Molecular Ecology Notes (2006) **6**, 194–196 doi: 10.1111/j.1471-8286.2005.01190.x

PRIMER NOTE

Polymorphic microsatellite markers in the painted dragon lizard, *Ctenophorus pictus*

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Abstract

We isolated eight polymorphic microsatellite markers from the painted dragon, *Ctenophorus pictus*, from a genomic library simultaneously enriched for a suite of di-, tri- and tetranucleotide repeat units. Genetic variation was assessed in 40 individuals from populations in Victoria and South Australia. The number of alleles per locus ranged from four to 45 with observed heterozygosity ranging from 0.23 to 0.95. These markers will be useful for studies of natural and sexual selection and the effects of habitat fragmentation in semiarid Australia.

Keywords: Agamidae, *Ctenophorus*, microsatellite, population genetics

Received 11 August 2005; revision accepted 12 September 2005

The painted dragon, *Ctenophorus pictus*, is a medium-sized diurnal dragon lizard (snout-vent length 70 mm) belonging to the family Agamidae. This species is found in dry sandy habitats across southeastern and south-central Australia (Cogger 1992). *Ctenophorus pictus* is sexually dimorphic, territorial and closely associated with burrows (Greer 1989). Pronounced sexual dichromatism is apparent in this species with breeding males showing bright blue, yellow or orange colours (Cogger 1992). The wide distribution of this species and the considerable variation in male colouration, both at a wider geographic scale and at a local level, lends itself to studies of population genetics and sexual selection.

Preliminary research into the genetic structure of this species in southeastern Australia using mitochondrial DNA data indicated that there is very little sequence divergence within the region, ranging between 0 and 0.45% (R. J. Rose, unpublished data). However, it is probable that these desert systems are now isolated units, with recent agricultural activities virtually eliminating suitable sandy habitats between national parks and reserves. To investigate current population genetic structure in *C. pictus* it was necessary to develop polymorphic nuclear markers. We developed eight polymorphic microsatellite loci. These are only the second microsatellite markers to be developed for species in the agamid subfamily Amphibolurinae (LeBas & Spencer 2000).

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Microsatellite markers were developed using a modified membrane-based capture method as described by Edwards *et al*. (1996). Genomic DNA was extracted using a standard proteinase K/phenol–chloroform protocol from a small piece of liver tissue from a single individual (NMVD71443, Museum Victoria) collected from Murray-Sunset National Park, Victoria. Approximately 250 ng of genomic DNA was digested with *Rsa*1 (Promega) and blunt-end ligated into a *Mlu*1 adapter consisting of annealed 21-mer and 25-mer oligonucleotides (Edwards *et al*. 1996). Ligated DNA was polymerase chain reaction (PCR)-amplified using the 21-mer oligonucleotide and concentrated sixfold by ethanol precipitation. Ten di-, tri- and tetranucleotide repeat oligonucleotides: $[CT]_{15}$, $[GT]_{15}$, $[CA]_{20}$, $[AAG]_{14}$, $[AAT]_{14}$, $[CAA]_{14}$, $[GATG]_{9}$, $[AAAT]_{9}$, $[GATA]_{9}$, $[GACA]_{9}$ (250 ng of each oligo), were simultaneously bound to 0.5 cm^2 of Hybond-N membrane (Amersharo). Enrichment of microsatellites was carried out by hybridizing amplified genomic DNA to membrane bound oligonucleotides overnight at 50 °C in the presence of 50% formamide, 3× SSC, 25 mM Na-phosphate and 2.5% SDS followed by five washes at 50 °C in 2× SSC, 0.1% SDS, three washes at 50 °C in 0.5× SSC, 0.01% SDS. DNA was eluted by boiling the membrane in 200 μL of sterile distilled water for 5 min, PCR-amplified using the 21-mer oligonucleotide and concentrated sixfold by ethanol precipitation. PCR products were cloned into

Table 1 Characteristics of microsatellite loci successfully amplified in *Ctenophorus pictus*

Locus	Repeat motif	Primer sequences (5′–3′)	Annealing temp. (°C)	Size range (bp)	No. of alleles	H_E	H_O
*Ctpi*1A1	$[AC]_{13}$	TET-AGAGAGCCAGCGGAGAGG GAGCCATGTCACCTGTGG	50	182–220	18	0.88	0.55
*Ctpi*1C2	$[GT]_{21}$	FAM-CCCTTCAAAAGAAATGGCTCT TTCCTCCCACTGTTTCTGCT	50	213–263	26	0.92	0.65
*Ctpi*1C7	$[GT]_{9}$	HEX-AGAGACACCTGCGTGGAGAT CTGCTAGGTTGCTGCTGTTG	55	125–171	9	0.80	0.95
*Ctpi*1D10	$[GAGT]_3[GA]_6$ $[TACA]_6[CA]_{18}$	TET-CATATTGAGATTTCTGTCTTGCT GGGAAGTTCCATAACCTGGAAT	50	92–188	45	0.98	0.90
*Ctpi*1F9	$[TG]_6AAAG$ $AG[TG]_7$	FAM-TGGGAACCACTGAGACTTGG AATTTTCCCTGAGGAATTTGG	55	99–123	10	0.65	0.63
*Ctpi*1F10	$[GTT]_5$	HEX-TCACAAGTGTAGCAAATAACAA GCCACGGAGAGTCGGACACG	55	144–153	4	0.29	0.22
*Ctpi*1G1	$[CA]_{22}$	FAM-TGAGTTTCACCCACAAGCAG CTGGAGATGGAGCGGACTAC	55	171–244	32	0.96	0.68
*Ctpi*1D1	$[AGGA]_5$ $[ACAA]_6[AC]_{31}$	FAM-TCCCCCACTTTATGAAGCAC GCTATGACTGTTTGGCCTCA	50	92–188	33	0.96	0.50

Allele size range, number of alleles and expected (H_E) and observed (H_O) heterozygosities were estimated from 40 individuals. GenBank Accession nos: DQ182296–DQ182303.

pGEM-T Easy vector (Promega) as per manufacturer's instructions and transformed into DH10B electrocompetent cells (Invitrogen). Individual colonies were picked and inserts were amplified using the vector primers M13(–20) forward and M13 reverse. Positive PCR products were sequenced using a commercial DNA sequencing service (Macrogen). Primers were designed from the flanking sequence of clones that contained unambiguous microsatellite repetitive elements.

Genomic DNA for PCR was extracted from tail tips using standard proteinase K/phenol–chloroform protocols. A set of 40 individuals from populations in northwestern Victoria (Little Desert, Wyperfield National Park, Murray Sunset National Park) and eastern South Australia (Ngarkat Conservation Park) were chosen to assess polymorphism and heterozygosity. Each locus was amplified individually via PCR in 12.5 μL volumes using 0.2 mM dNTPs, 0.2 mM of forward and reverse primers, 2 mM $MgCl_2$ 1× PCR Buffer (10 mM Tris-HCl, pH 9.0, 50 mM KCl, 0.1% Triton X-100, Promega) and 0.25 U *Taq* DNA polymerase (in storage Buffer B, Promega). Thermal cycling was carried out in a Corbett Research Palm-Cycler under the following conditions: 94 °C for 2 min, followed by 30 cycles of 94 °C for 20 s, 50–55 °C for 20 s and 72 °C for 45 s. Forward primers were fluorescently labelled with HEX, TET or FAM (Geneworks). PCR products were sized by a commercial genotyping service (AGRF) on an Applied Biosystems 377 DNA Sequencer using a TAMRA 500 size standard and genotypes assigned using GENOTYPER 2.1 (Applied Biosystems).

We sequenced 57 clones of which 24 (42%) contained microsatellite repeat regions, 22 contained no obvious repetitive DNA and 11 failed to yield clean sequence. Of the 22 sequences that did not contain microsatellite repeats, eight had high sequence similarity to previously published reptilian long interspersed nuclear elements (LINE, Kordis & Gubensek 1998; Zupunski *et al*. 2001). Primers were designed for 18 loci. Eight loci amplified consistently and were found to be polymorphic (Table 1). The number of alleles ranged from four to 45. Several loci with large numbers of alleles contained single-base allelic differences, which could not be attributed to sizing errors, suggesting indels in the flanking regions may be enhancing observed allelic diversity at these loci. Observed and expected heterozygosities and linkage disequilbrium (LD) between loci were calculated using GENEPOP version 3.4 (Raymond & Rousset 1999). There was no evidence of LD between the eight loci ($P > 0.05$). Four loci (*Ctpi*1A1, *Ctpi*1C2, *Ctpi*1D1 and *Ctpi*1G1) were found to have significant deviations ($P < 0.05$) from Hardy–Weinberg equilibrium within two or three of the four populations because of heterozygote deficiency. These results may be caused by null alleles or population substructure within each of the sampled regions. Chromatogram peak heights for homozygote and heterozygote individuals were manually checked in all cases, confirming that null alleles are an unlikely explanation. These microsatellite markers will provide important information on fine-scale population structure and migration in agamid desert communities in southern Australia.

Acknowledgements

This research was funded by an Australian Research Council Discovery Grant to JM. Additional support was provided by the Australian Society of Herpetology to RJR.

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