Molecular Ecology Resources (2008) **8**, 1408–1410 doi: 10.1111/j.1755-0998.2008.02206.x

PERMANENT GENETIC RESOURCES

Development of microsatellite markers in the St Lucia anole, *Anolis luciae*

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Abstract

***Anolis* lizards are important models in studies of ecology and evolution. Here we describe 13 polymorphic microsatellites for use in population screening in the St Lucia anole, *Anolis luciae*, that can be used as a natural replicate to *Anolis roquet* on Martinique to study processes involved in population differentiation and speciation. Genotyping of 32 individuals using M13 tails and FAM-labelled universal M13 primers showed that all loci were polymorphic with high genetic diversity, averaging at 16.8 alleles per locus. Genotypic frequencies conformed to Hardy–Weinberg expectations, and there were no instances of linkage disequilibrium between loci.**

Keywords: *Anolis luciae*, Caribbean anole, DNA, M13 tail, microsatellite, pigtail

Received 18 January 2008; revision accepted 9 March 2008

Anolis lizards have been extensively studied in the Caribbean and have had a major influence in the development of evolutionary ecology theory (Losos in press). Recently, studies on the Martinique anole, *Anolis roquet*, have highlighted the usefulness of Lesser Antillean species in the study of speciation (Ogden & Thorpe 2002; Thorpe 2005). The complex geological history and environment observed on Martinique allows for the study of allopatric effects and ecological determinism in population differentiation and speciation. Saint Lucia, adjacent to Martinique, is another Lesser Antillean island with complex geological history and similar environmental variation to Martinique. The endemic anole, *Anolis luciae*, is closely related to *A. roquet* (Thorpe & Stenson 2003) and can be used as a natural replicate to generalize observations from Martinique to shed light on aspects of speciation and parallel evolution in Lesser Antillean anoles. Here, we report 13 microsatellite loci for use in *A. luciae*. One (ABO-P4A9) is cross-amplified from the related *Anolis bonairensis* (Gow *et al*. 2006) while the remaining 12 are new and developed from *A. luciae*.

DNA samples were extracted from automotized tail tips collected from different locations across the species' geographical range. DNA samples were used to construct a genomic library enriched for tetranucleotide microsatellite repeats according to the protocol developed by Gardner *et al*. (1999) and modified by Gow *et al*. (2006). Purified polymerase chain reaction (PCR) products from 134 clones potentially containing microsatellites were sequenced by Macrogen (www.macrogen.com). From these clones, 27 had unique microsatellite motifs and had adequate flanking regions that were suitable for primer design using the program PRIMER 3 (Rozen & Skaletsky 1998).

An M13 tail was added to the forward primers allowing the use of a FAM-labelled universal M13 primer for amplification (Oetting *et al*. 1995). Reverse primers were pigtailed at the 5′ end (GTTT) to reduce stutter and improve reliability of allele scoring (Brownstein *et al*. 1996). Primers were first tested on four different individuals. Loci were amplified using 5 ng of genomic DNA, 1.5 mM $MgCl_2$, 0.1 mM of each dNTP, 0.05 μM of the forward primer, 0.5 mM of the reverse primer and of the labelled primer, and 0.5 U of *Taq* DNA polymerase with associated 5× buffer (Promega). PCR was performed with a denaturation time of 2 min at 95 °C, followed by 29 cycles (30 s at 95 °C, 30 s at 55 °C, and 30 s at 72 °C) and a final extension period of 5 min at 72 °C. Samples were run on an ABI 3130xl genetic analyser with the internal size standard LIZ-600 and analysed with the software GENEMAPPER version 4.0 (Applied Biosystems). Thirteen loci amplified and showed variation in the first four samples, and were screened on a further 32 individuals from one *A. luciae* population (Mon Repos, UTM coordinates

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Table 1 Polymorphic microsatellite loci for *Anolis luciae*. Locus name prefix indicates the species of origin: Abo, *Anolis bonairensis*; Alu, *A. luciae*. Final PCR conditions for all primers were: 1.5 mM $MgCl_2$, 55 °C annealing temperature. Primer name, repeat motif and GenBank Accession number of the sequenced clone are given alongside allele size range, number of alleles (N), observed (H_O) and expected (H_E) heterozygosities for the 13 microsatellites that were polymorphic within *A. luciae* (n = 32 from a single population from St Lucia)

Primer name	Repeat	Primer sequence (F, forward; R, reverse)	Allele size range	N	H_O	H_E	GenBank Accession no.
ABO-P4A9	$(CTAT)_9$	F: GTGACTATGAAGGGGAATCTTG R: GTTTGATGTAGGCTTTGCTGCTGT	347–407	15	0.88	0.88	DQ379373
ALU-MS02	$(AAAG)_{16}$	F: GAAATGCAGCTTCGATCACA R: GTTTATTGGGAGAAGTGGGTTGC	177–316	21	0.78	0.94	EU379658
ALU-MS04	$(AAAG)_{15}$	F: TCAGTCTAAGGGTGGGAGGA R: GTTTGCTCATTAGGATTTGGGACTT	272–327	14	0.84	0.89	EU379659
ALU-MS06	$(TAGA)_{10}$	F: CCTGATGCGCACAAAGAATA R: GTTTTCAAGTCTGGCAATGGA	240–284	12	0.87	0.86	EU379660
ALU-MS10	$(AAAG)_8$	F: GGCTCTTGGCACCTGATAAA R: GTTTCCAATCCTGGCAAAACATCT	252–351	10	0.78	0.82	EU379661
ALU-MS12	$(TACA)_5$	F: TACATACACCGTTGCCCACA R: GTTTATCAGCACACACCAGTCAGC	127–151	7	0.56	0.58	EU379662
ALU-P8A3	$(AAAG)_{13}$	F: GCTGGAAAGATTAACAAAGATGG R: GTTTCCCAACAAAAAGGATTCTGAC	213–268	17	0.78	0.89	EU379663
ALU-P8B10	$(AAAG)_{10}$	F: CAGAGAGTTCAAAAGGAATTGTCC R: GTTTACTGCCTTTCCCTTATGGTC	135–176	28	0.88	0.95	EU379664
ALU-P8C7	$(GT)_7$	F: TCAATGAATGGGCTGGTGT R: GTTTGGAAAGTGTTTCGCTTGA	194–312	14	0.81	0.83	EU379665
ALU-P8C9	$(AAAG)_{17}$	F: TCACTAAATGCCTCTAAGCTATTG R: GTTTCTCCCAAAGGCAAGGTTTC	217–274	16	0.81	0.91	EU379666
ALU-P8E12	$(AAAG)_{15}$	F: TCCTGGACCCATGTGAAAAG R: GTTTAAACAGGAGGCGAAGTTGG	91–125	21	0.94	0.92	EU379667
ALU-P8H7	$(AAAG)_{11}$	F: GGGGGTTCTGTGAATTGTTG R: GTTTCCAAGGTATTCTTCCATTTGC	98–152	17	0.94	0.92	EU379668
ALU-P8H8	$(AAAG)_{12}$	F: GGCATCTCCATTTTAACAAGAAAG R: GTTTGACAGATTTTCCTAGTTCCTCCTG	111–190	26	0.94	0.96	EU379669

easting 726279, northing 1533577, zone 20N). We also tested the utility in this species of the 10 loci previously isolated from different species of the *roquet* series. For these loci, the PCR conditions were described in Gow *et al*. (2006) and one of these (ABO-P4A9) amplified reliably in *A. luciae*.

In total, 13 loci amplified reliably, were polymorphic and did not show any evidence of null alleles. Allelic diversity was calculated for each locus, and ARLEQUIN version 3.01 (Schneider *et al*. 2000) was used to test for departures from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) (Table 1). Genetic diversity was high: number of alleles per locus ranged from seven to 28 and averaged 16.8 across all loci; mean expected heterozygosity was 0.87 with locus-specific values varying from 0.58 to 0.96. There were no significant departures from HWE, and none of the possible locus combinations showed significant genotypic LD, following sequential Bonferroni correction (Rice 1989). Extreme length variation is evident in two loci, ALU-MS02 and ALU-P8C7. However, there is no deviation from HWE in these loci and the 'outlying' alleles were found in different individuals. Thus, we are confident they are not artefacts. Furthermore, we have just studied a limited number of individuals in one population. Some microsatellite loci in the closely related anole *A. roquet* have more than 80 alleles across the entire distribution range. Thus, we expect to find alleles with intermediate sizes when scoring more individuals from different populations. Microsatellite loci in the Lesser Antillean anole, *A. luciae*, hence show comparative levels of genetic diversity to other Lesser Antillean anoles (Ogden *et al*. 2002; Gow *et al*. 2006), and can be used for intra-island population genetic screening.

Acknowledgements

This work was funded by BBSRC grant BB/C500544/1 to R.S.T., EC award MEIF-CT-2005–009981 to Y.S.G. and R.S.T., and a NERC studentship to H.J. (supervised by R.S.T.). We wish to thank St Lucia's Forestry Department for field permits.

References

Brownstein M, Carpten J, Smith J (1996) Modulation of nontemplated nucleotide addition of *Taq* DNA polymerase: primer

modifications that facilitate genotyping. *BioTechniques*, **20**, 1004–1010.

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.

Gow JL, Johansson H, Surget-Groba Y, Thorpe RS (2006) Ten polymorphic tetranucleotide microsatellite markers isolated from the *Anolis roquet* series of Caribbean lizards. *Molecular Ecology Notes*, **6**, 873–876.

Losos JB (in press) *Lizards in an Evolutionary Tree: Ecology and Adaptive Radiation of Anoles*. University of California Press, Berkeley, California.

Oetting WS, Lee HK, Flanders GL *et al*. (1995) Linkage analysis with multiplexed short tandem repeat polymorphisms using infrared fluorescence and M13 tailed primers. *Genomics*, **30**, 450–458.

Ogden R, Griffiths TJ, Thorpe RS (2002) Eight microsatellite loci in the Caribbean lizard, *Anolis roquet*. *Conservation Genetics*, **3**, 345–346.

Ogden R, Thorpe RS (2002) Molecular evidence for ecological speciation in tropical habitats. *Proceedings of the National Academy of Sciences, USA*, **99**, 13612–13615.

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

Rozen R, Skaletsky H (1998) *PRIMER 3*. Available from http://frodo.wi.mit.edu/primer3/input.htm.

Schneider S, Roessli D, Excoffier L (2000) *ARLEQUIN: A Software for Population Genetics Data Analysis*. Version 3.01. Genetics and Biometry Laboratory, Department of Anthropology, University of Geneva.

Thorpe RS (2005) Ecology: Population evolution and island biogeography. *Science*, **16**, 1778–1779.

Thorpe RS, Stenson AG (2003) Phylogeny, paraphyly and ecological adaptation of the colour and pattern in the *Anolis roquet* complex on Martinique. *Molecular Ecology*, **12**, 117–132.