

PRIMER NOTE

Isolation and characterization of novel microsatellite markers from the Australian water skink *Eulamprus kosciuskoi* and cross-species amplification in other members of the species-group

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Abstract

A panel of six samples from three *Eulamprus* species was used to screen 24 primer pairs developed from the Australian water skink *Eulamprus kosciuskoi*. We provide details of 10 microsatellite loci that exhibited the cleanest and strongest banding patterns. We also screened six of these microsatellite loci against 118 individuals from a population of *E. heatwolei*. These microsatellites exhibited large numbers of alleles per locus (4–19) and high heterozygosity (0.271–0.898) in this population. This suggests that they will be extremely useful for investigating mating systems and other facets of population biology in these lizards.

Keywords: *Eulamprus*, lizard, mating system, microsatellite, Sphenomorphus group

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The Australian water skinks comprise five species and are a subgroup generally referred to as the '*Eulamprus quoyi*' group of the genus *Eulamprus* that has 11 species in total (Greer 1989; Cogger 2000). Four species in the *E. quoyi* group (*E. heatwolei*, *E. kosciuskoi*, *E. tympanum* and *E. quoyi*) rank among Australia's best known skinks (reviewed in Greer 1989). We are using these species in detailed studies of social structure and mating systems and to this end we have developed microsatellite primers to assign paternity to offspring. Members of this species-group are closely related, so we have tested these primers against *E. kosciuskoi* and *E. tympanum*, and performed a large scale screening against 118 *E. heatwolei*.

E. kosciuskoi genomic DNA (gDNA) was extracted from an ethanol-preserved liver sample using a modified organic protocol (Sambrook *et al.* 1989). Approximately 20 µg of gDNA was cut using two 4 bp restriction endonucleases, *AluI* and *HaeIII* (Promega). DNA fragments ranging in size from 250 to 900 bp were excised and ligated into plasmid DNA (pUC 18 *SmaI*/BAP, Amersham-Pharmacia) then incubated for 48 h at 13 °C. Ligated products were transformed into electrocompetent *Escherichia coli* NM522.

Approximately 28 000 transformants were recovered and lifted onto Hybond nylon membrane (Amersham-Pharmacia). Clones were screened using a γ -dATP end-labelled oligonucleotide 'cocktail'. This cocktail comprised the following oligonucleotides: (GT)_{15'}, (GA)_{15'}, (TAT)_{10'}, (GCT)_{10'}, (GAA)_{10'}, (GAT)_{10'}, (GTA)_{10'}, (GCG)_{10'}, (GTT)_{10'}, (GAG)_{10'}, (GTG)₁₀ and (GTC)₁₀.

A total of 180 putative positive recombinants were picked, individually suspended in 100 µL of TE and then boiled for 5 min to lyse the cells and release the DNA. Cellular debris was pelleted by centrifugation (10 s) and 1 µL of supernatant was used as template for amplification using universal M13 primers. DNA amplification reactions were 40 µL in volume and contained 20 pmol of each primer, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 3 mM MgCl₂, 0.5 mM dNTPs and 2 units of *Taq*-DNA polymerase (*Amplitaq* DNA polymerase, Perkin-Elmer). Double-stranded product was amplified using a Corbett PC-960C cooled thermal cycler (Corbett Research). The following step-down cycling profile was used: initial denaturation at 94 °C for 5 min, followed by an annealing step at 65 °C for 20 s and extension at 72 °C for 1.5 min. This was followed by a further round of denaturation at 94 °C for 15 s, annealing at 65 °C for 20 s and extension at 72 °C for 1.5 min. The annealing temperature was then dropped by 5 °C in the next two rounds of

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Table 1 Attributes of 10 microsatellite loci developed for *Eulamprus* species. Values in the column 'Alleles (range) *E. heatwolei*' refer to results of a screen of 118 individuals from a population in Tidbinbilla Nature Reserve in the Australian Capital Territory, south-eastern Australia. Observed (H_O) and expected (H_E) heterozygosity values are noted. Values in the column 'Alleles (range) cross-species' refer to results of a screen of four microsatellite loci including two specimens each of *E. heatwolei*, *E. kosciuskoi* and *E. tympanum*. Values with an asterisk (*) refer to the expected size of the target fragment, but these loci were not screened in the cross-species trial

Locus (Accession no.)	Primers: 5' to 3'	Repeats	Alleles (range) <i>E. heatwolei</i>	H_O , H_E <i>E. heatwolei</i>	Alleles (range) cross-species
Ek8 (AF227540)	F: TGCCTTTTAAACGTGGAGGAC R: AAGCCATTTGATTCCACCTG	(GT) ₁₂	5 (166–176)	0.636, 0.645	6 (170–198)
Ek33 (AF227543)	F: GGGGGAGGAAAGAGAGAGC R: TGCCTTTCTGTGCAATCTACA	(GA) ₂ GC(GA) ₃	4 (143–158)	0.331, 0.314	7 (148–174)
Ek37 (AF227544)	F: TGAGTGCCAATTTACTTGATGC R: CCCTGCTCCTTATGCCATTA	(CT) ₂₆	17 (294–340)	0.271, 0.404	8 (274–340)
Ek39 (AF227545)	F: GGAACGCTGGTTCTATGCTC R: TTGAACTCCCAACCTTCTGG	(GA) ₂₀	4 (152–197)	0.831, 0.900	183*
Ek100 (AF227546)	F: GTAGAGCCTTGTGTCCATTCC R: CCAGAGAGAAAAGCAAGCCAC	(GT) ₁₈ (GA) ₁₃	19 (259–304)	0.898, 0.908	256*
Ek107 (AF227548)	F: TTGACACCATGCAAAACCTT R: CCTTTTCCTGCTGAAGATGC	(GAA) ₂₁	18 (253–319)	0.898, 0.909	9 (286–337)
Ek11 (AF227541)	F: CCCAACTCTCTTAGGGCACA R: GCCACACCAGCAATCACTAA	(CT) ₁₂			412*
Ek23 (AF227542)	F: TGAACGGAAGCACATGAGAG R: CATTTTGGGTCGTCCTCCTA	(CT) ₁₆			475*
Ek105 (AF227547)	F: TGTGTCTTCAGATGGTTGCTG R: AGAATCGCCCTGGTAGC	(GCT) ₁₀			161*
Ek108 (AF227549)	F: ATCATGACTTGAATGTGACTCG R: TTTGCACCACTGCTTATTGC	(TG) ₁₉ (AG) ₁₅			472*

cycling. This 'stepping-down' in annealing temperature was repeated until a final annealing temperature of 50 °C was reached. The next 25 cycles were then performed with this annealing temperature. A final extension step at 72 °C was done for 7 min. Polymerase chain reaction (PCR) products were gel purified using the BRESAClean kit (Geneworks) following manufacturer's instructions.

Aliquots (10 µL) of amplification products were separated on a 1% agarose gel (TAE), alongside size markers and linear pUC18 to determine insert size. A total of 125 amplified clones contained inserts ranging between 200 and 900 bp. The remaining 30 µL for each these products was electrophoresed (1.5% agarose, TAE), excised from the gel, purified and resuspended in 20–50 µL of deionized water (BRESAClean, GeneWorks). Two µL of each purified product was dotted on Hybond nylon membrane (Amersham-Pharmacia) and screened again using the γ -dATP labelled oligonucleotide cocktail. Following this second round screening a total of 54 positives were selected for sequencing.

Clones were sequenced in both directions using universal M13 forward and reverse primers. Sequencing reactions were performed using the ABI Prism BigDye terminator cycle sequencing kit (Applied Biosystems) according to manufacturer's instructions. Extension products were separated and visualized on an ABI 377 automated DNA

sequencer (Applied Biosystems). Sequences were aligned and edited using Sequencher 3.1.1 (Gene Codes Corporation). Of the 54 sequenced clones, 24 yielded sequence of appropriate length and were of sufficient quality for primer design. Primer pairs for amplification of microsatellite loci were designed using Primer 0.5 (Whitehead Institute of Biomedical Research). Primer pairs were designed to yield products in three size classes ('Short' 150–200 bp, 'Medium' 250–350 bp, 'Long' 400 + bp) in order to facilitate multiplex analysis of microsatellite loci.

A panel of six samples was used to screen each of the 24 primer pairs. This panel comprised two geographically distant samples each of *E. kosciuskoi*, *E. heatwolei* and *E. tympanum*. The amplification protocol mentioned previously was used to generate microsatellite fragments and these were visualized on 2% agarose gels. All 24 loci exhibited some degree of variability.

Table 1 shows attributes of 10 microsatellite loci that exhibited the cleanest and strongest banding patterns. Four of these loci were used to screen the same panel of six samples. These loci were labelled using [F]dUTPs (PE Biosystems) according to manufacturer's instructions. Extension products were separated and visualized on an ABI 377 autosequencer. Data were analysed using GeneScan Analysis 3.1 (PE Biosystems). At locus Ek8, none of the six alleles were shared among species, but heterozygotes were

only detected in *E. kosciuskoi*. For locus Ek33, one of the seven alleles (153 bp) was detected in single specimens for each of the three species. At locus Ek37 a single homozygote was observed (*E. kosciuskoi*) and two alleles were shared between species (allele 315 bp was shared between single specimens of *E. heatwolei* and *E. tympanum*, while allele 309 bp was shared between the other *E. heatwolei* sample and the heterozygous *E. kosciuskoi* sample). No alleles were shared within species. At locus Ek107, only one of nine alleles was shared between species (allele 291 bp was detected in single specimens of *E. kosciuskoi* and *E. tympanum*). Again, no alleles were shared within species.

Table 1 shows attributes of six microsatellite loci in a population of *E. heatwolei* ($n = 118$) from the Australian

Capital Territory. These six microsatellites exhibited large numbers of alleles per locus (4–19) and high heterozygosity (0.271–0.898). This suggests that they will be extremely useful for investigating mating systems and other facets of population biology in this closely related group of lizards.

References

- Cogger HG (2000) *Reptiles and Amphibians of Australia*. 6th edn. Reed Books, Chatswood, Australia.
- Greer AE (1989) *The Biology and Evolution of Australian Lizards*. Surry Beatty & Sons, Chipping Norton, Sydney.
- Sambrook J, Fritsch EF & Maniatis T (1989) *Molecular Cloning: a Laboratory Manual*. Cold Spring Harbor Laboratory Press, New York.