Molecular Ecology Notes (2006) **6**, 737–739 doi: 10.1111/j.1471-8286.2006.01326.x

PRIMER NOTE

Highly polymorphic tetranucleotide microsatellite loci for the eastern Canary Island lizard, *Gallotia atlantica*

P. BLOOR,* I. H.-B. DE LAGUNA† and S. J. KEMP‡

*School of Biological and Earth Sciences, Liverpool John Moores University, Byrom St., Liverpool L3 3AF, UK, †Departamento de Genética, Universidad Las Palmas de Gran Canaria, 35080 Las Palmas, Spain, ‡Animal Genomics Laboratory, School of Biological Sciences, The Biosciences Building, Crown St., University of Liverpool, Liverpool L69 7ZB, UK

Abstract

We describe 10 polymorphic tetranucleotide microsatellite loci from the eastern Canary Island lacertid lizard, *Gallotia atlantica*. Loci were isolated from a partial genomic library that had been enriched for AAAG repeat sequence. All loci were highly polymorphic (eight alleles or more) with observed heterozygosities from 0.75 to 1.00. At least four loci were successfully amplified and polymorphic in the Gran Canarian lacertid, *Gallotia stehlini*. These loci will be used to examine correlations between patterns of gene flow and recent volcanism on the island of Lanzarote.

Keywords: Canary Islands, Gallotia, microsatellite markers

Received 7 September 2005; revision received 22 November 2005; accepted 22 January 2006

The lacertid lizard, *Gallotia atlantica*, is endemic to the volcanic eastern islands and islets of the Canary archipelago. Eruptions less than 300 years ago covered the part of its range in western Lanzarote, where it is currently found on numerous small habitat 'islands', but appears to be absent from intervening flows (Bloor & Brown 2005). It remains to be seen whether or not dispersal and gene flow have been disrupted in this species. Microsatellite loci were isolated with the aim of testing this hypothesis.

Microsatellite loci isolated from the western Canary Island lacertid, *Gallotia galloti*, had low amplification success rates or low levels of polymorphism in the Gran Canarian lacertid, *Gallotia stehlini* (Richard & Thorpe 2000). The DNA phylogeny of *Gallotia* indicates that *G. stehlini* diverged from other lineages about 12.3 Ma, followed by *G. atlantica* about 9.3 Ma and *G. galloti* about 4.4 Ma (Carranza *et al*. 2004). The divergence times within the genus and the low cross-species amplification success rates in *G. stehlini* suggest that these loci are unlikely to be useful for *G. atlantica*. We describe the isolation and amplification of 10 highly polymorphic tetranucleotide microsatellite loci from *G. atlantica* and the results of cross-species amplifications in *G. stehlini*.

Microsatellite loci were isolated using an enrichment protocol modified from Gardner *et al*. (1999). A partial genomic library was constructed from size-selected fragments (400–1200 bp) of pooled *G. atlantica* DNA (Lanzarote) that had been digested with *Sau*3AI, adapter-ligated and enriched for AAAG repeat sequence [adapter: oligo S61 (5′-GGCCAGAGACCCCAAGCTTCG-3′) and the 5′-phosphorylated oligo S62 (5′-pGATCCGAAGCTT-GGGGTCTCTGGCC-3′); Refseth *et al*. 1997]. Subtractive hybridization was carried out with 200 pmol of 3′-biotinylated $(AAAG)_6$ repeat oligo bound to streptavidin-coated beads (Dynabeads M-280, Dynal). Nonspecific binding and unbound DNA were removed by several nonstringent and stringent washes. The repeat-enriched DNA was recovered by single-primer amplification in 50 μL volume polymerase chain reactions (PCRs) (ABgene Master Mix; 1.5 mM $MgCl_2$, 0.2 mM of each dNTP and 1.25 U *Taq* polymerase) with 0.5 μM of oligo S61 and 4 μL of bead-bound biotinylated hybrid suspension [cycling profile: 94 °C for 1 min, followed by 30 cycles at 94 °C for 30 s, 58 °C for 30 s and 72 °C for 1 min and a final step at 72 °C for 10 min]. The purified (PCR purification kit, QIAGEN), enriched DNA was ligated into pGEM-T vector (Promega) and cloned into JM109 *Escherichia coli* competent cells (Promega). Recombinants were identified by black/white screening on S-gal (Sigma) agar/ampicillin plates. Microsatellite-containing clones were identified by the presence of two or more amplification products after direct PCR amplification from bacterial colonies using oligos S61

Correspondence: Paul Bloor, Fax: +(44) 151 2073224; E-mail: pbloor@gmail.com

Table 1 Description of the 10 microsatellite loci isolated from *Gallotia atlantica* based on an island-wide sample of 16 individuals. H_E and H_O correspond to expected and observed heterozygosities, respectively

Locus (Accession no.)	Primer sequence (5′–3′)	Repeat array in clone	Size of cloned allele (bp)	Number of alleles	Allele size range (bp)¶	H_E	H_O
Gat1 (DQ166825)	ACACAGCCTCATTTTCCATTTC ATTTCTCCCACTTCTGCCATAG†	$(GAAA)_{13}$	179	15	186–250	0.92	0.88
Gat2 (DQ166826)	CTTTGGGAGATTTGTCTACCTCT* CCTGAAATGGCAGAAATTAGG	$(GAAA)_{16}$	187	8	183–209	0.88	0.88
Gat3 (DQ166827)	TGCTGCTACCATCAATCTGC§ TTCAATGGAACAGGTAAGTCCA	$(GAAA)_4AA(GAAA)_{17}$	158	17	103–215	0.93	0.88
Gat4 (DQ166828)	CTCGCCTTTAGTATAGGTTGTCTT‡ AGTATGCTGCTTTGATTTGATGT	$(GAAA)_{21}$	205	13	161–308	0.90	0.81
Gat5 (DQ166829)	GGGAGACCCTCATATCTGCT CTTTCATGCAGTACCCAATCC*	$(GAAA)_{18}$	428	15	397–442	0.94	0.81
Gat6 (DQ166830)	GCAGCTGTTCTGACTGTTTAGG TACCTGCAGGGAGAAGTGATAG‡	$(GAAA)_2AA(GAAA)_{15}$	347	16	329–396	0.96	0.94
Gat7 (DQ166831)	TTTCGGAAGCTTACTCTGTGC† CATGGCGAATGGTACAAATG	$(GAAA)_{14}$	250	17	215–274	0.91	0.88
Gat8 (DQ166832)	GGAATATCCAACCCAGCAAGTTC TACAGGCTAGCCACCCCAAGTA*	$(GATA)_2(GAAA)_{21}(GATA)$	212	16	168–214	0.94	0.81
Gat9 (DQ166833)	AAGCCCAGACGTATTATCCAG§ TATCATTTTGCACTCACTACTTCC	$(GAAA)(GCAA)_2(GCAC)(GAAA)_{21}$	196	9	164–233	0.92	0.75
Gat10 (DQ166834)	TACTGCACCTGCTACACTGGA§ AAGCAGCAGGAGATAACGAAC	$(GAAA)_{16}(GAGA)(GGGA)_3$	250	11	226–274	0.96	1.00

*6-FAM-labelled primer, †NED-labelled primer, ‡VIC-labelled primer and §PET-labelled primer.
¶Based on the GeneScan-500 (LIZ) size standard.

and $(AAAG)_6$. Amplifications were carried out in 20 μL volume PCRs (ABgene Master Mix; 1.5 mM $MgCl_2$, 0.2 mM of each dNTP and 0.5 U *Taq* polymerase) with 0.25 μM of the $(AAAG)_6$ repeat oligo and 0.5 μM of oligo S61 [cycling profile: 94 °C for 5 min, followed by 30 cycles of 94 °C for 30 s, 56 °C for 30 s and 72 °C for 45 s]. Positive clones were sequenced on an ABI PRISM 377 sequencer (Applied Biosystems). Primer pairs were designed from unique microsatellite sequences using the program PRIMER 3 (Rozen & Skaletsky 2000). Forty-three positive clones were identified from 192 colonies. Thirty-nine clones (20.3% of the colonies screened) contained AAAG repeat sequence of 10 repeats or more. Twenty-seven sequences were excluded because they contained one of two distinct minisatellite repeat sequences (i.e. individual inserts were different but highly conserved).

Primer pairs were tested by typing an island-wide sample of 16 individuals of *G. atlantica* from Lanzarote. One primer from each pair was fluorescently end-labelled with 6-FAM, NED, VIC or PET (see Table 1 for more details). Amplifications were carried out in 10 μL volume PCRs (Bioline: 1× NH_4 reaction buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP and 0.5 U *Taq* polymerase) with 0.1 μM of each primer [cycling profile: 1 min at 94 °C, followed by 30 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s and a final step at 72 °C for 30 min]. Fluorescently labelled fragments were run on an ABI PRISM 3100 DNA Sequencer (Applied Biosystems) with the GeneScan-500 (LIZ) size standard. Five loci (Gat1, 2, 3, 4 and 5) were combined to produce a multiplex PCR based on their allele sizes and coamplification. Additional polymorphism data were collected from the multiplexed loci by typing a further 48 individuals of *G. atlantica* from two sites on Lanzarote [*n* per site = 24, site labels from Bloor & Brown (2005)]. Multiplex amplifications were carried out in 10 μL volume PCRs (Bioline: 1.2× NH_4 reaction buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP and 0.5 U *Taq* polymerase) with 0.1–0.45 μM of each primer [cycling profile: 94 °C for 1 min, followed by 30 cycles at 94 °C for 30 s, 55 °C for 30 s and 72 °C for 45 s and a final step at 72 °C for 30 min].

Ten loci were successfully amplified and highly variable (eight alleles or more) in *G. atlantica*, with observed heterozygosities from 0.75 to 1.00 (Tables 1 and 2). Allele sizes not explained by the gain or loss of repeat units were observed for all loci, with the exception of Gat2. These were probably due to the occurrence of insertions/deletions in the microsatellite-flanking sequence. No evidence of null alleles (tests of heterozygote deficiency) or linkage were found [$P > 0.05$, after sequential Bonferroni correction (Rice 1989)]. All calculations were performed using the program GENEPOP version 3.4 (Raymond & Rousset 1995).

Cross-species priming of the multiplex PCR was assessed by typing 32 individuals of *G. stehlini* from two sites on

Table 2 Polymorphism data for the multiplexed loci (Gat1, 2, 3, 4 and 5) based on 48 individuals of *Gallotia atlantica* from two sites (S5 and S9) on Lanzarote [*n* per site = 24, site labels from Bloor & Brown (2005)] and 32 individuals of *Gallotia stehlini* from two sites (A and C) on Gran Canaria (*n* per site = 16: A, Mogán; C, Arucas). H_E and H_O correspond to expected and observed heterozygosities, respectively

	G. atlantica (*n* = 48)					*G. stehlini* (*n* = 32)				
Locus	Site	No. of alleles	Size range (bp)*	H_E	H_O	Site	No. of alleles	Size range (bp)*	H_E	H_O
Gat1	S5	15	190–242	0.92	0.96	A	NA			
	S9	20	182–259	0.95	0.92	C	NA			
	All	21	182–259	0.93	0.94	All	NA			
Gat2	S5	11	171–303	0.89	0.88	A	6	135–185	0.74	0.63
	S9	13	171–217	0.91	0.88	C	10	135–198	0.85	1.00
	All	14	171–303	0.90	0.88	All	11	135–198	0.80	0.81
Gat3	S5	16	111–203	0.93	0.88	A	7	96–128	0.72	0.56
	S9	17	114–170	0.93	0.83	C	7	96–120	0.84	0.81
	All	21	111–203	0.93	0.85	All	8	96–128	0.80	0.69
Gat4	S5	14	157–203	0.91	0.92	A	2	131–135	0.12	0.13
	S9	17	165–219	0.91	0.88	C	2	131–135	0.39	0.50
	All	20	157–219	0.92	0.90	All	2	131–135	0.27	0.31
Gat5	S5	16	397–445	0.92	0.92	A	2	364–368	0.06	0.06
	S9	13	401–479	0.91	1.00	C	1	368		
	All	18	397–479	0.91	0.96	All	2	364–368	0.03	0.03

*Based on the GeneScan-500 (LIZ) size standard; NA, no amplification.

Gran Canaria (*n* per site = 16; A, Mogán; C, Arucas). PCR conditions were the same as those used for *G. atlantica*, with the exception of annealing at 54 °C. Four loci (Gat2, 3, 4 and 5) were successfully amplified and variable (two to 10 alleles) in *G. stehlini*, with observed heterozygosities from 0.03 to 1.00 (Table 2). This suggests that several of the remaining loci may also be useful for this species.

Acknowledgements

The isolation work was carried out by Paul Bloor (supervisor: Richard Brown) supported by a Liverpool John Moores University studentship in Stephen J. Kemp's laboratory. We thank the Cabildo Insular de Lanzarote and the staff of the Parque Nacional de Timanfaya for granting fieldwork permits and transportation within the National Park and José Pestano for his contribution to one of the trips to Lanzarote.

References

Bloor P, Brown RP (2005) Morphological variation in *Gallotia atlantica* from the volcanic island of Lanzarote: subspecies designations and recent lava flows. *Biological Journal of the Linnean Society*, **85**, 395–406.

Carranza S, Arnold EN, Amat F (2004) DNA phylogeny of Lacerta (Iberolacerta) and other lacertine lizards (Reptilia: Lacertidae): did competition cause long-term mountain restriction? *Systematics and Biodiversity*, **2**, 57–77.

Gardner MG, Cooper SJB, Bull CM, Grant WN (1999) Isolation of microsatellite loci from a social lizard, *Egernia stokesii*, using a modified enrichment procedure. *Journal of Heredity*, **90**, 301–304.

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

Refseth UH, Fangan BM, Jakobsen KS (1997) Hybridization capture of microsatellites directly from genomic DNA. *Electrophoresis*, **18**, 1519–1523.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223–225.

Richard M, Thorpe RS (2000) Highly polymorphic microsatellites in the lacertid *Gallotia galloti* from the western Canary Islands. *Molecular Ecology*, **9**, 1919–1920.

Rozen S, Skaletsky H (2000) PRIMER 3 on the WWW for general users and for biologist programmers. In: *Bioinformatics Methods and Protocols* (eds Misener S, Krawetz S), pp. 365–386. Humana Press, Totowa, New, Jersey.