Molecular Ecology Notes (2003) **3**, 400–402 doi: 10.1046/j.1471-8286.2003.00459.x

PRIMER NOTE

Characterization of dinucleotide microsatellite markers in the parthenogenetic mourning gecko (*Lepidodactylus lugubris*)

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Abstract

We present 16 variable dinucleotide microsatellite markers to quantify genetic variation in the parthenogenetic gecko, *Lepidodactylus lugubris*. Genetic diversity at these loci was unusually high for an asexual species. Subsets of individuals produced identical genotypes across all loci. Individual loci revealed evidence of polyploidy and marked differences between observed and expected heterozygosity, indicating the presence of null alleles. These patterns conform to prior expectations based on the distant genetic relationships between the presumed sexual progenitors of *L. lugubris*. Comparisons with sexual relatives will be required to determine the sources of the observed genetic variation.

Keywords: asexual, dinucleotide, *Lepidodactylus*, microsatellite, parthenogen, SSR

Received 2 February 2003; revision received 2 April 2003; accepted 2 April 2003

The relatively common occurrence of parthenogenesis in gecko lizards presents an opportunity to study the relative advantages of sexual and asexual reproduction. *Lepidodactylus lugubris* is an all-female parthenogen that occurs throughout the tropical Pacific. Although it is now being displaced by other invading geckos (Petren *et al*. 1993; Case *et al*. 1994; Petren & Case 1996), the broad range of *L. lugubris* greatly exceeds the ranges of related sexual species (Radtkey *et al*. 1995). This success may be attributable to their mode of reproduction, greater ecological versatility (Hanley *et al*. 1994), lower prevalence of parasites (Hanley *et al*. 1995), or a combination of factors. Microsatellite loci will help us distinguish the relative contribution of these factors by enabling us to quantify clonal variation and genetic history.

To quantify genetic variation in *L. lugubris*, we constructed a genomic library with DNA from eight individuals representing a range of dorsal colouring patterns that reflect genetic differences among clones (Moritz *et al*. 1993). All specimens were collected from two different locations on Oahu, Hawaii, USA: Sunset Beach Elementary School and Windward Community College in Kaneohe. Total DNA was extracted from tail tissue taken from field specimens stored in 70% EtOH (Qiagen: Tissue Easy Kit). We isolated microsatellites generally following the methods of Petren (1998). Total DNA was digested with Sau 3a, and fragments (200–700 bp) were ligated into a pUC18 vector. Competent cells (Stratagene, SURE) were transformed by electroporation and colonies (> 60 000) were screened twice by colony-lift hybridization using an equimolar mixture of two ^{32}P-ATP end-labelled oligonucleotides $(CA)_{15}$ and $(GA)_{15}$. Positive colonies (N = 40) were sequenced, and primer pairs were developed (N = 20) for colonies that contained pure repeat motifs at least eight units long with sufficient flanking regions for primers. Polymerase chain reactions (PCRs) (in 10 μL) contained 50 mM KCl, 10 mM Tris-Cl (pH 8.3), 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.5 μM of each primer, 0.5 units *Taq* polymerase (ABI, Amplitaq Gold) and ~25 ng of genomic DNA. Forward primers were labelled with a fluorescent dye (ABI), and 33 PCR cycles were performed (Hybaid, PCR Express): 40 s at 94 °C; 40 s at the annealing temperature listed in Table 1; and 40 s at 72 °C. An initial single step of 94 °C for four minutes, and a final single step of 72 °C for five minutes were also included. Reactions for three to six loci were combined, and allele sizes were quantified using an ABI 3100.

Sixteen loci were polymorphic among the 48 *Lepidodactylus lugubris* genotyped (Table 1). Four loci yielded no clear PCR products at four different annealing temperatures

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Table 1 Microsatellite loci isolated from parthenogenetic *L. lugubris*, including GenBank accession numbers, primer sequences, and expected and observed heterozygosity among individuals ($N = 48$) from Oahu, Hawaii. For all loci, the forward primer was labelled and PCR conditions were the same (2.5 mM $MgCl_2$), except for the annealing temperature (shown). All but one locus showed significant deviations from Hardy–Weinberg equilibrium (*, $P < 0.001$; †, $P < 0.05$)

Locus	Core motif	Anneal temp. (°C)	Size range	No. of alleles	H_E	H_O	Primer sequence (5′ → 3′)
Ll01	$(CT)_{25}$	56	121–133	6	0.64	0.85†	F-ATGTTGTTTTTCCCCCATGT
AY221135							R-AGAGACACAGGCATGTTTACG
Ll02	$(GT)_{8.5}$	56	144–152	5	0.72	1.00*	F-CAAAGGCATCTATGCAGACG
AY221136							R-CCTGCACACCAGCTTATGAAG
Ll03	$(GT)_{11}(CA)_{11}$	54	193–203	2	0.49	0.85*	F-TTTATCTGCCCTCCAGCTTC
AY221137							R-GCTGTATGCTCTTGTTTGAGAAAA
Ll04	$(AG)_{15}$	54	183–198	4	0.68	0.97*	F-TCAACATGGCCAAATCTGC
AY221138							R-GCTTAGCAGAAGGCATTCATTT
Ll05	$(GT)_{13}(AT)_8$,	56	134–180	10	0.83	0.76*	F-ACAAGGGAGTATGGTAAGTTC
AY221139	$(GT)_8$						R-GCATCATGCAATTAGGTTCCA
Ll06	$(CA)_{14}$	56	184–200	4	0.65	0.04*	F-CCCAAGTCTGCAGGAAAATC
AY221140							R-CCAGATGAAAAGTGGCAGGT
Ll07	$(CA)_{12}(GT)_{15}$	62	236–262	10	0.82	0.90*	F-ATGGCTCAGAAACACCCTGT
AY221141							R-GCAGGAAAAGAGGAAGACTCA
Ll08	$(CA)_{21.5}$	56	264–284	8	0.77	0.14*	F-GGACACCTGAACAGGTGAG
AY221142							R-TCTTTTCCCTGGGTGTTTTG
Ll09	$(AC)_{18}$	54	105–119	5	0.51	0.17*	F-AGGGTTTGGGTTTTCAGCTT
AY221143							R-GGTCACAGCTGATTTTTGGTG
Ll10	$(GT)_{11.5}$	56	162–200	9	0.73	0.38*	F-GATCCCCAAGGGGATTGGTG
AY221144							R-ATCGCTCCGTAGTGCATTG
Ll11	$(GT)_{13}$	54	198–220	6	0.53	0.32*	F-ATCATGTACTGACCGGAAGGG
AY221145							R-GTGATCTCGGAAGCTAAGCAG
Ll12	$(CA)_9$	56	155–188	2	0.51	0.93*	F-TGACCTTGATGTTGGTTGGA
AY221146							R-ACTGTTTTCCCCGCTTTTCT
Ll13	$(GATA)_{13}$	54	152–193	9	0.78	0.11*	F-TGAAAAAGGAGAGTAAATGTTGGA
AY221147							R-AAAAATTAAAACACAGCACAAGTTT
Ll14	$(GT)_{11}$	54	206–242	4	0.42	0.10*	F-TGGAGATGGTGGATAACTTGG
AY221148							R-GGTGAAAATTTGCCTCATGC
Ll15	$(AC)_{18}$	56	99–123	6	0.71	0.82	F-AAGCTAAGGGAGGCTCATGC
AY221149							R-GTGAAGGGCGGGGTATAAAT
Ll16	$(CA)_{13}$	57	110–126	5	0.67	1.00*	F-GTGTGCGCATCATCCAAA
AY221150							R-AGACACAACTGTGGGCGAGT

(48 °C to 58 °C). As expected, there was genetic uniformity across all loci among subsets of individuals. Substantial genetic differences were detected among subgroups, indicating the presence of distinct clonal lineages. Some individuals ($N = 26$) produced clear triploid haplotypes at two or more loci, in agreement with previous reports of triploid karyotypes in *L. lugubris* (Moritz *et al*. 1993).

Every locus but one (LL15) showed significant deviations from Hardy–Weinberg equilibrium (Table 1) based on Markov-chain estimated *P*-values (Raymond & Rousset 1995), even with triploid genotyopes excluded from the analysis. Previous evidence suggests that *L. lugubris* was formed through the hybridization of two sexual species (*L. moestus* and an undescribed *Lepidodactylus* species) that co-occur in the Marshall Islands (Radtkey *et al*. 1995). Because these species are genetically distinct (cytochrome *b* sequence divergence > 8%), *L. lugubris* may have inherited null (nonamplifying) alleles from either progenitor species, and a single PCR product may not always indicate homozygosity. Null alleles inherited from one progenitor may account for the observed heterozygosity deficit at some loci (e.g. Ll 05, 06, 08–11, 13, 14), while the heterozygosity excess observed at other loci (e.g. Ll 01–04, 07, 12, 15, 16) may be attributed to the inheritance of alleles from progenitors that are nearly fixed for different alleles.

Direct comparisons with the sexual progenitors will be required to determine if the high levels of genetic diversity observed in *L. lugubris* is due to separate hybridization events, backcrosses, mutations, or genomic rearrangements. This parthenogen may provide a rare opportunity to explore the relative advantages of sexual and asexual reproduction without the confounding affects of reduced genetic variation typically encountered in asexual lineages.

Acknowledgements

We thank T. J. Case, D. T. Bolger, R. N. Fisher and K. A. Hanley for providing some of the specimens used for this study. Funding was provided in part by the University of Cincinnati Research Council.

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