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TECHNICAL NOTE

Microsatellites isolated from the North American ground skink (*Scincella lateralis*)

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Abstract Thirteen polymorphic microsatellite markers were developed in the North American ground skink, *Scincella lateralis*, for use in landscape and population genetic research. Loci were genotyped for a sample of 37 lizards from southeastern Louisiana. The number of alleles per locus ranged from 5 to 25 and observed heterozygosity ranged from 0.23 to 0.97. The high levels of polymorphism observed in these loci will be useful for a variety of applications.

Keywords Southeastern United States · Scincidae · Lizard · Primers · Population genetics

The North American ground skink, *Scincella lateralis*, is a small, mesic-adapted, leaf-litter-dwelling lizard endemic to the southeastern United States. Although *S. lateralis* is one of the most abundant reptiles in the southeastern United States (Dundee and Rossman 1989; Conant and Collins 1998), a recent phylogeographic study has shown that a very high level of cryptic fragmentation exists across the species range and that some groups have been isolated to geographically-restricted areas along the Gulf Coast (Jackson and Austin 2010). To better understand the evolutionary significance of these groups as well as the nature of the dispersal limitation that created them, we have developed 13 microsatellite loci to be used in a series of landscape and population genetic studies geared toward exploring the effects of current and ancient landscape features on ground skink dispersal and the genetic consequences of secondary contact between distinct lineages. These loci will allow us to both identify the potential consequences of ongoing landscape alteration on the connectivity of ground skink populations and to better characterize the vast cryptic diversity exhibited by this species such that its evolutionary potential can be properly managed and conserved.

To develop these loci, we first doubly-enriched a microsatellite library from a single *S. lateralis* sample (from Stone County, Arkansas) following the protocol of Glenn and Schable (2005). Described briefly, genomic DNA was extracted using a Qiagen DNeasy kit (Qiagen, Valencia, CA) and then digested using restriction enzyme *Rsa*I (New England Biolabs) and ligated to double-stranded SuperSNX linkers SuperSNX24 Forward (5′-GTTTAAGGCCTAGC TAGCAGCAGAATC) and SuperSNX24 Reverse (5′-GAT TCTGCTAGCTAGGCCTTAAACAAAA). Linker-ligated DNA was denatured, hybridized to three separate mixes of biotinylated microsatellite probes (mix 2 = $(AG)_{12}$, $(TG)_{12}$, $(AAC)_6$, $(AAG)_8$, $(AAT)_{12}$, $(ACT)_{12}$, $(ATC)_8$; mix 3 = $(AAAC)_6$, $(AAAG)_6$, $(AATC)_6$, $(AATG)_6$, $(ACAG)_6$, $(ACCT)_6$, $(ACTC)_6$, $(ACTG)_6$; mix 4 = $(AAAT)_8$, $(AACT)_8$, $(AAGT)_8$, $(ACAT)_8$, $(AGAT)_8$), and captured on streptavidin beads (Dynal). Unhybridized DNA was then washed away while the remaining DNA was eluted and amplified using polymerase chain reaction (PCR). PCR products were enriched a second time using the process described above, after which doubly-enriched amplicons were ligated to

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vectors and cloned using a TOPO-TA Cloning Kit (Invitrogen, Carlsbad, CA).

Cloned inserts from 192 colonies were isolated and amplified using M13 primers, followed by purification and cycle-sequencing with a BigDye Terminator kit v3.1 (Applied Biosystems, Foster City, CA). Sequences were electrophoresed on a 3130xl Genetic Analyzer (Applied Biosystems).

Primer design was carried out using MSATCOMMANDER v0.8.1 (Faircloth 2008) with the stipulation that one primer within each pair contains a 5′ engineered oligo (CAG tag 5-CAGTCGGGCGTCATCA-3′) that can anneal to a fluorescently-labeled (6-FAM) probe during amplification, facilitating visualization of amplicons on a capillary sequencer. Unlabeled primers were also tagged with 5′ GTTT to promote non-template A addition during PCR. Thirty primer pairs were used to optimize microsatellite amplification for a panel of four to eight individuals (depending on locus) across a gradient of annealing temperatures, ranging from 50 to 65°C. PCRs were performed in 20 µl volumes using 2.5 µl genomic DNA (10 ng/µl), 2 µl of 10× buffer with $MgCl_2$ (15 mM), 2 µl BSA (250 µg/mL), 0.4 µl of dNTP solution (10 mM of each nucleotide), 0.12 µl *Taq* polymerase (5 U/µl, Sigma-Aldrich, St. Louis, MO), 0.54 µl 6-FAM-labled CAG tag (10 µM), 0.6 µl unlabeled primer (10 µM), 0.6 µl labeled primer (1 µM), and 11.24 µl purified H_20. Reactions were carried out by denaturing for 3 min at 96°C followed by 35 cycles of denaturation (30 s at 94°), primer annealing (45 s at 50–65°), and extension (1 min at 72°), ending with a 6-min final extension at 72°. PCR products were electrophoresed on a 3100 Genetic Analyzer and scored using Genemapper v3.7 (Applied Biosystems) against a Naurox size standard (DeWoody et al. 2004).

Thirteen loci that demonstrated reliable amplification, good genotype readability, and polymorphism were

Table 1 Specifications for 13 microsatellite loci screened in 37 *Scincella lateralis* samples

Locus/GB	Primer sequence 5′–3′	Repeat motif	T_a (°C)	Size range (bp)	*N*	*k*	H_0	H_1	PIC
P1-20	F: *CAG*-AGCAACAGCAAGCATGGG	(AGT)^23	53	226–290	37	21	0.9189	0.9493	0.9329
HM627393	R: GTTTAAGGCATTGTGTTGGGCAC								
P1-26	F: *CAG*-TCTAGCAGCATTGGGGAGC	(GTTT)^7	53	222–246	37	6	0.2973*	0.7242	0.6706
HM627394	R: GTTTGCTGTGTGTCTGTGCCATC								
P1-45	F: *CAG*-TTGGGTCACGAGTTGTCCG	(ATCT)^23	60	211–315	37	25	0.9189	0.9611	0.9457
HM627395	R: GTTTTCACATCATCGGTCTTTGCC								
P2-C03	F: GTTTTGTGTTCCATATCACAAGCCC	(CATT)^18	60	164–228	37	15	0.7838	0.8963	0.8738
HM627396	R: *CAG*-TGCATTCCTTCAGTCGAAAGC								
P2-D11	F: *CAG*-ACCTATGCCGTCAGCCAC	(AAAC)^8	63	221–239	37	6	0.6216	0.6479	0.571
HM627397	R: GTTTACAAATCTGGGGTAGTGGGG								
P2-E02	F: *CAG*-GGGATCAAGCAGGGTCAG	(AAAG)^23	55	155–250	37	19	0.973	0.9315	0.9138
HM627398	R: GTTTGGGGAGGAAATGGGGAAGG								
P2-E08	F: *CAG*-TTGGCATAGGAAAGGCGGG	(GAT)^18	63	176–210	37	12	0.6486	0.8545	0.825
HM627399	R: GTTTCACCACTATTGCCCACCAC								
P2-E12	F: *CAG*-TCCCCTCAAACACCATGC	(ATGT)^28	55	218–295	37	16	0.8919	0.9108	0.8902
HM627400	R: GTTTCAGCAATGACCAGAACCCC								
P2-F11	F: GTTTAGCCTCTTTGCTCATTTGC	(AATG)^10	60	208–272	37	13	0.6216*	0.8823	0.8571
HM627401	R: *CAG*-GCCATCCTGGAAACTGC								
P1-08	F: *CAG*-CAGTGGCGTAGCTAGGTG	(CATT)^11	53	165–201	37	8	0.3784*	0.8245	0.7878
HM627402	R: GTTTTTGTTGCTCTGCATCAGCC								
P1-76	F: *CAG*-GGCTTGGGTCAAGTAAGAAC	(CATT)^15	60	156–208	37	14	0.9189	0.9126	0.8919
HM627403	R: GTTTTTGCAGATAGGGGTTGCAG								
P2-C08	F: GTTTGCAAATGCAGTCTGTCCCC	(GAT)^16	60	220–241	37	7	0.7297	0.8138	0.7743
HM627404	R: *CAG*-CCTTTTAGAGCAGGACCCAG								
P2-G08	F: *CAG*-TTGGTTGGTTGGTTGGCTG	(GATT)^9	60	249–265	35	5	0.2286*	0.7093	0.6449
HM627405	R: GTTTATGGATTGGGCTGCAAGTC								

GB Genbank accession number; T_a PCR Annealing temperature; *N* Number of individuals per locus; *k* Number of alleles per locus; H_0 and H_1 Observed and expected heterozygosities, respectively; and *PIC* Polymorphic information content. * indicates loci not in HWE after Bonferroni correction. Primers indicated with 5′ *CAG* were engineered with a CAG tag sequence (5′-CAGTCGGGCGTCATCA-3′) as explained in the text

selected to be amplified for 37 *S. lateralis* individuals sampled from near False River, a Mississippi River oxbow lake near New Roads, Louisiana. Genotyping was carried out as described for optimization and the results are listed in Table 1. Allelic richness (*k*), polymorphic information content (PIC), and proportions of observed versus expected heterozygosity were then calculated for these samples using Cervus v3.0.3 (Marshall et al. 1998). Exact tests for Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium among loci were carried out using GENEPOP v3.4 (Raymond and Rousset 1995). No significant genotypic disequilibrium was detected among loci after a Bonferroni correction, although, four loci exhibited significant deviation from HWE (Table 1). These deviations may be due to a substantive incidence of null alleles at these loci or to likely genetic substructure within the sample. A previous study has observed mixed clades and populations at this sample locality based on population genetic analysis of mitochondrial and multilocus nuclear data, respectively (Jackson and Austin 2010). These microsatellite loci will be useful in studying the scope, origins, and behavior of this zone of population overlap in southeastern Louisiana as well as in the investigation of a variety of other landscape and population genetic phenomena that govern connectivity, diversification, and maintenance of populations within this North American lizard.

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