

PRIMER NOTES

Highly polymorphic microsatellites in the lacertid *Gallotia galloti* from the western Canary Islands

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Microsatellite loci are increasingly used in population genetic and evolutionary studies, see Schlötterer & Pemberton (1994) and Jarne & Lagoda (1996) for reviews. Although primers may amplify a homologous microsatellite locus in related taxa (Schlötterer *et al.* 1991; Primmer *et al.* 1996) they tend to be specific to species/species groups and have to be isolated *de novo*.

We report here the isolation and characterization of five highly polymorphic microsatellite loci for phylogenetic and population genetic use in *Gallotia galloti*, which have also been tested on the related species, *G. stehlini*. A *G. galloti* genomic library was established in XL-1 Blue MRF' (Stratagene) transformed with *AluI*/*HaeIII* fragments (300–600 bp), ligated into the *SmaI* site of pUC18 (Pharmacia). A total of 2500 recombinant clones were transferred on plates (2× TY medium) and replica plated

onto nylon membranes. Colonies were screened with end-labelled oligonucleotides (CA)_n, (CT)_n, (GATA)_n and (GACA)_n. From 100 selected positive clones, primers were designed for 10 loci using OLIGO 5.0 (National Biosciences, INC. Plymouth, MN, USA). At least five of the primer sets produce clear, highly polymorphic amplification products of the expected size range (Table 1).

Tissue samples come from noninvasive biopsies (tail tips naturally autotomized) taken from individuals of *G. galloti* from several geographically distinct localities in Tenerife and from *G. stehlini* in Gran Canaria. An average number of 30 individuals per population has been analysed. DNA extractions were performed using a standard protocol (Sambrook *et al.* 1989). Radioactive polymerase chain reaction (PCR) amplifications were carried out in 10 µL of a mixture containing 15–30 ng of DNA, 200 nM of each primer (100 nM of one of them labelled with [³²P]-dATP), 30 µM of each dNTP, 1.5 mM MgCl₂, standard MgCl₂-free BRL 1× reaction buffer (50 mM KCl, 20 mM Tris-HCl pH 8.4) and 0.4 U of *Taq* DNA polymerase (BRL). Initial denaturing step of 3 min at 94 °C was followed by 35 cycles [94 °C for 30 s, 55 °C (53 °C for locus A348 for 30 s and 72 °C for 15 sec) and 2 min at 72 °C. PCR products were run through 6% denaturing sequencing polyacrylamide gels and visualized by autoradiography. Allele lengths were determined by comparison to the original clone and those previously scored. Observed (H_O), and expected (H_E)

Table 1 Characteristics of *Gallotia galloti* microsatellites including, GenBank accession nos, core sequences, product length in bp (length of cloned alleles, and primer sequences. Polymorphism data are from three populations on Tenerife (codes from Thorpe & Brown 1991 and Thorpe *et al.* 1996), with the range in size of the repeat region in bp (Range), number of alleles (A), observed (H_O) and expected (H_E) heterozygosity and P -values for departure from Hardy–Weinberg equilibrium ($*P < 0.05$) derived from an exact test using a Markov chain (length: 100 000)

Locus	Accession no.	Core sequence	Length	Primers	Pop code	Range	A	H_O	H_E	P
A348	AF070978	(AC) ₁₉	228	AATGCTGCTCTCTGTGTCC TTTGTGTCTCTGTCTTTTC	63	20–42	11	0.622	0.722	0.024*
					48	20–40	9	0.590	0.690	0.359
					51	20–42	12	0.763	0.740	0.725
					All	20–42	12	0.658	0.717	
A49	AF070979	(CA) ₁₀	198	AGAGGAAGTGGTAATA GATAGAGGATGGGTGAT	63	16–46	14	0.944	0.893	0.459
					48	16–38	11	0.816	0.873	0.015*
					51	16–40	11	0.763	0.885	0.61
					All	16–46	15	0.841	0.883	
B81	AF070980	(TC) ₁₉	163	GGCAGGTAGAGGAAATC ATAGGGAATGAACAGG	63	14–44	11	0.838	0.877	0.176
					48	12–42	12	0.872	0.845	0.752
					51	14–44	14	0.763	0.836	0.145
					All	12–44	16	0.824	0.853	
B821	AF070981	(AC) ₁₂	261	CCAGAGAGAGGTTTGAC GGTTTGAAGATAGAGAA	63	22–44	11	0.838	0.876	0.440
					48	22–42	11	0.770	0.826	0.554
					51	22–42	11	0.658	0.750	0.062
					All	22–44	12	0.755	0.818	
B967	AF070982	(GT) ₃ AT(GT) ₁₀	149	CACTGCTGTTCCAAAAGACCAC CCCTCCCCTCCACTCACC	63	16–46	15	0.919	0.907	0.738
					48	16–44	12	0.795	0.882	0.218
					51	22–48	13	0.816	0.887	0.250
					All	16–48	17	0.843	0.892	

Table 2 *Gallotia stehlini* alleles sequences for the two loci which exhibited primers conservation and polymorphism within both species. The sequences are compared with the sequence of the original cloned allele from *G. galloti*

Locus	Species	Sequences	
B821	<i>G. galloti</i>	CTCCTACTGG CAGCAGTGTG AAITGCAGCA TGATGACAGC CTTACATTTT TAGGTATATA	
	<i>G. stehlini</i>T...	
	<i>G. galloti</i>	GAGGA[AC] _n GGATGGATAT TGTACTAGG GGAAAACAAG AAITGCAAGA GGGTCCTTCA	
	<i>G. stehlini</i>[AC] _n '	
	<i>G. galloti</i>	GATTAGAAGT TTGTCAGAGG ATCTGGTGTG TGACATGGA GCAGCCCAAT TTAATCCACA	
	<i>G. stehlini</i>G...	
	<i>G. galloti</i>	TTCCAGATTA AGGAACATCT ATCACCAA	
	<i>G. stehlini</i>	
	A348	<i>G. g</i> clone	AGCCATTTC A TAGAATCGG TCTAAAGCAG TATTCACCAA CTTTGGGGG CAAGTGGGCT
		<i>G. s</i> allele2	.A.....
<i>G. s</i> allele4		.A.....	
<i>G. s</i> allele5		.A.....	
<i>G. g</i> clone		CATTTGGAAT TTTGAGAAAA TG---TATGT GCCAGTCACA AATTGGCTAC TGGGGAGATG	
<i>G. s</i> allele2		T.....	
<i>G. s</i> allele4		T.....	
<i>G. s</i> allele5		T.....	
<i>G. g</i> clone		TGACTAACAC ATAATGGCTG CCACATATTT AAA [CA]19 -- length = 188 bp	
<i>G. s</i> allele2		
<i>G. s</i> allele4		
<i>G. s</i> allele5		

heterozygosity and departure from Hardy–Weinberg equilibrium were calculated (Raymond & Rousset 1995).

There are from 9 to 17 alleles per locus in the sampled Tenerife localities (Table 1) and high gene diversity (0.717–0.822). There was no significant evidence of null alleles from heterozygote deficit. Four out of the 30 possible combinations of loci and populations showed a significant departure from independence, but as these combinations revealed no consistent linkage, the loci were considered to be in linkage equilibrium. Two loci (A348, B821) could be amplified in *G. stehlini* (Table 2) and they both exhibit some size polymorphism. For B821 the sequence is highly conserved as only four transitions are observed between the original *G. galloti* cloned allele and *G. stehlini* observed alleles. The polymorphism is very low in *G. stehlini* with only three alleles, one of which has a frequency of 0.90 in our sample. A348 size polymorphism is not only due to different numbers of repeats; deletion and insertion of short sequences are also found (Table 2). For the other loci, we found major sequence differences between the two species. There was no amplification for some individuals, or multiple amplification products for some others.

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Characterization of microsatellite loci in western redcedar (*Thuja plicata*)

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