

PRIMER NOTE

Five polymorphic microsatellite markers for the Great Plains toad, *Bufo cognatus*

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Abstract

We characterize five polymorphic microsatellite loci for the Great Plains toad, *Bufo cognatus*, which are being used as markers in a study of the genetics and structure of toad populations inhabiting playa lakes on the Llano Estacado plateau of Texas. Observed heterozygosity (H_O) estimates for the toads ranged from 0.56 to 0.90, and expected heterozygosity (H_E) estimates ranged from 0.85 to 0.95.

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Amphibians are integral parts of wildlife communities; they serve as prey for vertebrates and consume large quantities of invertebrates (Bury *et al.* 1980; Smith *et al.* 2003). However, since 1960 world-wide amphibian biodiversity has markedly declined (Carey *et al.* 2001; Alexander & Eischeid 2000). Anurans (frogs and toads) are especially vulnerable to environmental stressors, due in part to their permeable skin. As such, they may serve as sentinel species for environmental disturbances in specialized habitats (e.g. ephemeral wetlands). Anuran success in a particular ecosystem may therefore serve as an indicator of how other species in similar environments adapt to physiological stresses, such as pollutants (Pechmann & Wilbur 1994).

The Great Plains toad, *Bufo cognatus*, is found from extreme southern Canada, to northern and western Texas and westward to southeast California and northern Mexico (James 1998). The demographics of *B. cognatus* in north-western Texas are affected by both the structure and location of wetlands and by anthropogenic factors (Gray 2002). Herein we describe five novel polymorphic microsatellite loci characterized as part of a study examining population structure and subdivision in *B. cognatus*.

Toads ($n = 119$) were collected from eight playas (shallow, depression wetlands with variable hydroperiods) located in three northwest Texas counties (Hale, Floyd, and Castro)

using drift fences and pitfall traps. Toe clips were collected from each toad and stored in 1.5-mL tubes at -70°C . Following the manufacturer's protocol, genomic DNA was isolated from toes using PUREGENE DNA kits (Gentra Systems). We used these DNAs to construct a library enhanced for microsatellites by adapting the protocol of Schable *et al.* (2001). Flanking sequences of the 100 screened genomic inserts were used to develop primers allowing amplification of the five microsatellite loci. PCR amplification was performed using an Eppendorf Mastercycler (Eppendorf) with the following protocol: one 30 s 94°C denaturation step followed by 30 cycles of 94°C for 15 s, optimal annealing temperature (Table 1) for 30 s; extension at 72°C for 50 s. PCR amplification reactions included *Taq* polymerase (0.5 units), optimal MgCl_2 (Table 1), dNTPs (10 μM), unlabelled forward primer (10 μM), fluorescently labelled reverse primer (10 μM), ~5 ng DNA, and 2.5 μL of PCR buffer (10 \times , Promega); H_2O was added to a final volume of 25 μL .

Fragment length (Table 1) was determined using an ABI 310 Genetic Analyser (Perkin-Elmer) and the size standard Gensize Tamra 500. Allele sizes were estimated using Genemapper 3.0 (Perkin-Elmer) and verified by eye. Heterozygosity estimates for all loci (Table 1) were determined using ARLEQUIN 2.0 (Schneider *et al.* 2000). Tests of linkage disequilibrium (LD) and deviation from Hardy-Weinberg equilibrium (HW) were tested using GENEPOP 3.3 (Raymond & Rousset 1995).

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Table 1 Characterization of five *Bufo cognatus* microsatellite loci

Locus	Repeat motif	Primer sequences (5'-3')	T_A (°C)	MgCl ₂ (mM)	Allele size (bp)	No. of alleles	H_O	H_E	GenBank Accession no.
IDDD	(AC) ₁₆	F: CCAATATCCCCAACTTTTGTG R: AATGAACAGCGATTTTGTGTG	56	2.0	200	51	0.56	0.95	AY338402
IHHH	(AC) ₁₁	F: TCTGCTGAATCTGTTTGAGAAAC R: TTCTCTATTGCAACTGCATCC	60	2.0	162	33	0.84	0.94	AY338403
IYY	(GT) ₁₇	F: GCGATTACTCACCAGTGTGCC R: TAAGGAACCTTCCCGGACAC	60	0.5	187	24	0.90	0.90	AY338400
ICCC	(CA) ₂ A(CA) ₉	F: GTGACAGCGACCGTAAAA R: CTCCAAGAAGACCCCAAAAG	57	1.0	194	22	0.79	0.90	AY338401
IKK	(AC) ₁₆	F: GCCCAGGGGTGTATACTCA R: TCCCAAAAATGTCAGGGGTA	60	2.0	112	20	0.89	0.85	AY338399

T_A , optimized annealing temperature; H_O , observed heterozygosity; H_E , expected heterozygosity; F, forward; R, reverse.

Loci are characterized in Table 1. Following Bonferroni correction, highly significant LD tests were shown for only two loci (IDDD, IKK). At four of the loci, observed (H_O) and expected heterozygosity (H_E) values did not conform to HW expectations. One locus (IKK) displayed a heterozygote excess, whereas three loci (IDDD, IHHH, ICC) displayed heterozygote deficits. These deviations may be due to bottlenecks in the populations or to the presence of null alleles. All eight populations inhabit playas that become intermittent during extreme drought, presumably isolating individuals and leading to reduced dispersal. These markers will allow us to further characterize these populations, including monitoring temporal and spatial changes in population structure.

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References

- Alexander AM, Eischeid JK (2000) Climate variability in regions of amphibian declines. *Conservation Biology*, **15**, 930–942.
- Bury RB, Dodd CK, Jr, Fellers CGM (1980) Conservation of the amphibians of the U.S.: a review. *US Fish and Wildlife Service Research Publication*, **134**, 1–34.
- Carey C, Heyer RW, Wilkinson J *et al.* (2001) Amphibian declines and environmental change: Use of remote-sensing data to identify environmental correlates. *Conservation Biology*, **15**, 903–913.
- Gray MJ (2002) *Effect of anthropogenic disturbance and landscape structure on body size, demographics and chaotic dynamics of Southern High Plains amphibians*. PhD Dissertation. Texas Tech University, Lubbock, Texas.
- James JD (1998) *Status of the Great Plains Toad (Bufo Cognatus) in Alberta*. Wildlife Status Report no. 14, Alberta Environmental Protection, Wildlife Management Division, and Alberta Conservation Association, Edmonton, Alberta.
- Pechmann JHK, Wilbur HM (1994) Putting declining amphibian populations in perspective: Natural fluctuations and human impacts. *Herpetologica*, **50**, 65–84.
- Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact test and ecumenicism. *Journal of Heredity*, **86**, 248–249.
- Schable AN, Fischer RU, Glenn TC (2001) Tetranucleotide microsatellite DNA loci from the dollar sunfish (*Lepomis marginatus*). *Molecular Ecology Notes*, **2**, 509–511.
- Schneider DR, Roessli D, Excoffier L (2000) *ARLEQUIN ver. 2.000: a Software for Population Genetics Analysis*. Genetics and Biometry Laboratory, University of Geneva, Switzerland.
- Smith LM, Gray MJ, Quarles A (2004) Diets of newly metamorphosed amphibians in West Texas playas. *Southwestern Naturalist* (in press).