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PRIMER NOTE

Microsatellite loci for Australian agamid lizards

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Abstract

We characterize 15 microsatellite loci from two microsatellite libraries developed from the Australian agamid lizards *Amphibolurus muricatus* and *Ctenophorus pictus*. All loci were tested for amplification in four other agamids: *Ctenophorus fordi*, *Ctenophorus decresii*, *Chlamydosaurus kingii*, and *Physignathus lesueurii*. These loci were highly polymorphic within and across species, with nine to 12 loci amplifying for each species tested.

Keywords: Agamidae, cross-amplification, lizard, microsatellite, paternity, simple-tandem-repeat

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Because of their diversity in life-history traits, agamid lizards provide ideal vertebrate models for addressing many concepts in evolutionary biology. We have developed microsatellite markers for two agamid lizards: the jacky dragon (*Amphibolurus muricatus*) and the painted dragon (*Ctenophorus pictus*). The jacky dragon is a common lizard found primarily in coastal heathland habitat of southeastern Australia. The painted dragon is a small to medium-sized agamid lizard found in arid regions of southern Australia (Cogger 2000). Both species provide excellent models for addressing questions of mate choice, sperm selection and competition, the evolution of colour polymorphism, and temperature-dependent sex determination (Warner & Shine 2005).

Here, we describe 15 highly polymorphic microsatellites for paternity analyses in these two agamids. Additionally, we screened these loci for amplification and polymorphism in four other agamids: *Ctenophorus fordi*, *Ctenophorus decresii*, *Chlamydosaurus kingii*, and *Physignathus lesueurii.*

Microsatellites from *A. muricatus* were isolated using a modified enrichment technique (Fisher & Bachmann 1998; Saltonstall 2003). DNA from three jacky dragons was digested with *Rsa*I and *Hae*III (Boehringer Mannheim) and ligated to two oligo adaptors (Edwards *et al*. 1996). Two biotinylated oligo probes (GA_{10} and CA_{10}) were hybridized to the digested DNA and isolated using Streptavidin magnetic particles (Promega). This subsample of GA and CA repeat-rich DNA was used as template for polymerase chain reaction (PCR) using one of the oligo adaptors as a primer. The product from the first PCR was used as template to repeat the enrichment process. The enriched library was gel-purified with the UltraClean Kit (MoBio Laboratories), ligated into pCR 2.1-TOPO vector (Invitrogen), and transformed into TOP10 cells (Invitrogen). Inserts from 25 clones were PCR-amplified, gel-purified with the UltraClean kit (MoBio Laboratories), and sequenced using dye terminator chemistry (Applied Biosystems). Of these 25 clones, 18 (72%) contained a repeat region. Primers were designed for 10 of these loci using PRIMER *3* (Rozen & Skaletsky 1997). Seven of these loci amplified consistently in *A. muricatus* (Table 1).

The development of a microsatellite-enriched library from *Ct. pictus* was outsourced to ecogenics GmbH (Zurich, Switzerland). Briefly, genomic DNA was digested, size-selected, and ligated to a TSPAD-linker (Tenzer *et al*. 1999). Magnetic bead separation and biotin-labelled probes containing CA_{13} and $ACAG_7$ repeats were used to enrich the library (Gautschi *et al*. 2000a, b). Of the 384 recombinant clones screened through hybridization with the fluorescently labelled probes $ACAG_{30}$ and CA_{63}, 93 (24%) were positive for a hybridization signal and 48 of these were sequenced. Primers were designed for 12 loci and 8 were tested for polymorphism (Table 1).

PCRs contained 0.05 U HotStart *Taq* (QIAGEN), 0.22 mM dNTPs, 250 pmol of each primer, 1 × PCR buffer (QIAGEN) containing Tris-Cl, KCl $(NH_4)_2SO_4$, 15 $MgCl_2$, pH 8.7 (final concentration of 1.5 mM $MgCl_2$), and 20 ng of DNA in a

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Table 1 Characteristics of microsatellite loci in the species from which they were developed. AM loci were developed in *Amphibolurus muricatus* and CP loci were developed *Ctenophorus pictus*. Values reported are the number of alleles (N_A), observed (H_O) and expected (H_E) heterozygosities, and amplification conditions (annealing temperature and final mM of $MgCl_2$)

Loci	Repeat motif	Primer sequence (5′–3′) with fluorescent label	Accession no.	Size (bp)	N_A	H_O	H_E	PCR
AM01	$(GA)_{35}$	F: GGACAGGACTTCCTAGTTTTGC R: PET-TGCATGGCTATATGGCAGTAAC	EF042966	128–240	27	0.879	0.952	56 : 2.0
AM10	$(GA)_{10}$	F: FAM-TCCCTTCATTGTCCCCATTA R: CTTGACAGTTTCAGTCCCAGTG	EF042967	183–238	13	0.806	0.842*	52 : 2.5
AM16	$(CA)_{14}$ $(GA)_{32}$	F: FAM-TTTCCAAAATTATACTGACCAATAAGG R: TCTCAATAAACATGAATAAGATTGTGC	EF042968	145–207	15	0.889	0.878	54 : 3.0
AM25	$(CT)_5/(GT)_8$ $(GA)_{12}$	F: FAM-ACCTCTGCCAAGAATGTAAGG R: ATTGTTGTGAGCCCTATGTGG	EF042969	290–434	16	0.679	0.886	54 : 2.0
AM41	$(GA)_{28}$	F: GCTATGCTGTGGAAATGTGG R: NED-ACACTGGTGGAGGCAAAGC	EF042970	84–126	11	0.531	0.855*	56 : 2.0
AM52b	$(GT)_{16}$ $C(GT)_{14}$	F: VIC-AAAGAGCGGAGCATTCTAGG R: GAGGAAGGAATAGTGGAAACAGG	EF042971	86–196	26	0.750	0.964*	52 : 3.0
AM53	$(CA)_{11}$	F: CCACCACTGAAAAGAAAATCC R: FAM-ATCCCCAGTGTCTACGATGC	EF042972	376–380	3	0.143	0.232*	54 : 2.0
CP01	$(TCTG)_{19}$	F: PET-CTGTCACCACATCTCTGCATC R: GCCCCCAGGCTCACATAG	DQ680807	138–240	13	0.882	0.866	60 : 1.5
CP02	$(TCTG)_{10}$	F: 6-FAM-TAAATTTCCAGGCAACATTCAG R: GTCAGCCTGTCCTACCAGTACC	DQ680808	143–317	16	0.794	0.918	60 : 1.5
CP06	$(CT)_{17}$ $(CA)_{26}$	F: NED-CATGCTCAGAGGCACTCTTTC R: CCTTCCTCGCCGCAATAG	DQ680809	149–240	29	0.971	0.964	54 : 1.5
CP10	$(CA)_{13}$ $(GA)_{38}$	F: VIC-AGTATGTGCAGCAAAAGGTGTC R: GATGGGACAGCTTCCTTGTC	DQ680810	119–228	23	0.853	0.952*	56 : 2.5
CP11	$(CA)_3$ $CCAA(CA)_{17}$	F: PET-TGAAGAGCCATACTTCATCACC R: ATTTCCCAAAGCATCCTGAAC	DQ680811	139–159	10	0.794	0.831	56 : 1.5
CP17	$(TG)_{27}NGC$ $(GT)_5$	F: *NED*-ACTTAGTTCCTTCACTGAACAATTTAC R: ATGGAAGGGGAGGAAAGATAAG	DQ680812	104–188	25	0.828	0.917	56 : 2.5
CP22	$(CA)_{17}$	F: 6-FAM-GTAGAGCAAGTGGTGGGGAAAC R: CTTCTGGAAGCTGGAAAGAATG	DQ680813	94–151	19	0.546	0.932*	56 : 2.5
CP23	$(CA)_{36}$	F: VIC-TCTATTTTTAAGAGGCGGTTGC R: AGCTGCCCCAGACCCCCAGT	DQ680814	130–187	11	0.618	0.847	56 : 2.5

*Out of Hardy–Weinberg equilibrium.

7 μL reaction. Cycling conditions included a hot start denaturation of 95 °C for 15 min; 30 cycles of 95 °C for 30 s, annealing temperature for 30 s, 72 °C for 30 s, and a final extension of 72 °C for 30 min. Cross-species amplification used the same conditions, but varying annealing temperatures (50–60 °C), and $MgCl_2$ (1.5–3 mM) (Table 2).

The 15 loci were screened for polymorphism on 29–34 individuals of *A. muricatus*, *Ct. pictus*, and *Ct. fordi* (Tables 1 and 2). Linkage disequilibrium was tested in GENEPOP (Raymond & Rousset 1995). ARLEQUIN 3.01 was used to calculate observed and expected heterozygosities and exact tests for Hardy–Weinberg equilibrium (Excoffier *et al*. 2005). Significance values were corrected for multiple tests using the sequential Bonferroni correction (Rice 1989). Only one locus pair was significantly linked: CP10 and CP22 in *A. muricatus*. Results of tests for Hardy–Weinberg equilibrium are shown in Table 1.

The loci were tested on 4–8 individuals of *Ct. decresii*, *Ch. kingii*, and *P. lesueurii* (Table 2). While not an extensive search for heterozygosity in these species, these tests provide a starting point for other researchers interested in using these loci for molecular analysis on these and other related agamid species. The loci reported here along with those previously reported (Austin *et al*. 2006) provide a suite of DNA markers that will be useful for both population and parentage analyses in many Australian agamid lizards.

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Table 2 Result for the cross-species amplification trials. For the successful amplifications the PCR conditions (annealing temperature and final mM of $MgCl_2$), number of alleles (N_A), and the size range in bp are given. For the species with more than 29 samples, the observed (H_O) and expected (H_E) heterozygosities are also reported. Dashes represent unsuccessful amplifications

	A. muricatus† N = 33				*Ct. pictus* N = 34				*Ct. fordi* N = 29				*Ct. decresii* N = 8			*Ch. kingii* N = 4			*P. lesueurii* N = 6		
	H_O:H_E	PCR	N_A	Size	H_O:H_E	PCR	N_A	Size	H_O:H_E	PCR	N_A	Size	PCR	N_A	Size	PCR	N_A	Size	PCR	N_A	Size
AM01					0.71 : 0.94*	56 : 2.5	23	152–227	—	—	—	—	52 : 2.5	11	179–251	56 : 2.5	3	142–176	—	—	—
AM10					—	—	—	—	0.92 : 0.96	52 : 2.5	24	186–244	—	—	—	—	—	—	52 : 2.5	1	190
AM16					0.85 : 0.94	52 : 3.0	24	133–208	—	—	—		52 : 2.5	5	141–173	52 : 3.0	1	143	50 : 2.5	1	130
AM25					0.68 : 0.97*‡	52 : 2.5	25	266–544	—	—	—	—	—	—	—	52 : 30	2	295–296	52 : 3.0	2	268–270
AM41					0.82 : 0.94	56 : 2.5	25	93–190	0.83 : 0.92	54 : 2.5	18	96–148	54 : 2.5	10	90–148	56 : 2.5	5	92–118	—	—	—
AM52b					—	—	—	—	0.18 : 0.20	52 : 3.0	2	54–74	52 : 3.0	1	74	52 : 3.0	3	83–89	52 : 3.0	1	73
AM53					—	—	—	—	—	—	—	—	—	—	—	52 : 2.5	3	388–394	—	—	—
CP01	—	—	—	—					0.91 : 0.95	54 : 2.5	22	116–279	54 : 2.5	11	142–238	—	—	—	56 : 2.5	1	134
CP02	0.67 : 0.94*	52 : 2.5	22	194–382					0.96 : 0.96	54 : 2.5	27	260–410	52 : 2.5	7	245–329	56 : 2.5	5	197–316	—	—	—
CP06	—	—	—	—					—	—	—	—	54 : 2.5	11	173–239	56 : 2.5	3	181–185	58 : 2.0	1	182
CP10	0.94 : 0.95*	54 : 2.0	23	132–248					0.34 : 0.34	52 : 2.5	6	109–137	52 : 2.5	12	113–145	52 : 2.5	2	222–300	56 : 2.5	5	119–154
CP11	0.48 : 0.57*	54 : 2.0	5	130–176					0.79 : 0.71	52 : 2.5	7	142–155	52 : 2.5	5	141–153	56 : 1.5	2	142–144	56 : 1.5	1	125
CP17	—	—	—	—					0.81 : 0.96	54 : 2.5	22	114–189	54 : 2.5	3	94–98	—	—	—	56 : 2.5	3	132–148
CP22	0.73 : 0.90*	52 : 2.5	23	95–208					0.41 : 0.36	54 : 2.5	19	148–252	54 : 2.5	8	101–168	56 : 1.5	3	104–123	54 : 2.5	1	189
CP23	—	—	—	—					0.36 : 0.39	54 : 2.5	5	141–152	54 : 2.5	7	99–121	—	—	—	—	—	—

*Out of Hardy–Weinberg equilibrium, possibly null alleles.
†Captive population that may include half-siblings and individuals from multiple wild populations.
‡Alleles longer than 550 bp may be present but were unable to be detected due to methodological constraints.

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