



Eight microsatellite loci in the Caribbean lizard, *Anolis roquet*

R. Ogden, T.J. Griffiths & R.S. Thorpe

Brambell Building, School of Biological Sciences, University of Wales, Bangor, LL57 2UW, UK

(*author for correspondence: E-mail: bsp631@bangor.ac.uk)

Received 9 September 2001; accepted 9 October 2001

Key words: *Anolis roquet*, lizards, microsatellite

The islands of the Lesser Antilles in the Eastern Caribbean are often used in studies of inter-island speciation, with the radiation of the lizard genus *Anolis* being highlighted as a natural evolutionary model (Roughgarden 1995). Even within small islands, populations may be differentiated due to both natural selection and past allopatry (Thorpe and Malhotra 1996) and these island anoles typically show such intraspecific differentiation (e.g. Malhotra and Thorpe 2000). Moreover, the introduction of lizard species such as anoles (Giannasi et al. 1997) and iguanas (Malhotra and Thorpe 1999) from other islands in the archipelago raises the issue of de-differentiating hybridisation between endemic and introduced forms (Malhotra and Thorpe 1999). Hence, the development of genetic markers is important for the study of both natural and artificial contact between populations and species and is pertinent to both conservation genetics and evolution.

The island of Martinique in the central Lesser Antilles supports the endemic species, *Anolis roquet*, which displays strong population structure based on morphological and mtDNA markers (Giannasi 1997). In an attempt to investigate the exact nature of this differentiation and its causes, the following eight pairs of microsatellite primers have been developed for *A. roquet*, enabling fine-scale genetic changes to be observed between populations.

A genomic library was established by cloning size-selected (200–1000 bp) fragments of pooled *A. roquet* DNA, generated by the restriction enzyme Tsp509 I (New England Biolabs), into the EcoR I site of the phagemid pBluescript KS(+) (Stratagene). Competent cells of *E. coli* XL1-BlueMRF⁺ (Stratagene) were transformed with the recombinant clones

and incubated on LB agar. The resulting colonies were screened with ³²P-end-labelled dinucleotide repeat probes [(AG)₁₂, (AT)₁₂, (GC)₁₂, (GT)₁₂]. 26 primer pairs were designed from 53 sequenced cloned inserts using OLIGO 5.0 (National Biosciences, INC. Plymouth, MN, USA). Of these, eight pairs of primers produced reproducible polymorphic bands, with the remainder forming monomorphic, multiple or undetectable products.

Tissue samples in the form of autotomised tail tips were taken from animals captured throughout Martinique and stored in 75% ethanol. DNA was extracted using the Nucleospin kit (Machery-Nagel GmbH & Co.), resulting in concentrations of 50–100 ng DNA μl^{-1} . Fluorescent end-labelled primers (Life Technologies) were used with coloured dyes FAM, TET and HEX chosen from Applied Biosystems filter set C. Primers were assigned colours based on locus fragment size in order to enable a single multiplex gel to be run using all eight loci simultaneously (Table 1).

PCR reactions were carried out using a GeneAmp PCR System 9700 (Applied Biosystems). 10 μl reaction volumes were used consisting of 1x PCR buffer (50 mM KCl, 20 mM Tris-HCl pH 8.4), each dNTP (120 μM), each primer (1 μM), *Taq* DNA polymerase (0.75 U, Life Technologies), magnesium chloride (variable amount – see Table 1) and 50–100 ng of *A. roquet* DNA. Following an initial denaturing step of 3 minutes at 94 °C, the reactions underwent 35 thermal cycles of: 30 s at 94 °C, specific annealing time and temperature (Table 1), 30 s at 72 °C. An extension period of 5 minutes at 72 °C followed by 30 minutes at 60 °C was included to promote non-specific adenylation. PCR products were subsequently separated on

Table 1. Polymorphic microsatellite loci for *A. roquet*. GenBank accession numbers, primer sequences, dye labels, allele sizes, repeat regions and PCR conditions are listed, together with observed and expected heterozygosities averaged across 40 populations of 15 individuals

Locus	Accession number	Primers (5' to 3')	Dye label	Cloned allele size range (bp)	Repeat region	Anneal T. (°C)	[MgCl ₂] (mM)	No. of alleles	Mean H _E	Mean H _O
Ar014	AF415158	AATTTCTTAGAGAGATGTGTG CCATTGAATCCAATGTCCTG	FAM	120–138	(TG) ₁₀	57	1.5	10	0.6915	0.6702
Ar031	AF415159	TCAGTTTCTGCAATGCCTCTCT TTGTTTCAAGCATCCAGCATTG	HEX	217–241	(GT) ₇	63	1.5	12	0.4997	0.4748
Ar035	AF415160	TTCCTTCTTTTTTACAGCTGTC TATTTCCAGCCCGTCTTAG	HEX	126–154	(TG) ₁₃	55.5	1.5	13	0.6619	0.5896
Ar062	AF415161	GAGAGCGAAAGAGACATCAGA ATGTGCTGAACTACGGAAGAG	TET	162–178	(AG) ₉	64.5	1.5	9	0.5655	0.5520
Ar065	AF415162	TCTGTCTTTCCAGTGTGCTAC CTGGCTTCTTTTCATTTCCCTTG	FAM	65–101	(GT) ₁₅	64.5	1.5	19	0.8237	0.7960
Ar068	AF415163	AATTAATGTTTGTTCGTGTGT GTAGCATTGTTGGCATAGTT	TET	118–132	(GT) ₁₀	57	1.5	5	0.4291	0.4020
Ar120	AF415164	CAGTCCTTACATTGTGTCCTATC TGTTACCAAACAACCTCCTCTCT	FAM	154–172	(AC) ₁₄	64	1.5	10	0.7714	0.7653
Ar126	AF415165	GGGAAGAACTGGTCTGAATC CCTTTAAAGGAATACGTGGCA	HEX	110–130	(GT) ₅	61.5	3.0	11	0.7906	0.7774

a denaturing polyacrylamide gel (BMA) and detected using an ABI 377 automated sequencer.

The eight loci have all been used to screen populations of *A. roquet* along five transects on Martinique. The transects incorporate localities representing all *A. roquet* haplotypes (Giannasi 1997) and range over the entire island, excluding the south western peninsular and northern tip. A total of 600 individuals were analysed with between 5 and 19 alleles detected at each locus (Table 1). Allele frequencies were analysed using GENEPOP v3.1 (Raymond and Rousset 1995) to calculate expected heterozygosities (H_E) and to test for departures from Hardy-Weinberg equilibrium. Expected heterozygosities ranged between 40% and 78% (Table 1) and observed heterozygosities (H_O) showed no significant differences from these levels. Linkage disequilibrium tests (GENEPOP v3.1) showed no significant evidence of linkage between any loci pair combination.

Acknowledgements

This work was funded by BBSRC studentship 99/A2/G/5481 (RO), Wellcome Trust grant number 057257/z/99/z (RST) and The Linnean Society (RST). We would like to thank Fauna and Flora Interna-

tional and Herpetofauna Consultants International Ltd. for BBSRC CASE sponsorship (RO) and Dr Andy Stenson and Ross McEwing for technical advice.

References

- Giannasi NC, Thorpe RS, Malhotra A (1997) Introductions of *Anolis* species to the island of St. Lucia, West Indies: Testing for hybrids using multivariate morphometrics. *Journal of Herpetol.*, **31**, 586–589.
- Giannasi NC (1997) *Morphological, Molecular and Behavioural Evolution of the Anolis Roquet Group*. PhD Thesis, University of Wales, Bangor, UK.
- Malhotra A, Thorpe RS (1999) *Reptiles and Amphibians of the Eastern Caribbean*. Macmillan Education Ltd, London.
- Malhotra A, Thorpe RS (2000) The dynamics of natural selection and vicariance in the Dominican anole: patterns of within-island molecular and morphological divergence. *Evolution*, **54**, 245–258.
- Raymond M, Rousset F (1995) *Genepop* Ver 3.0, Institut des Sciences de l'Evolution. Université de Montpellier, France.
- Roughgarden J (1995) *Anolis Lizards of the Caribbean*. Oxford Series in Ecology and Evolution. Oxford University Press, Oxford.
- Schneider S, Kueffer J-M, Roessli D, Excoffier L (1997) *Arlequin ver. 1.1: A Software for Population Genetic Data Analysis*. Genetics and Biometry Laboratory, University of Geneva, Switzerland.
- Thorpe RS, Malhotra A (1996) Molecular and morphological evolution within small islands. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.*, **351**, 815–822.