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Isolation and characterization of 11 polymorphic microsatellite loci in collared lizards (*Crotaphytus collaris*)

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Abstract

We identified 11 polymorphic microsatellite loci in collared lizards (*Crotaphytus collaris*). Polymorphism assessment in 512 individuals from 52 populations sampled across much of the species distribution revealed a fairly high degree of genetic diversity (six to 20 alleles per locus) and a wide range of average expected heterozygosity values (0.143–0.530). We found no evidence for linkage, very few deviations from HW expectation (two of 572 possible population/locus analyses) and thus no evidence for null alleles. There was a tendency for reduced polymorphism towards the northern periphery.

Keywords: *Crotaphytus collaris*, genetic variation, microsatellites, peripheral populations, population structure

Collared lizards (*Crotaphytus collaris*) are medium-sized heliothermic predators that inhabit rocky outcrops throughout their extensive range from the southwestern US and northern Mexico eastward through Oklahoma into the Flint Hills of Kansas and the Ozark Plateau of southern Missouri and northern Arkansas (McGuire 1996). Environmental conditions important to collared lizard survival and reproductive fitness vary widely across this geographical expanse. As a result, populations face varying selective and demographic forces in different parts of the range—making this an interesting group within which to investigate genetic structure (Hranitz & Baird 2000; Campbell & McCoy 2002), regional patterns of variability (Hutchison 2003) or the effects of habitat degradation (Templeton *et al.* 2001; Brisson *et al.* 2003). These animals also make excellent subjects for investigating social behavior or reproductive success due to dramatic sexual dimorphism in coloration, behavior, and territoriality (Baird *et al.* 1996).

Access to polymorphic microsatellite loci would greatly enhance the effectiveness of any of these investigations. To this end, we isolated 20 µg of whole genomic DNA from a single collared lizard sampled in southwestern Missouri using standard phenol-chloroform extraction (Bruford *et al.* 1992). This DNA was

used to construct two libraries, one nonenriched and one enriched. For the nonenriched library (described in Hutchison 1997), the DNA was digested with *Bam*HI, size sorted (200–600 bp), ligated into pUC19 vector and transformed into DH5α cells. Subsequent colonies were screened twice with CA₈, CAC₅, and GACA₄ oligonucleotides to yield loci Orig6, Orig7, Orig11, Orig21, Orig24, Orig25, and Orig26 (from the original screening) and N5 (from the second screening). Construction of the enriched library followed a protocol designed by S. Cummings based on instructions provided with the streptavidin-coated magnetic beads (Promega™) with frequent reference to Sambrook *et al.* (1989). Whole genomic DNA was digested with *Sau*3AI, ligated into pUC19 vector and subjected to polymerase chain reaction (PCR) with pUC19 primers 1224 and 1233. Product was mixed with streptavidin-coated magnetic beads attached to oligonucleotides CAG₅ or CCT₅ to locate PCR products with these motifs. After separation from the beads, the products were again digested with *Sau*3AI, ligated back into pUC19 vectors and transformed into DH5α cells. Resulting “enriched” colonies were harvested and sequenced to identify loci Enri3, Enri21 and Enri48. For all 11 loci, primers flanking the microsatellite repeats (Table 1) were designed using oligo shareware and guidelines in Hoelzel & Green (1992).

Table 1. Temp (PCR annealing temperature), Buffers (PCR buffer; see below), Notes (locus-specific information; see below), *n* (number of individuals surveyed for variability); H_O and H_E (observed and expected heterozygosity values, respectively, averaged across all populations)

Locus	Primer Sequence (bp)	Motif	Temp (°C)	Buffer	# alleles (size range)	Notes (1–4)	<i>n</i>	H_O	H_E	GenBank #
Orig6	For: AGGACACAAACATTCTTATC (20) Rev: TCTGAGTATTCCTTGCTT (18)	CA ₉ perfect	46	C	7 (138–150)	1	441	0.265	0.348	AY567818
Orig7	For: AGTTGTGGTCTGTAGCA (18) Rev: GGATCTGTCCCAATAATG (18)	CA ₂₄ imperfect	55	B	16 (199–229)	2	208	0.222	0.270	AY567819
Orig11	For: CACTATAAGATGTAGCACTTGC (22) Rev: GGTAAGCACCAAGATTGAGC (19)	CA ₈ perfect	56	D	7 (169–182)		256	0.091	0.162	AY567820
Orig21	For: GGGGTTGGAGCAAAGAG (17) Rev: AAGCCCCGGAAGAAAAG (17)	CA ₁₁ perfect	TD (62–50)	C	17 (126–160)	3	190	0.160	0.223	AY567821
Orig24	For: AGTTGAACCTCGATGCTCT (18) Rev: ATTGAACCCATGGTAAAG (18)	GACA ₁₀ imperfect	52	D	16 (144–204)	4	276	0.323	0.400	AY567822
Orig25	For: AGCCTTTACTGTGAGACCCA (20) Rev: TCATGAAATGAAGCAAAATTAATAATA (27)	ATT ₁₀ perfect	51	D	12 (109–154)	1,4	313	0.354	0.417	AY567822
Orig26	For: TATACATTCTTGAGTTCCGC (20) Rev: CAATGAGGGATATAAGGTGG (20)	CA ₈ perfect	54	J	20 (99–139)		329	0.294	0.324	AY567823
Enr3	For: CCTCAGCAGAAGCACCAGC (19) Rev: TATTGAGCAGGACAGGAAG (20)	CAG ₆ perfect	60	D	6 (101–116)	1	311	0.175	0.209	AY567824
Enr21	For: AGGAAGGCTCAAAGGTAC (19) Rev: ATTCACACAGGAGGAGGAGG (21)	CCT ₆ perfect	60	D	6 (99–114)		453	0.378	0.350	AY567825
Enr48	For: TTTGTTCTTATTTTACTTGCGT (22) Rev: AGGTATTGTGCTGTCTGAGG (20)	CA ₁₃ perfect	50	A	11 (107–127)		394	0.412	0.530	AY567826
N5	For: CTGCCATATCATGAAGGGTG (20) Rev: AATCAGCACACTGGGAAAG (19)	AT ₁₆ perfect	56	J	17 (166–198)	2	106	0.070	0.143	AY567827
Buffers:	A – 200 mM Tris (pH 8.4), 250 mM KCl, 15 mM MgCl ₂ B – 200 mM Tris (pH 8.4), 250 mM KCl, 25 mM MgCl ₂ C – 200 mM Tris (pH 8.4), 500 mM KCl, 15 mM MgCl ₂ D – 200 mM Tris (pH 8.4), 500 mM KCl, 25 mM MgCl ₂ J – 200 mM Tris (pH 9.2), 250 mM KCl, 25 mM MgCl ₂									
Notes:	1 = Poly A (add 30 minute, 72°C extension) 2 = Stutters, but readable since one peak much higher 3 = TD refers to touchdown PCR 4 = Physically linkage between loci 25 and 25 (no evidence of linkage disequilibrium)									

Microsatellite loci were amplified using locus-specific conditions optimized for buffer, annealing temperature, and amplification cycles (Table 1). Fragments were amplified with fluorescently labelled forward primers and visualized using a BaseStation DNA Fragment Analyzer (MJ Research). Polymorphism was assessed using genomic DNA isolated from 512 collared lizards sampled at 52 sites across the species range (mean = 8.13 animals/population). All 11 loci reported here were polymorphic (six to 20 alleles per locus) and amplified reliably, although there was variability in their performance. Loci Orig6, Orig25 and Enr3 exhibited a tendency to form poly-A tails. Fortunately, this is remedied by adding a 30 minute extension of 72°C at the end of the PCR run. Loci Orig7 and N5 stuttered, but in each case the true band is much greater in amplitude than false bands. Population analyses with the GDA computer program (Lewis & Zaykin 1996), revealed a range of average heterozygosity values (0.143–0.530) across all 52

populations. Furthermore, no linkage disequilibrium was evident between any of the loci, despite the fact that Loci Orig24 and Orig25 are only separated by 138 base pairs. Bonferroni analyses (Rice 1989) showed only two populations, of 572 possible population/locus combinations, deviated significantly from Hardy-Weinberg expectations, thus giving little evidence for null alleles (Chakraborty *et al.* 1992).

Workers interested in using these loci should be aware of potential geographical trends in polymorphism in some of the loci. For example, although all loci were variable across the species range, nine (all but Orig25 and N5) exhibited lower levels of heterozygosity towards the northern periphery of the range. However, correlation analyses between levels of heterozygosity and latitude showed this trend was significant (5% level) for only six loci (Orig6, Orig11, Orig21, Orig24, Orig26, and Enr21), while only three remained significant after Bonferroni analyses (Orig11, Orig24, and Orig26).

Thus, these three loci may prove less useful than others as measures of genetic variability in peripherally located populations.

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Crotaphytus collaris, Stegall Mountain, Missouri (Alan Templeton & National Park Service)