Molecular Ecology Notes (2006) **6**, 233–236 doi: 10.1111/j.1471-8286.2005.01204.x

PRIMER NOTE

Isolation and characterization of di- and tetranucleotide microsatellite loci in the yellow-spotted night lizard *Lepidophyma flavimaculatum* (Squamata: Xantusiidae)

ELIZABETH A. SINCLAIR,*‡ REBECCA SCHOLL,* ROBERT L. BEZY,† KEITH A. CRANDALL* and JACK W. SITES JR*

**Department of Integrative Biology, Brigham Young University, Provo, Utah, 84602, USA and †Natural History Museum of Los Angeles County, Los Angeles, California, 90007, USA*

Abstract

We report the development of 17 di- and tetranucleotide microsatellite markers in the Yellow-Spotted Night Lizard *Lepidophyma flavimaculatum*. Levels of heterozygosity ranged from 0 to 100%. Several loci also amplify in other *Lepidophyma* species and several species of the sister genus *Xantusia*. High levels of variation at some loci indicate that they will be useful for parentage assessment and population genetic studies, whereas the 15 loci that amplify across multiple *Lepidophyma* species suggest these will be useful in determining the origin of parthenogenetic populations.

Keywords: *Lepidophyma*, microsatellites, nuclear DNA markers, parthenogenesis, *Xantusia*

Received 11 August 2005; revision accepted 05 October 2005

The Yellow-Spotted Night Lizard, *Lepidophyma flavimaculatum* (Xantusiidae), is unique among vertebrates consisting of all-female populations (presumed to be parthenogenetic and found in lower Central America), and populations containing males (gonochoristic and found from Mexico to northern Costa Rica) (Bezy 1989; Bezy & Camarillo 2002). Studies of karyotypes (Bezy 1972), morphology (Bezy 1989) and allozymes (Bezy & Sites 1987) show no differences between gonochoristic and parthenogentic populations of *L. flavimaculatum* and no fixed heterozygostity, consistent with a nonhybrid ('spontaneous') origin for the unisexual populations (Bullini 1994). Taxonomic sampling was limited in these studies, and for those taxa that were sampled, some chromosome and allozyme markers were insufficiently diagnostic in gonochoristic taxa to test for the fixed heterozygosity in parthenogens that is taken as evidence for a hybrid origin (Dawley 1989). Here, we describe the development of polymorphic di- and tetranucleotide microsatellite DNA markers to further explore the origin of parthenogenesis in *Lepidophyma*.

DNA was phenol/chloroform extracted from *L. flavimaculatum* and sent to Genetic Identification Services Inc. (http://www.genetic-id-services.com/) for the development of four enriched microsatellite libraries: $CA_{(19)}$, $GA_{(19)}$, $TACA_{(31)}$, $TAGA_{(30)}$. A total of 82 microsatellite loci were found in 100 cloned sequences, with primers designed for 55 of these, using DESIGNERPCR version 1.03 (Research Genetics, Inc.). Polymerase chain reaction (PCR) conditions were optimized for 17 loci, and screened for variation in four species of *Lepidophyma* (four or five individuals each), and five species from the sister genus *Xantusia*. Microsatellites were amplified in 20 µL reaction volumes containing 2.0 µL 10× PCR buffer (HotMaster), 3.2 µL 10 mM dNTPs, 0.15 µL HotMaster *Taq* (Brinkmann), 0.5 µL 20 µM primers, 2.0 µL of DNA template and water to 20 µL. The cycle consisted of 3 min denaturation at 96 °C, followed by 35 cycles of 40 s denaturation at 94 °C, 40 s annealing at 55–57 °C (Table 1) and 30 s extension at 72 °C, followed by 5 min final extension at 72 °C. One primer from each pair was fluorescently end-labelled (Table 1). The cycling was performed in an MJ thermal cycler (Bio-Rad). PCRs were diluted (1:10), and multiple (predetermined) loci were combined and run on the ABI DNA automated sequencer. Allele sizes were determined using GS500 ROX standard (Applied Biosystems) and scored using the GENOTYPER software (Applied Biosystems).

Between 15 and 16 loci were amplified across all four *Lepidophyma* species (Table 2). Numbers of alleles ranged

Correspondence: Elizabeth A. Sinclair, ‡Present address: Botanic Gardens, Park Authority/Frasel/Avenue, West Perth, Western Australia 6005, Australia. Fax: 61 8 9480 3641; E-mail: esinclair@iinet.net.au

© 2006 Blackwell Publishing Ltd

Table 1 Characteristics for 17 microsatellite loci isolated from *Lepidophyma flavimaculatum* ($n = 5$)

Locus	Repeat motif	Primer sequences 5′–3′	Anneal temp. (°C)	Mg conc.	Size range	No. of alleles	% Het.	Genbank Accession no.
A102-F	$(CA)_{12}$	CCTTCAACCTACATTTTCCTTC	55	3.5	194	1	0	DQ078669
A102-R		6FAM-ATGCTACAGGGGATTGTCTG						
A104-F	$(CA)_{21}$	HEX-CTCAGCAACATCCCTCCTC	55	3.5	132–134	2	0	DQ078670
A104-R		GCAAAGCATTGGAACCTG						
A107-F	$(CA)_{12}$	ACAGGCAAGGTCAAGATAAAC	57	2.5	144–151	2	0	DQ078671
A107-R		6FAM-TTTTGCTAAAAGTCTTCTCTGG						
B102-F	$(CT)_{20}$	NED-GAAGGGGCTACACAGAGTGC	55	3.5	248–257	2	0	DQ078672
B102-R		GAGGCATTTGCTTGATGTTG						
B111-F	$(CT)_{20}(CGCA)_5(CAGG)_6$	6FAM-AACAGGTTTATGCAACACACG	55	3.5	—	—	—	DQ078673
B111-R		AACTTCCGGTCAGCTTATGAG						
C101-F	$(TACA)_{13}$	NED-GATGTTGGTTGATTGGAAGTC	57	3.5	166–174	3	0	DQ078674
C101-R		CCTACCTGTGTGCCATAAGTC						
C102-F	$(TGTA)_{11}$	HEX-AAATGCAAACACTGTGAGATC	55	3.5	195	1	0	DQ078675
C102-R		CTGCTCCAATTTTTCAGATG						
C104-F	$(TACA)_{14}$	6FAM-ACTGCTTGGGTCTCTTTATATG	55	1.5	175–183	3	25	DQ078676
C104-R		TGCATCTGACAGTAACTTCTTG						
C105-F	$(AGAC)_6(AGAT)_6(TACA)_{15}$	TGGAATATGGTTTTATGTCTCC	55	1.5	289	1	0	DQ078677
C105-R		HEX-CCTCAATATCCTTTCTGAGATG						
C109-F	$(TAAA)_4(TACA)_{11}$	TGGTTTTTCTTGACCTTTCTC	55	3.5	273–282	2	0	DQ078678
C109-R		NEX-CCTGATTCCCAGTAGTTTACC						
C110-F	$(TTAA)_4(TACA)_7$	GACCAGGATTGTTGTAAAGAAC	55	1.5	218–222	2	0	DQ078679
C110-R		NED-GGGTGTATGATAAGGAGGTTG						
C112-F	$(CATA)_8$	HEX-TCTTTTCACTGGTTTCATTGTG	55	1.5	204–220	2	0	DQ078680
C112-R		ACCCATTCATAGGCAGAGG						
D102-F	$(TATC)_{29}$	GTCCAAACGACTGCTAAGG	57	3.5	275–344	4	0	DQ078681
D102-R		6FAM-TAGGCTTCTCTATCATGGAGAG						
D103-F	$(TATC)_{16}$	AGGAGGTCATACGGTAAATGG	55	1.5	210–225	5	0	DQ078682
D103-R		NED-AAGCGGCATACAAATGTTTC						
D110-F	$(TCTA)_{15}$	ATGCTGTGTGGATGGAAATAC	55	1.5	119–167	7	100	DQ078683
D110-R		6FAM-CCCTTCTCCTATGCTGGTTAG						
D120-F	$(AGGC)_8(ATAG)_{20}$	GCCCTTGTTACTGTGAGAATAG	55	3.5	282–305	2	0	DQ078684
D120-R		HEX-TGCTACAATGTGCATTCAATAC						
D124-F	$(GATA)_{18}$	NED-GCCAATCCATTTTGCTTC	55	3.5	232–256	3	0	DQ078685
D124-R		GACCAACCAGGAGACACC						

© 2006 Blackwell Publishing Ltd, *Molecular Ecology Notes*, 6, 233–236

Table 2 Allelic diversity and heterozygosity in another three *Lepidophyma* species and two species of *Xantusia*

	L. micropholis (*n* = 4)			*L. reticulatum* (*n* = 5)			*L. sylvaticum* (*n* = 4)			*X. riversiana* (*n* = 7)			*X. vigilis* (*n* = 5)		
Loci	Size range	No. of alleles	% Het.	Size range	No. of alleles	% Het.	Size range	*A*	% Het.	Size range	No. of alleles	% Het.	Size range	No. of alleles	% Het.
A102	125–186	3	25	194	1	0	184–186	2	25	193	1	0	194–196	2	20
A104	125	1	0	122	1	0	122–143	4	75	125–154	2	0	127	1	0
A107	140–149	2	0	147–156	2	20	142–158	5	75	—	—	—	140	1	0
B102	231–248	2	0	253–262	5	75	231–245	2	50	242	1	0	239	1	0
B111	—	—	—	—	—	—	—	—	—	95–252	11*	75	102–209	4*	80
C101	126–138	2	100	182–186	2	0	134	1	0	—	—	—	—	—	—
C102	167–234	3	50	122–212	3	20	204–254	2	25	133–229	5	66	—	—	—
C104	140–150	3	25	145	1	0	145–154	3	50	123	1	0	123	1	0
C105	330–351	3	66	289–305	4	80	257–361	3	50	243–351	2	100	—	—	—
C109	204–234	2	0	273–300	4	60	234	1	0	97	1	0	97	1	0
C110	190–223	8*	100	190–234	4	20	201–213	3	0	154–156	2	50	156	1	0
C112	209–232	3	75	209	1	0	187–236	4	25	201	1	0	201	1	0
D102	232–299	7*	100	277–310	6	40	279–309	6	100	225–275	6	100	218–254	3*	100
D103	154–214	4*	50	154–261	5	40	154–206	2	50	—	—	—	—	—	—
D110	97–157	10*	100	131–163	5*	100	97–165	10*	100	92–163	10*	86	88–135	7*	60
D120	—	—	—	293–297	2	60	—	—	—	—	—	—	—	—	—
D124	174–187	2	33	245–260	4	0	174–242	3	50	182–217	2	80	174	1	0

*denotes the detection of multiple alleles (beyond diploid count) in single individuals.
PCR products were also obtained in *X. arizonae* (*n* = 2): A102, A107, B102, B111, C101, C104, C105, C109, C110, C112, D110, D124.
X. bezyi (*n* = 2): A102, A107, B102, B111, C104, C109, C110, C112, D110, D124.
X. sierrae (*n* = 1): A102, A107, B102, C104, C109, C110, D110, D124.

© 2006 Blackwell Publishing Ltd, *Molecular Ecology Notes*, 6, 233–236

between 1 (fixed) and 10 for the most polymorphic locus, with heterozygosities between 0 and 100%. However, the low level of variation at some loci (e.g. A102, A104 and B102 in *Lepidophyma micropholis*) is likely an artefact of the small number of individuals screened. Tests for Hardy–Weinberg and linkage disequilibrium (LD) were performed in GENEPOP (Raymond & Rousset 1995) for all diploid data, for the six species sampled ($n \geq 4$). Heterozygote deficits were significant for six loci each in *Lepidophyma reticulatum* and *L. flavimaculatum*, and two loci in *Lepidophyma sylvaticum*. However, five of these tests (two in *L. flavimaculatum* and three in *L. reticulatum*) were at a marginal level of significance ($P < 0.045–0.048$) given the small sample sizes, and the others may be due to decay of heterozygosity, the presence of null alleles, or inbreeding in small isolated populations. There was no evidence of significant LD between any combinations of loci in any species.

Cross amplification was performed in the sister genus *Xantusia*, and between eight (*Xantusia sierrae*) and 13 (*Xantusia riversiana*) loci amplified in five species tested (Table 2). *Xantusia riversiana* was highly polymorphic for four loci and moderately polymorphic (50–75%) for three others, showing that some of these loci are variable across two of the three genera in this family.

The 17 microsatellite loci characterized for *L. flavimaculatum* are sufficiently variable to be suitable for population genetic and parentage studies, and the detection of shared alleles in hybridization studies. Four loci (C110, D102, D103, D110) segregated three or four alleles in some individuals of *L. sylvaticum*, *L. micropholis*, *L. reticulatum* and *X. riversiana*, and because karyotypes (Bezy 1972) suggest that all species are diploid, it is unlikely that multiple alleles represent polyploidy (readily detected using microsatellites; Julian *et al*. 2003). All species in this study are viviparous, and the most plausible explanation is that multiple alleles result from embryonic 'leakage'; shared embryonic/maternal blood vessels in the placenta (e.g. Perez-Sweeney *et al*. 2005). This can be tested in future studies of Xantusiidae as single and multiple (2 to 8) young are documented (Bezy & Camarillo 2002). More importantly, this set of markers offers the possibility of collecting multilocus genotypes for gonochoristic and unisexual *Lepidophyma* populations from throughout their ranges, and with newer methods now available (Halkett *et al*. 2005), critically testing the hybrid vs. nonhybrid origin alternatives for these enigmatic lizards.

Acknowledgements

This project was funded by the Department of Integrative Biology and Monte L. Bean Life Science Museum at Brigham Young University, and the National Science Foundation (award DEB-0132227 JWS and RLB).

References

Bezy RL (1972) Karyotypic variation and evolution of the lizards in the family Xantusiidae. *Contributions in Science*, **227**, 1–29.

Bezy RL (1989) Morphological differentiation in unisexual and bisexual xantusiid lizards of the genus *Lepidophyma* in Central America. *Herpetological Monographs*, **3**, 61–80.

Bezy RL, Camarillo JL (2002) Systematics of xantusiid lizards of the genus *Lepidophyma*. *Contributions in Science*, **493**, 1–41.

Bezy RL, Sites JW Jr (1987) A preliminary study of allozyme evolution in the lizard family Xantusiidae. *Herpetologica*, **43**, 280–292.

Bullini L (1994) Origin and evolution of animal hybrid species. *Trends in Ecology & Evolution*, **10**, 422–426.

Dawley RM (1989) An introduction to unisexual vertebrates. In: *Evolution and Ecology of Unisexual Vertebrates* (eds RM Dawley, JP Bogart), pp. 1–18. University of State of New York — New York State Museum, Albany, New York.

Halkett F, Simon J-C, Balloux F (2005) Tackling the population genetics of clonal and partially clonal organisms. *Trends in Ecology & Evolution*, **20**, 194–201.

Julian SE, King TL, Savage WK (2003) Novel Jefferson Salamander, *Ambystoma jeffersonianum*, microsatellite DNA markers detect population structure and hybrid complexes. *Molecular Ecology Notes*, **3**, 95–97.

Perez-Sweeney BM, Valladares-Padua C, Burrell A *et al*. (2005) Dinucleotide microsatellite primers designed for a critically endangered primate, the black lion tamarin (*Leontopithecus chrysopygus*). *Molecular Ecology Notes*, **5**, 198–201.

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.