

PRIMER NOTE

Population genetic differentiation and multiple paternity determined by novel microsatellite markers from the Mountain Log Skink (*Pseudemoia entrecasteauxii*)

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A total of 15 microsatellite primer pairs were developed from the Mountain Log Skink, *Pseudemoia entrecasteauxii*. Nine were used to screen 46 individuals from four populations, and a representative from *P. spenceri* and *P. pagenstecheri*. Seven of the loci exhibited large allele variation (16–30) and high heterozygosity (0.24–0.82), and the three populations were genetically differentiated. The markers were also used to screen 36 clutches of known maternity and identified high levels of multiple paternity clutches (57%). The primers developed will provide useful markers for the study of population biology and mating system of these lizards.

Keywords: lizard, mating system, squamates

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The genus *Pseudemoia* comprises six species of small, diurnal skinks (Cogger 1996). One member of the genus, *Pseudemoia entrecasteauxii*, is currently involved in a sexual selection and behavioural ecology study. Consequently, we set out to develop microsatellite primers that will be useful in paternity assessment and population differentiation.

Pseudemoia entrecasteauxii genomic DNA (gDNA) was extracted from an ethanol-preserved liver sample using a modified protocol (Sambrook *et al.* 1989) and a partial genomic library was made following the procedure described previously (Scott *et al.* 2001). An estimated 24 000 transformants was recovered and transferred onto Hybond nylon membrane (Amersham-Pharmacia). Using a γ -dATP end-labelled oligonucleotide mixture, clones were screened for microsatellites of the following repeats: (ACTC)₇, (AACG)₇, (ACAG)₇, (AGAT)₇, (GA)₁₅, (GT)₁₅, (GTG)₁₀, (GTC)₁₀, (GTT)₁₀, (GTA)₁₀, (GCC)₁₀, (GAA)₁₀, (GAT)₁₀, (GAG)₁₀, (AGC)₁₀ and (ATA)₁₀.

Six hundred putative recombinants were picked, suspended in 100 μ L of TE and boiled for 5 min. Each sample was used as template for amplification using M13 primers.

Two hundred products of appropriate size were dotted on Hybond nylon membrane and secondarily screened with the same γ -dATP end-labelled oligonucleotide mixture. Following this, 54 positives were selected for sequencing. Clones were purified using BRESAclean (GeneWorks) and sequenced using the ABI Prism Big Dye terminator (Applied Biosystems). Products were separated and visualized on an ABI 377 automated DNA sequencer (Applied Biosystems). Of those cloned, 22 contained bp repeats with sequences useful for primer design, and 15 microsatellite primers were identified using PRIMER 3.0 (Whitehead Institute of Biomedical Research) (Table 1).

A panel of 46 individuals of *P. entrecasteauxii*, and a single representative of *P. spenceri* and *P. pagenstecheri*, was screened with nine of these primer pairs. Each M13 forward primer was labelled with a different fluorescent dye (FAM, HEX or NED, Gibco BRL Life Technologies) and used in amplification reactions of a total volume of 40 μ L. Reactions contained 10 ng of gDNA, 10 μ M of each primer, 3 mM MgSO₄, 2 mM dNTPs, 1 \times PCR amplification buffer, 1 \times PCR Enhancer and 1 U of PLATINUM® *Taq*-DNA polymerase (Gibco BRL Life Technologies). The reactions were amplified using a step down PCR protocol of initial denaturation at 94 °C for 5 min; the following cycle was then repeated twice, beginning

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Table 1 Characteristics of loci with forward and reverse primers, repeat motif, fragment length of PCR product and allele size range for nine loci that were screened with 45 *P. entrecasteauxii* individuals

Locus accession no.	Primer: 5' to 3'	Repeats	Fragment length (bp)	Allele range (bp)
Pe24 AY212889	F: CAATGAGAGCATCTATAAA R: GGCATAGCTTCAACACACACA	(TC) ₂₆	120	109–209
Pe31 AY212890	F: TTGCCACAACAGTTTCAACA R: GCCCGTCTACTCAGAAGCAG	(CT) ₁₇ (AC) ₁₃	200	324–408
Pe38 AY212891	F: TCATCCTGGTGGATTGTGTA R: CATGTGTGCATGCAAGTGAG	(GT) ₉	146	ND
Pe40 AY212892	F: TCTTGAACCGTATGTTGTTCCCTT R: GCCCATCCTGAGTTATTCCA	(TC) ₃₇	224	NA
Pe48 AY212893	F: CCTCACTGCCCTTACTCTGG R: TCAGGGGGTTGGACTAGATG	(AGAC) ₇	236	ND
Pe73 AY212894	F: AGGTTGTTGTGTGTATGGCG R: ATTGCAAACCTCGCAAATTC	(GT) ₂₂ (GA) ₂₅	242	ND
Pe80 AY212895	F: TCAAGGAAACATGGTCAGGC R: TGAATTTTCCACGGAATC	(GA) ₂₄	282	240–362
Pe90 AY212896	F: GCCAGTACATACAGGCAGCA R: GCCGTAACCTGCTCTGGATTG	(CT) ₂₁	147	150–218
Pe124 AY212897	F: ACGTCTCCACATTACCAGC R: GCCTGCCTGGAGAGGAT	(AGC) ₉	152	160–178
Pe132 AY212898	F: ATCTTGTGGTCCCTACGGC R: AAAGGCTTTCTGTGACAAA	(AC) ₃₅ (GT) ₂₆ (GA) ₃₁	298	266–332
Pe134 AY212899	F: TAACCTATGCACAAAGAGAGAG R: AAGGGGGCAGAGCAGTTAGT	(AG) ₃₁	184	162–224
Pe178 AY212900	F: CTGTGATTATTCAGTTACCGAGAG R: CCCCCAAGTATTCTGCATTG	(GA) ₂₈	232	ND
Pe187 AY212901	F: AATCTGAGCCAGAGTGCATT R: TGGATGATGCACACACACAC	(TG) ₇	294	ND
Pe197 AY212902	F: CTGTGATATTTCTTCATGTTGCTAGA R: ACAATCTGCAGGCTCTTGCT	(AG) ₂₁	293	ND
Pe242 AY212903	F: TCCTAGCCACATTAGACCTGA R: TCCAACATTGTGTTGCCAGT	(CT) ₂₆ (GT) ₁₅ (GA) ₁₃	219	206–300

ND represents loci that were not screened. NA refers to loci that did not amplify.

with 94 °C for 30 s, 70 °C for 15 s and 72 °C for 1.5 min. The cycle was subsequently repeated six times dropping the annealing temperature by 5 °C each cycle. The final stage consisted of 94 °C for 30 s, 35 °C for 15 s and 72 °C for 1.5 min, which was repeated 30 times, followed by a final 5 min extension step at 72 °C. A maximum of three products of different colour and size were multiplexed into a single lane, separated and visualized on an ABI 377 autosequencer. The loci were scored using GENOTYPER 2.5 and allele frequencies calculated using CERVUS 2.0 (Marshall).

Of the nine loci screened across the panel, eight amplified clean product (allele size ranges listed in Table 1). All loci demonstrated high allelic variation (9–30) and five exhibited high expected heterozygosity (Table 2). Two loci deviated significantly from the Hardy–Weinberg equilibrium (Pe90, Pe124) and these loci have null allele frequencies of 0.42 and 0.22, respectively

(CERVUS). Using the eight loci, F_{ST} scores for each population were calculated, and these demonstrated genetic differentiation between the three populations [*AMOVA* (*GenAlEx* 5.4); $P < 0.05$]. All eight loci amplified product in *P. pagenstecheri*, and all but Pe80 amplified product in *P. spenceri*.

Three of the primer pairs (Pe31, Pe132 and Pe242) were used to screen 122 offspring from 36 clutches of known maternity. Animals were sampled from the same populations noted earlier and the number of nonmaternal alleles was calculated. Several clutches exhibited high levels of multiple paternity (for clutches of ≤ 3 offspring, mean multiple paternity = 57%). A total of 10 clutches was collected from population 1; two had ≤ 3 offspring and one had a minimum of two fathers. There were 17 clutches from population 2, 10 of which had a clutch size of ≤ 3 (maximum = 6) and four of which had a minimum of two fathers. Nine clutches were obtained from population 3,

Table 2 Number of alleles, observed (H_O) and expected (H_E) heterozygosity estimated from 45 individuals of *P. entrecasteauxii* from three populations [Population 1 (P1) Shanahan's Mountain $n = 16$, (P2) Boroomba Rocks $n = 21$ and (P3) Shannon's Flat $n = 9$] and deviations from Hardy–Weinberg equilibrium (HW)

Locus	No. of alleles				H_O				H_E				HW Total
	P1	P2	P3	Total	P1	P2	P3	Total	P1	P2	P3	Total	
Pe24	15	16	11	29	0.91	0.75	0.87	0.82	0.95	0.91	0.94	0.94	NS
Pe31	12	20	12	29	0.38	0.65	1.00	0.60	0.94	0.94	0.96	0.95	NS
Pe80	11	10	9	22	0.23	0.22	0.25	0.24	0.91	0.77	0.92	0.90	NS
Pe90	10	16	4	16	0.50	0.18	0.14	0.34	0.89	0.81	0.58	0.80	**
Pe124	5	4	3	9	0.39	0.35	0.13	0.36	0.54	0.53	0.34	0.53	**
Pe132	15	16	13	27	0.53	0.55	0.87	0.64	0.94	0.94	0.97	0.96	NS
Pe134	14	16	11	24	0.75	0.82	0.75	0.79	0.94	0.94	0.91	0.95	NS
Pe242	19	21	10	30	0.77	0.78	0.75	0.77	0.98	0.96	0.93	0.97	NS

NS, denotes no significant deviation from H-W equilibrium, ** denotes significant deviation from H-W equilibrium

five with ≤ 3 offspring (maximum = 5) and four containing three to four nonmaternal alleles. No null alleles were identified during this analysis.

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