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PRIMER NOTE

Nine polymorphic microsatellite loci for the northern leopard frog (*Rana pipiens*)

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Abstract

We describe the cloning and characterization of nine microsatellite loci from the northern leopard frog. Seven loci consist of tetranucleotide repeats, one locus consists of a dinucleotide repeat and one locus consists of a GT repeat juxtaposed with a GATA repeat. In a sample of 36 frogs from a natural population, polymorphism at these loci ranged from two to 13 alleles per locus with expected heterozygosities ranging from 0.5 to 0.91. These loci will be useful to researchers since this species is used for a broad range of studies.

Keywords: anuran, biotin enrichment, leopard frog, microsatellites, *Rana pipiens*

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Northern leopard frogs are used to study a broad range of biological processes including anatomy, physiology, endocrinology and embryology (Hillis 1988). Furthermore, throughout the 20th century, northern leopard frogs have been used as a model organism for the study of genetic and phenotypic polymorphism (Hoffman & Blouin 2000). Recently, conservation concerns have been raised about the northern leopard frog. Studies conducted in the 1990s indicated that the northern leopard frog has suffered population declines observed in other amphibians on a global level (Leonard *et al*. 1999). In this study, we characterize nine novel microsatellite loci in northern leopard frogs using a biotin enrichment procedure.

Microsatellites were isolated following the protocol of Ardren *et al*. (2002) with minor modifications. Briefly, DNA was isolated from northern leopard frog toe tissue following a standard phenol/chloroform procedure (Sambrook *et al*. 1989). Degenerate oligonucleotide-primed polymerase chain reaction (DOP-PCR), using the K6-MW primer developed by Macas *et al*. (1996), was used to generate small DNA fragments from throughout the genome with known flanking sequences. The PCR reaction cocktails were identical to those of Ardren *et al*. (2002) but reactions were carried out in a Perkin Elmer 9600 with the following temperature profiles: 95 °C for 2 min; five cycles of 95 °C for 30 s, 30 °C for 1.5 min, ramp at 1 °C/s to 72 °C and 72 °C for 3 min; 29 cycles of 95 °C for 30 s, 56 °C for 1.5 min, 72 °C for 3 min; and 72 °C for 20 min. This reaction produced amplified genomic fragments of approximately 200–2000 bp in length.

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The DOP-PCR-generated genomic fragments were enriched for microsatellites using a biotin enrichment procedure. A $(GATA)_8$ repeat motif biotinylated on the 3′-end bound to streptavidin-coated magnetic particles (Promega) was the basis for the enrichment. Hybridization conditions were identical to those of Ardren *et al*. (2002) with the following exceptions. First, our temperature profile to hybridize the biotinylated $(GATA)_8$ oligonucleotide to the PCR product was 98 °C for 5 min, cooled at 1 °C/s to 65 °C and held for 25 min and second, the final two washes were carried out at 73 °C.

Repeat-enriched DNA was made double stranded and cloned following the protocol of Ardren *et al*. (2002). Colonies containing $(GATA)_n$ repeats were identified using a PCR screening procedure described in Cabe & Marshall 2001). Of 658 colonies screened, 27 (4%) appeared as positives and were sequenced. Sixteen sequences contained repeat motifs, 11 of which were unique.

We designed primer pairs for the 11 unique microsatellite loci. The PCR reactions were carried out in 25-μL reactions containing 1× PCR buffer (10 mM Tris-HCl, 50 mM KCl and 0.1% Triton X-100), 1.5 mM $MgCl_2$, 0.1 mM of each dNTP, 0.8 mM of each primer, with the forward primer fluorescently labelled, and 0.5 U *Taq* DNA polymerase (Promega). Nine primer pairs were polymorphic in a screening of individuals from a natural population near Duluth (Minnesota, USA). Two loci contained dinucleotide repeats and eight loci contained tetranucleotide repeats

Table 1 Microsatellite primer pairs for northern leopard frogs (*Rana pipiens*)

Locus	*n*	Sequences (5′–3′)	Annealing temp. (°C)	Repeat	Size range (bp)	No. alleles	H_O	H_E	GenBank accession no.
Rpi100	36	*GGACTGGGGAGTTTCATCC AAGTCCTATCCCTAGTATGATACAC	62	$(GATA)_{10}$-$(GATA)_2$-$(GATA)_2$-$(GATA)_2$	174–222	9	0.86	0.85	AY157646
Rpi101	36	*AACGCACAGCAAAGGAGTAA CAAGGGATGACTTAGAAAGGG	62	$(GATA)_{13}$	161–201	9	0.86	0.84	AY157647
Rpi102	9	*GTGTGTGTGTTTATTTACTG CTTCCATTTTAATTGTGT	54	$(GATA)_{18}$	118–152	5	0.22	0.73	AY157648
Rpi103	36	*TTGAACAGGTATATCTAATAAAGT TGCTTCCATTTTAATTGTGTC	56	$(GT)_{20}$-$(GATA)_{18}$	135–211	10	0.83	0.78	AY157648
Rpi104	36	*CAGGGCAATGTGGAATGTGGA AGGACCACTCAGGTACAAAATGTTCT	62	$(AGAC)_{10}$-$(GATA)_{14}$	226–230	2	0.44	0.50	AY157649
Rpi105	14	*CAAGGCAAACTATCTATTTAT CCTTCCATGAACTTTTAAT	47	$(GATA)_{11}$-$(GATA)_{14}$-$(GATA)_5$	406–567	13	1.0	0.91	AY157650
Rpi106	18	*ACAGGGGTAAACAAAAATACTT GGGCTAAAAAGGACATCAA	50	$(GATA)_{15}$-$(GACA)_6$	307–607	11	0.72	0.90	AY157651
Rpi107	36	*GTGGTCTTATTACATTTCTTAC GCCAGTGAGTGTAGATAGAT	57	$(GATA)_{16}$-$(GATA)_{10}$-$(GACA)_5$-$(GATA)_{12}$	161–223	8	0.56	0.62	AY157652
Rpi108	36	AAATAACTCCTGGGAAATGT *CATCCCAAAGAGTCATATC	57	$(GT)_{11}$-$(GT)_4$-$(GT)_4$	272–298	7	0.78	0.67	AY157653

*5′ fluorescent-labelled primer.
Rpi103 contains a GT repeat juxtaposed with Rpi102.
n, Number of individuals screened from the population.

(Table 1). One locus had a GT dinucleotide repeat motif juxtaposed with a GATA repeat and primers were designed to include the entire locus (Rpi103) and just the tetranucleotide repeat (Rpi102). The remaining two loci amplified inconsistently or produced no product. The polymorphic loci had between two and 13 alleles per locus and expected heterozygosities ranged from 0.5 to 0.91 (Table 1). Microsatellite data were analysed with GENEPOP version 3.3 (Raymond & Rousset 1995) to test for Hardy–Weinberg equilibrium (HWE; exact probabilities) and to test for genotypic disequilibrium for pairs of loci within the population (exact probabilities). Both tests were corrected for multiple comparisons by applying a sequential Bonferroni correction (Rice 1989). Exact tests indicated that only one locus (Rpi102) might have null alleles, as indicated by a significant deviation from HWE ($P = 0.001$) and a considerable heterozygote deficiency. Genotypic disequilibrium tests provided no evidence for linkage among loci at a 5% significance level. However, the test of Rpi105 and Rpi106 provided no information and Rpi102 was removed from the analysis owing to its juxtaposition with Rpi103, which has a larger sample size. These loci provide the first published microsatellites derived directly from *Rana pipiens* DNA libraries.

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