer.

Primers were developed using OLIGO 6.0 software (Molecular Biology Insights, Inc.), and the upper primer of each pair was 5′-fluorescently labelled with either FAM or HEX (MWG Biotech). Primer pairs were optimized, and PCR was performed with 25 μL total volumes containing at least 50 ng template DNA, 0.24 μM of each primer, 800 μM dNTPs, 1× PCR buffer, 1.0 U *Taq* (Sigma), and a primer-specific $MgCl_2$ concentration (Table 1). All PCR reactions began with a 94 °C denaturization (5 min), followed

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Table 1 Characterization of eight polymorphic microsatellite loci in the Florida scrub lizard, *Sceloporus woodi*

Locus (GenBank ID)	Primer sequence (5′–3′)	Repeat unit	Allele size range (bp)	T_a (°C)	$MgCl_2$ (mM)	No. of cycles	n	N_a	H_O	H_E
SW614–A1 (AY450600)	**U-F**TTATGCTTGTTGCTTGCC **L**-AGGTGTTGGTAGTTCAGGGTAAA	$(CA)_2CTA(CA)_{14}$	388–406	62.6	1.5	33	91	12	0.626	0.772
SW614–A4 (AY450601)	**U-F**CACTAAGGACCACACTCG **L**-GGAAGAACCACCAGAGTAAC	$(CA)_{14}$	376–391	61.7	1.5	30	87	8	0.575	0.799
SW614–A6 (AY450602)	**U-H**AAAATCCAGCAAACCAAC **L**-TTCTCATCTGACTGGCTATT	$(CA)_{17}$	263–280	60.8	3.0	34	89	10	0.719	0.860
SW614–A7 (AY450603)	**U-H**CCATAGGAAAACCAGAACATC **L**-ATAACACTCTAATAGCCAA	$(GT)_{17}$	238–264	59.3	3.0	32	89	20	0.573	0.840
SW614–B1 (AY450604)	**U-F**GCAGAGGAGGAAACCAGTTA **L**-GGGAGGACATTCTTATTAGC	$(GT)_5TT(GT)_{13}$	326–355	63.2	1.5	32	88	14	0.830	0.906
SW614–B6 (AY450605)	**U-F**ACTCTACGGTCTACTTCC **L**-TGCCTATGTTGCTTACTTAC	$(GT)_{15}$	295–328	60.8	1.5	30	82	15	0.415	0.874
SW614–B10 (AY450606)	**U-H**AAAAGTAGCAACATCAAACA **L**-GTGAGACAAAACTAAAAGC	$(CA)_{24}$	189–220	61.6	3.0	34	95	20	0.716	0.885
SW614–B12 (AY450607)	**U-H**GCTGGTGTATGCTGCTATGGA **L**-GCCGACATCTCTCATACCC	$(GT)_{20}$	267–289	65.1	1.5	30	75	13	0.320	0.895

U = upper primer; **L** = lower primer.
Fluorescent primer labels: **F** = Fam; **H** = Hex.
T_a is the annealing temperature; n = sample size; N_a = number of alleles; H_O and H_E are observed and expected heterozygosities, respectively.

by a primer-specific number of cycles at 94 °C (30 s), primer-specific annealing temperature (30 s), 72 °C (50 s), and a final extension of 72 °C (20 min). The number of PCR cycles and the annealing temperatures are presented in Table 1. All PCR products were run on an ABI 377 automated sequencer using ROX 500 size standard and analysed using GENESCAN and GENOTYPER software (Applied Biosystems).

Of the 17 primer pairs tested, eight were ultimately optimized and found to be polymorphic. Each polymorphic locus was screened with a minimum of 75 individuals, and observed and expected heterozygosities were calculated using POPGENE version 1.31 (Yeh *et al*. 1999). The number of alleles at each locus ranged from eight to 20 and observed heterozygosities ranged from 0.320 to 0.830 (Table 1).

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