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PRIMER NOTE

Isolation and characterization of dinucleotide microsatellite loci in communally breeding Guira cuckoos (Aves: Cuculidae)

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Abstract

Guira cuckoos (*Guira guira*) are communal nesting birds endemic to South America that show high levels of conflict between members of the group over the contribution to the clutch. Adults eject eggs and even nestlings from the communal nest, sometimes leading to the loss of the entire brood. We developed seven polymorphic microsatellites for Guira cuckoos using an enrichment protocol. The number of alleles ranged from 5 to 14 (mean 9.86) and the heterozygosity ranged from 0.41 to 0.89 for the eight to 88 individuals screened. These loci will allow parentage assignments and population analysis in this species.

Keywords: communal breeding, enrichment, *Guira guira*, microsatellites, parentage analysis

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Guira cuckoos (*Guira guira*, Cuculidae) live in groups of up to 15 adults in open habitats throughout South America (Macedo 1992). Their mating system is polygynandrous with no complete monopoly of reproduction by one individual of either sex (Quinn *et al*. 1994) and, after chicks hatch, some adults provide more parental care than others (Macedo 1994; Pacheco 2002). High rates of egg ejection and infanticide are other components of the evident reproductive conflict within groups (Macedo *et al*. 2001; Pacheco 2002).

Highly variable genetic markers are needed in order to assign parentage of chicks when one or both parents are unknown and individuals are likely to be closely related. We present here a set of seven highly polymorphic microsatellite loci, specific to the Guira cuckoo, isolated from enriched libraries.

The DNA was extracted from blood samples using standard organic procedures (Sambrook *et al*. 1989). DNA (1 μg) pooled from 10 unrelated adults (six males and four females) from Brasília, Brazil, was digested with *Mbo*I (Promega) and ligated to 600 ng of annealed *Sau*LA and *Sau*LB linkers (Armour *et al*. 1994) with 3 U of T4 DNA ligase and 1 × ligase buffer (Promega; 10 mM Tris-HCl, pH 7.8, 10 mM $MgCl_2$, 5 mM DTT and 1 mM ATP) in 55 μL. Two microlitre of the ligation reaction was amplified in 50-μL reactions containing 1 × Bioline buffer [16 mM $(NH_4)_2SO_4$, 67 mM Tris-HCl, pH 8.8, and 0.01% Tween-20], 0.01 mM dNTP, 2.0 mM $MgCl_2$, 20 pmol of *Sau*LA linker and 1.5 U Bio*Taq* (Bioline) at 72 °C for 2 min, 94 °C for 3 min then 20 cycles of 94 °C for 30 s, 57 °C for 30 s and 72 °C for 1 min and a final elongation step of 5 min at 72 °C in a thermocycler (PTC-100; MJ Research).

For the enrichment, $(CA)_{24}$ oligonucleotides were cross-linked to 0.4-cm^2 nylon filter squares (Hybond-N+; Amersham) following the method of Becher *et al*. (2002). The membranes were prehybridized in 2 × SSC/0.5% sodium dodecyl sulphate (SDS) at 45 °C for 12 h. The amplification product was denatured at 98 °C for 5 min and hybridized to the membranes in 2 × SSC/0.5% SDS with 1 mg *Sau*LA linker at 45 °C overnight. The membranes were washed five times in 500 μL of 2 × SSC/0.01% SDS and three times in 0.5 × SSC/0.01% SDS each at 45 °C. The bound DNA was eluted from the membranes in 250 μL of H_2O at 98 °C for 5 min. The DNA was ethanol precipitated and dissolved in 25 μL of H_2O. A volume (2 μL) of this enriched DNA was amplified in a second round of polymerase chain reaction (PCR) as before, but without the initial 72 °C step. The PCR product was used in a second round of enrichment,

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Table 1 Characteristics of polymorphic microsatellite loci isolated from *Guira guira*-enriched genomic library

Locus	Repeat motif	Primer sequence (5′–3′)	T_a (°C)	No. of alleles	Product size range (bp)	N	H_O	H_E	PIC
GUIRA1	$[TG]_8[TC]_2[TG]_5$ CCTC $[TG]_8$	GCT CAG TCT TGC TGG GAT GT TTA AAG GCA TTG GGA CTT GC	60	12	134–174	51	0.863	0.873	0.851
GUIRA30	$[AC]_{14}$	GGC ACT CTG GTC TGC CTT TA AGC AGC CAT CGT CAC TCT TC	60	12	178–208	88	0.716	0.869	0.849
GUIRA398	$[AC]_{16}$	CCT ACA GCC AAA CAG CCT CT CTC CCC TAC CAT GTC TGC TC	60	14	207–241	38	0.895	0.861	0.834
GUIRA405	$[CA]_{15}$	CCT CAC CAC CCA TAT TTT GC GCG GAT TGG TGA GAC TTC TG	61	7	180–216	38	0.579	0.540	0.502
GUIRA463	$[CA]_{15}$	CTC ATG CTT CTC ATG CTC CT GAA ACC ATC TGG GTC CTC TG	57	9	200–240	8	0.875	0.883	0.810
GUIRA963	$[CA]_{14}$	CTG AAG CCT TCC AGG AGG TA GCA GGT CTT TGG CTC ACA A	58	10	214–236	37	0.730	0.887	0.875
GUIRA1037	$[CT]_7[CA]_{11}$	TGC GAG CCT CGT ATC AGT TA CGG GGC ATT CTG TAA GAA AC	60	5	206–214	39	0.410	0.466	0.434

T_a, Annealing temperature; N, no. of individuals tested. H_O, Observed heterozygosity; H_E, expected heterozygosity and PIC, polymorphism information content, were calculated using the program CERVUS 2.0 (Marshall *et al*. 1998). GenBank accession nos AY123771–AY123777.

subtraction, elution and precipitation. A final PCR was performed as previously with 2 μL of this twice-enriched library. The DNA was then digested with *Mbo*I (Promega) to remove the linkers for 2 h at 37 °C in a 100-μL reaction containing 1 × buffer C (6 mM Tris-HCl, pH 7.9, 10 mM $MgCl_2$, 50 mM NaCl and 1 mM DTT), 100 μg/mL bovine serum albumin and 10 U of *Mbo*I. The library was purified with Wizard PCR Prep purification columns (Promega) and the purified fragments were then ligated into 'Ready-to-go' *Bam*HI-digested pUC18 (Pharmacia) following the manufacturer's protocol. After transformation in Epicurian XL1 Blue MRF' supercompetent cells (Stratagene) and plating onto Luria-Bertani agar plates with 50 μg/mL ampicillin, the colonies were screened with $(CA)_{24}$ oligonucleotides end-labelled with [γ^{32}P] dATP (T4 Polynucleotide kinase RTG kit; Pharmacia).

We isolated and amplified 73 positive clones with M13 primers to determine insert length. Colonies with inserts of 300–500 bp were preserved and 28 clones selected for sequencing on an ABI 377 sequencer. All but one of these contained a microsatellite repeat. We selected the 11 clones that had repeats of more than 28 bp, either pure or broken, and sufficient flanking region. Primers were designed using PRIMER 3 (Rozen & Skaletzky 2000).

Approximately 20 ng of genomic DNA were amplified in 15-μL PCR reactions containing 1 × buffer (as above), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 μM of each primer (both unlabelled) and 0.5 U of Bio*Taq* (Bioline). The reactions were denatured at 95 °C for 3 min followed by 26 cycles of 90 °C for 30 s, primer-specific annealing temperatures (Table 1) for 30 s, 72 °C for 30 s and final elongation at 72 °C for 5 min. The PCR products were separated on 6% polyacrylamide gels, visualized by silver staining and allele lengths scored from a 10-bp DNA ladder (Invitrogen).

Seven loci were polymorphic (Table 1). Two of these, Guira30 and Guira963, were significantly out of Hardy–Weinberg equilibrium ($P = 0.002$ and $P < 0.001$, respectively; GENEPOP 1.6; Raymond & Rousset 1995) and both had null allele frequencies estimated at 9% (CERVUS 2.0; Marshall *et al*. 1998). In the case of Guira30, where 88 individuals were genotyped, an excess of homozygotes was found for one of the two most common alleles and this may be attributable to the existence of a null allele. Guira963 had two alleles present homozygously in more than one of the 37 individuals typed (one in two and the other in four individuals). More extensive genotyping and analysis of parentage will indicate whether apparent homozygotes in these loci are attributable to null alleles.

The mean expected heterozygosity for all loci was 0.77 (CERVUS 2.0; Marshall *et al*. 1998). Since the parental exclusionary power of these loci combined is 0.99 when neither parent is known they will be useful for analysing the social organization and reproductive behaviour of the communally breeding Guira cuckoo.

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