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PERMANENT GENETIC RESOURCES

Polymorphic microsatellite markers for the lizard *Psammodromus algirus* (Squamata: Lacertidae)

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Abstract

Eight microsatellite loci are described for the lizard *Psammodromus algirus*, a species widely used as a model in behavioural and ecological studies. All loci were highly polymorphic (six alleles or more per locus) in a sample of 24 individuals from a single site near Navacerrada (central Spain). Observed heterozygosity ranged from 0.29 to 0.96. These markers will be used to study mating strategies and determinants of reproductive success in this species.

Keywords: microsatellite, multiplex PCR, *Psammodromus algirus*, tetranucleotide

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Psammodromus algirus (Linnaeus 1758) is a small-medium sized lizard that inhabits the Iberian Peninsula (including southern France) and North Africa (Carretero *et al*. 2002). It has been widely used as a model in behavioural and ecological studies of mating success (e.g. Martin & Forsman 1999; Salvador & Veiga 2001; López *et al*. 2003), largely because males have conspicuous breeding colours that are amenable to experimental manipulation. Two new species (*Psammodromus manuelae* and *Psammodromus jeanneae*) have recently been proposed for Iberian populations on the basis of differences in morphology, allozymes and mitochondrial DNA (Busack & Lawson 2006; Busack *et al*. 2006). Population samples used here come from an area close to the type locality of *P. manuelae* but because its distribution has yet to be determined, we continue to use the species name *P. algirus*. Microsatellite markers were developed with the aim of studying mating strategies and determinants of individual reproductive success in a natural population of *P. algirus* that has been followed over three breeding seasons.

In constructing an enriched microsatellite library, we have followed Hamilton *et al*. (1999). Briefly, a partial genomic library was constructed from size selected fragments (250–700 bp) of *P. algirus* DNA (from a single individual caught near Navacerrada, Comunidad de Madrid, central Spain) that had been digested with *Nhe*I, *Rsa*I and *Hae*III, blunt ended, dephosphorylated, ligated to SNX linker (Hamilton *et al*. 1999) and enriched for AAAG, GATA and GAA repeat sequences. Hybridization capture of repeat sequence was carried out using biotinylated $(GATA)_7$ $(AAAG)_7$ and $(GAA)_8$ oligonucleotides and streptavidin-coated magnetic beads (Dynabeads M-280) following the manufacturer's instructions. Repeat-enriched DNA was amplified using the forward SNX linker as primer, digested with *Nhe*I and ligated into *Xba*I-cut pBluescript II SK+ vector (Stratagene) that was then used to transform competent *Escherichia coli* cells (ElectroTen-Blue electroporation, Stratagene). Recombinant clones were identified by blue/white screening on agar/ampicillin plates. Clones were screened for repeat sequence by filter hybridization using DIG-labelled repeat oligonucleotides and chemiluminescence (CDP-Star detection kit, Sigma). Fifty positive clones were sequenced on an ABI PRISM 3100 Sequencer (Applied Biosystems). Primer pairs were designed for 14 unique repeat-containing sequences using the program PRIMER3 (Rozen & Skaletsky 2000).

All primer pairs were initially tested in single reactions. Polymerase chain reactions (PCRs) were performed in 10-μL reaction volumes (Biotools: 1× standard reaction buffer, 2.0 mM $MgCl_2$, 0.2 mM of each dNTP and 0.25 U *Taq* Polymerase) with 0.25 μM of each primer. Cycling conditions were 1 min at 94 °C, then 35 cycles of 30 s at 94 °C, 30 s at 56 °C and 45 s at 72 °C with a final step of 30 min at 60 °C. One primer from each pair was fluorescently labelled (FAM, VIC, NED or PET) so that the corresponding PCR products could be distinguished from one another by size

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Table 1 Summary data for microsatellite loci isolated from *Psammodromus algirus*. Polymorphism data are based on 24 individuals of *P. algirus* from a single site near Navacerrada (see text for details). H_E and H_O correspond to expected and observed heterozygosities, respectively; *P* values (*P*) are given for the Hardy–Weinberg tests of heterozygote deficiency

Locus (accession no.)	Reaction (Primer, μM)	Primer sequence (5′–3′)	Repeat array in clone	Size of cloned allele (bp)	No. of alleles	Allele size range (bp)*	H_O (H_E)	*P*
Psal1	1	ACCACCACCATTCTGACTCC†	$(GATA)_{11}$	99	9	95–127	0.75	0.16
(EU000299)	(0.3)	GACGAAGAAGTATCTGAAGTGCTC					(0.85)	
Psal2	1	ATCCCTCTGTAAAGGTGTGGAA¶	$(TAGA)_{11}$	300	8	292–312	0.67	0.49
(EU000300)	(0.4)	TTTCTTTCCTTATGCCATCAATC					(0.70)	
Psal3	1	AAATGAATGCTGTGTTAATGCAA§	$(CTAT)_{18}$	330	19	327–419	0.92	0.60
(EU000301)	(0.5)	AAATTTTTATCCTGCCCTAATCCT					(0.92)	
Psal4	1	TCCAAGTTGTCTGTTATGCTCTG	$(CTAT)_{18}$	228	6	178–237	0.29	1.00
(EU000302)	(0.5)	AAAACACCACATTGAGGACCTA§					(0.27)	
Psal5	2	CTGCCCTGTGATCTTTGGAT	$(TAGA)_{16}$	203	15	187–235	0.88	0.29
(EU000303)	(0.2)	AGGAACAGCCAACAACCATC‡					(0.88)	
Psal6	2	TGGAAAATATTCTTTCTCATCCCTA¶	$(CTAT)_{14}(CATCTATCTA$	224	19	162–255	0.96	0.59
(EU000304)	(0.2)	CACTTGCTCTGAGAGGTTGC	$TCTAT)_2(CTAT)_{11}$				(0.94)	
Psal7	2	GGGAAGAGGCTGGGAGATAG†	$(GATA)_{18}$	222	13	206–236	0.96	0.91
(EU000305)	(0.2)	GCACCAGGAGGTTAGGACAT					(0.91)	
Psal8	2	TCCATTCTGAACATGGAACA†	$(CTAT)_4CAT(CTAT)_{12}$	358	14	324–376	0.96	0.92
(EU000306)	(0.5)	ATATTGGCCCCTGAAGCAT					(0.91)	

†6-FAM-labelled primer; ‡PET-labelled primer; §NED-labelled primer; ¶VIC-labelled primer; *based on the GeneScan-500 (LIZ) internal size standard run on an ABI PRISM 3700 DNA Sequencer; 5′ ends of unlabelled primers were modified by addition of the sequence GTTTCTT to provide conditions for essentially full terminal nucleotide addition (see Brownstein *et al*. 1996).

difference or by different fluorescent dyes (see Table 1). Eight primer pairs produced amplification products consistent with single-locus products, and without evidence of null alleles. For these, primer pairs were combined to allow their amplification in two multiplex PCRs (reactions 1 and 2; see Table 1). Multiplex reactions were performed in 10-μL reaction volumes (Biotools: 1× standard reaction buffer, 2.0 mM $MgCl_2$, 0.2 mM of each dNTP and 0.75 U *Taq* Polymerase) with 0.2–0.5 μM of each primer (see Table 1). Cycling conditions were the same as those used for single PCRs. Results from the multiplex reactions were the same as those from single reactions. Fluorescently labelled PCR products were run on an ABI PRISM 3700 DNA Sequencer (Applied Biosystems) with the GeneScan-500 (LIZ) internal size standard. The fluorescent labelling scheme used here allows all eight loci to be pooled and run simultaneously.

Twenty-four individuals of *P. algirus* (12 males, 12 females) from the same site as the individual used for library construction were typed to determine levels of polymorphism (Table 1). Number of alleles per locus ranged from six to 19. Observed heterozygosity ranged from 0.29 to 0.96. Microvariant alleles (alleles differing from one another by lengths other than the repeat length) were present in six loci: Psal2, Psal3, Psal5, Psal6, Psal7 and Psal8. These were due to the presence of insertions or deletions in the microsatellite flanking region. There was no evidence of null alleles (Table 1; tests of heterozygote deficiency) or linkage (although the significance of the test of linkage disequilibrium between Psal7 and Psal8 was 0.051). All calculations were performed using the program GENEPOP version 3.4 (Raymond & Rousset 1995). The levels of polymorphism uncovered at these loci suggest that they should be useful for the study of determinants of individual reproductive success in this species as well as detailed analyses of the validity and utility of the proposed species.

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