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PRIMER NOTE

Characterization of microsatellite markers for the endemic sand dune lizard, *Sceloporus arenicolus*

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Abstract

Here we characterize and report on the genetic variability of eight microsatellite loci for the endemic sand dune lizard, *Sceloporus arenicolus*, that amplified consistently for individuals throughout the species' range. The number of alleles per locus was high (mean = 13.25) and observed heterozygosities ranged from 0.354 to 0.808. With the exception of a single locus, the loci did not deviate from Hardy–Weinberg expectations. This set of markers is currently being used to examine population structure and landscape genetics in *S. arenicolus*, a habitat specialist with a restricted range and patchy distribution.

Keywords: conservation, habitat specialist, landscape genetics, Phrynosomatidae, population genetics, shinnery oak

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The sand dune lizard, *Sceloporus arenicolus*, is a small-bodied terrestrial lizard found exclusively in the shinnery oak (*Quercus havardii*) dominated sand dune habitat of eastern New Mexico and western Texas (Degenhardt *et al*. 1996). This species is a habitat specialist, found in deep and open sand dune blowouts with particular thermal and physical characteristics (Fitzgerald *et al*. 1997). *Sceloporus arenicolus* is listed as endangered by the New Mexico Department of Game and Fish and is categorized by the US Fish and Wildlife Service as a candidate for federal listing with a priority number of 2. Threats to this species include habitat destruction due to the removal of shinnery oak by herbicide spraying and habitat fragmentation resulting from oil and gas development. To examine historic patterns of genetic connectivity among subpopulations, identify potential consequences of fragmentation, and indirectly measure current levels of dispersal in this patchy landscape, we developed a set of eight microsatellite markers for population genetic analyses.

Whole genomic DNA was extracted from liver tissue of an adult preserved in 100% EtOH (voucher: MSB 57615) using lysis buffer and proteinase K followed by organic clean up with phenol–chloroform (Sambrook & Russell 2001). We digested 300 ng of DNA with the restriction enzymes *Alu*I and *Hae*III (New England Biolabs) in two separate reactions and ligated SNX linkers to the fragments using T4 DNA ligase (Hamilton *et al*. 1999). We enriched the pool of ligated DNA fragments by hybridization with biotin-labelled di-, tri-, and tetrameric oligonucleotides in reactions consisting of a 10 min incubation at 97 °C followed by a 60 min incubation at 52, 60, or 65 °C. We recovered single-stranded fragments containing microsatellite repeats using streptavidin-coated magnetic beads (New England Biolabs) and conducted a polymerase chain reaction (PCR) using the SNX primer to make fragments double-stranded. PCR products were cut with *Nhe*I (New England Biolabs) and the digested product was run on a 1% TAE agarose gel for size selection. Fragments from 300 to 900 bp in length were excised, purified using a QIAquick Gel Extraction Kit (QIAGEN), and ligated to pUC19 plasmids and transformed into DH5α T1 phage resistant competent cells (Invitrogen). We plated cells on Luria-Bertani (LB) agar/ampicillin plates and transferred colonies to nylon membranes that were hybridized to ^{32}P radio-labelled probes with the same repeat motifs used in the enrichment and visualized on autoradiographs (Sambrook & Russell 2001). Clones were cultured overnight in 2-mL Luria broth and DNA was extracted with a QIAprep Spin Miniprep Kit (QIAGEN).

We sequenced 96 positive clones using the primer M13F (5′-GTTGTAAAACGACGGCCAGTG-3′) and BigDye

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Table 1 Primer sequences, amplification conditions, and diversity in eight microsatellite loci for *Sceloporus arenicolus*. Significant departure from HWE is indicated by an asterisk (*)

	Locus (Genbank no.)	Primer (5′–3′)	Repeat	T_a	N/N_a	Size	H_O/H_E
1	sar80 (DQ922786)	F: PET – ATGCCCTCAGAAACACAACC R: GTTTCTGCGGAACCAAATACACCT	$(ATAG)_{12}(ACAG)_6$	58	99/21	349–402	0.737/0.924
2	sar84 (DQ922788)	F: PET – AATTTCCCAATTGCAGTGGA R: GTTTTGTTGCCCTTTGATGTGTGT	$(CA)_{16}$	56	99/11	109–147	0.515/0.680
3	SA52.20 (DQ922793)	F: VIC – TTTCTGGCATTCCGAGGTGAGG R: TAATGCATCCAGTATCCCCGTCTTG	$(TTG)_9$	60	99/4	103–112	0.545/0.547
4	SA52.29 (DQ922794)	F: NED – GCGGTCTATAACGCACCTTTCTGTC R: ATCCCCCTGTGCATTTCTTTCTCTAC	$(TTTC)_{15}$	59	99/16	185–249	0.808/0.910
5	SA52.33 (DQ922790)	F: 6-FAM – TGGCGGAATGGGTGAATGTAAAA R: TTGTAGAACTGTCCCCCTGTCCTGA	$(AGAT)_{13}$ – 23 – $(AGAT)_4(AGAC)_3$	64	99/17	344–446	0.697/0.856
6	SA60.02 (DQ922791)	F: NED – CTTTCATAGCAGAGGTTAGAGTCAGA R: ACTGCTTATTAAATGCTGCTGTTG	$(GT)_{10}$	60	99/8	117–133	0.455/0.772
7	sar82 (DQ922787)	F: VIC – ATGTGGAAGGCTTTGGTGAC R: GTTTAACAGGCCACTGATCCAGAC	$(GGAG)_3(GGAA)_{13}$	64	82/16	255–319	0.354/0.902*
8	SA60.12 (DQ922789)	F: PET – CTTCCAACTTTTCCATTCTTTACTGACA R: AATATACACCACTCGCTCTTTCTCT	$(GT)_{24}$	Touchdown	99/13	122–148	0.758/0.873

F, forward primer; R, reverse primer; T_a, annealing temperature; N, number of individuals scored; N_a, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity.

termination sequencing chemistry (PerkinElmer) on an ABI PRISM 377 DNA sequencer (Applied Biosystems). We trimmed the pUC19 and SNX linker and aligned all sequences containing microsatellites to identify duplicate clones. We found 57 unique sequences containing microsatellites for which we designed primer pairs using PRIMER 3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) or PRIMERSELECT (DNASTAR).

Sceloporus arenicolus tail or toe clips were extracted in 150 μL of a 5% Chelex solution (Chelex-100, Bio-Rad) with 19 μg proteinase K by incubation at 55 °C for 180 min and 99 °C for 10 min. We performed PCRs on a PTC-100 thermocycler (MJ Research) at a total volume of 10 μL with 1 μL of the supernatant from the Chelex extraction as template DNA, 1× buffer (10 mM Tris-HCl, 50 mM KCl; Roche), 1.5 mM $MgCl_2$, 0.4 mM dNTPs, 0.2 μM of each primer (forward and reverse), and 0.25 U of *Taq* polymerase (Roche). Locus SA52.29 amplified optimally at 1.0 mM $MgCl_2$. Primer pairs that reliably yielded product of high concentration were labelled with a fluorescent tag (VIC, PET, 6-FAM, or NED) for visualization on an ABI 3100 Automated Capillary DNA Sequencer using the GeneScan-500 LIZ size standard (Applied Biosystems). Sequences of original clones are accessioned in GenBank DQ922786–DQ922794.

We chose eight loci that were polymorphic and produced clean peaks for further data collection (Table 1). PCR cycles for five loci consisted of an initial denaturation at 94 °C for 5 min followed by 35 cycles of 1 min at 94 °C, 1 min at a primer specific annealing temperature (see Table 1), and 1 min at 72 °C followed by a final extension for 5 min at 72 °C. For locus sar82, a similar profile was used with each step of the internal cycle held for 30 s instead of 1 min. For locus SA60.12 we used a touchdown protocol with an initial denaturation at 94 °C for 5 min, 35 cycles of 94 °C for 30 s, touchdown annealing temperature for 30 s, and 72 °C for 30 s, and a final extension of 72 °C for 5 min. The touchdown consisted of five cycles at each of three annealing temperatures (60 °C, 57 °C and 54 °C) followed by 20 cycles with a 52 °C annealing temperature. After amplification, we pooled PCR product for individual loci into two multiplex groups (loci 1–6 and Loci 7–8, Table 1) and combined 1 μL of pooled PCR product with 19 μL Hi-Di formamide and 0.1 μL GeneScan-500 LIZ for electrophoresis on the ABI 3100 sequences.

We chose 99 individuals from seven populations throughout the species range for tests of variation at the eight loci. We tested for evidence of linkage disequilibrium and departures from Hardy–Weinberg equilibrium using the software GENEPOP ON THE WEB version 3.4 (Raymond & Rousset 1995). A Markov chain method (Guo & Thompson 1992) with 2500 dememorization steps and 100 batches of 5000 iterations per batch was used to determine significance. We did not find evidence of linkage disequilibrium among any pairs of loci. Overall observed heterozygosities were lower than expected heterozygosities (Table 1) which may be due to a Wahlund effect and the pooling of otherwise distinct populations. Within population, tests of departure from HWE were nonsignificant for most loci and most populations. The only locus that consistently

showed a large excess of homozygotes in all populations was locus sar82. The heterozygote deficiency and failed amplification in approximately 15% of individuals suggests that departure from HWE may be due to a null allele.

Our pilot data suggest that these eight microsatellite loci are adequate for detecting and characterizing population genetic structure in *Sceloporus arenicolus* at fine and range-wide geographical scales. Given the patchy distribution of *S. arenicolus* in a landscape experiencing high rates of anthropogenic change, these markers will also be important for identifying barriers to movement, quantifying dispersal parameters, and designing effective conservation strategies.

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