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PRIMER NOTE

Characterization of polymorphic microsatellite loci in Castelnau's Antshrike, *Thamnophilus cryptoleucus* (Aves: Thamnophilidae)

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Abstract

In order to study the biogeography and population genetic structure of the obligate river-island species *Thamnophilus cryptoleucus* (Thamnophilidae) we isolated four microsatellite loci. Number of alleles ranged from six to 11, heterozygosities from 60% to 89%, and individual populations were found in Hardy–Weinberg equilibrium. Cross-species amplification of the loci was tested in eight species of the family Thamnophilidae and all loci successfully amplified in five or more related species.

Keywords: antbird, microsatellite, polymorphism, Thamnophilidae, *Thamnophilus cryptoleucus*

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Castelnau's Antshrike (*Thamnophilus cryptoleucus*) is a medium sized antbird specialized on the undergrowth and middle-storey of *Cecropia* stands growing along primary successional vegetation of riparian forest in Western Amazonia (Rosenberg 1990). It inhabits older and middle-aged islands of the Amazonas River and its larger white-water tributaries (Rosenberg 1990; Zimmer & Isler 2003). The riverbanks and floodplains are affected by periodic and asynchronous inundation events related to the seasonal distribution of rainfall in areas surrounding the basin (Irion 1997). Such fluctuation in water level effects different degrees of forest disturbance along riverbanks and floodplains (Salo *et al*. 1986). There is little information on the dispersal capabilities and migratory movements of river-island specialists (Rosenberg 1990), or about the ecological and biogeographical mechanisms that shape their distributions. For this reason, we have developed species-specific polymorphic genetic markers that will help to estimate levels of gene flow and population genetic structure and, to test the role of the flood pulse acting as a barrier in the dispersal of individuals of this species.

Blood samples (20–50 μL each) from a total of 45 individuals were obtained by puncture of the brachial vein and mixed immediately with 500–700 μL of lysis buffer (100 mM Tris pH 8.0, 100 mM EDTA, 10 mM NaCl, 0.5% SDS; Longmire *et al*. 1988). Genomic DNA was extracted using phenol–chloroform procedure or dialysis in 1× TEN buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA, 100 mM NaCl; Sambrook & Russell 2001). Microsatellite loci were isolated from a single female individual following the protocol of Ciofi & Bruford (1998) with 5′-end biotinylated oligonucleotide probes $(CA)_{12}$, $(CA)_{14}$, $(CT)_{12}$ and $(GATA)_7$ in a 50 μL hybridization reaction volume under polymerase chain reaction (PCR) conditions (Lieckfeldt *et al*. 2001). The hybridization procedure followed the stepping-down program described in Williams & Dewoody (2004). The hybridization products were incubated in 100 μL of 0.5× SSC with streptavidin-coated paramagnetic beads (Promega) for 20 min at room temperature to allow binding of biotinylated probes to the avidin matrix. Unbound particles were selectively washed out four times with 300 μL 0.1× SSC for 15 min, twice at room temperature and at 45 °C and 60 °C to increase stringency. Recovered hybridized DNA was amplified by PCR in a 25-μL reaction using SAULA as primer (Ciofi & Bruford 1998). PCR products were cloned using pCR4-TOPO vector and transformed into *Escherichia coli* one-shot chemically competent cells (TOPO TA Cloning Kit, Invitrogen). Colonies were selected and amplified using universal M13 primers. Positive clones were directly sequenced with a fluorescent BigDye Terminator Kit (ABI) in an ABI 3130xl Genetic Analyser.

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Table 1 Characterization of four microsatellite loci developed for Castelnau's antshrike *Thamnophilus cryptoleucus* (n = 45). Expected (H_E) and observed (H_O) heterozygosities have been calculated using TFPGA (Miller 2005)

Locus	Repeat motif	Accession no.	Primer sequence (5′–3′)	T_a (°C)	Cloned allele size (bp)	Allele size range	No. of alleles	H_O	H_E
ThamnoMic A11	$(CW)_{43}$	DQ 202210	F: 6-FAM — AAGAAAATTGTGGAGGCATGTCTGC R: ACCTTAGGGACTGGAGAGGCAC	59	304	274–306	9	0.753	0.775
ThamnoMic D07	$(CA)_{14}$	DQ 202207	F: 6-FAM — GCCATGCTGGCTGCATCCTTGTG R: CAGAGAGTTCGGTTCTGAAGCAATG	61	187	181–191	6	0.632	0.596
ThamnoMic D09	$(GAGT)_{11}$	DQ 202206	F: HEX — ACAGTTAATGTGACTCCCCTAG R: CACAGGGAACTGTCAACAAGC	55	202	190–230	8	0.796	0.778
ThamnoMic G03	$(GGAT)_{10}(AGAY)_3$ $(GGAT)_{11}$	DQ 202208	F: 6-FAM — TGAAAGTGTTGTCTAAATGCTACC R: ATCTCTCAATCTCCCCTCAGAC	60	350	302–370	11	0.889	0.848

Table 2 Results obtained from cross-species amplification tests on other species of antbirds of the family Thamnophilidae

Species	ThamnoMic A11	ThamnoMic D07	ThamnoMic D09	ThamnoMic G03
Thamnomanes ardesiacus	+	+/−	+	+
Thamnomanes caesius	+	−	+	+
Thamnophilus schistaceus	+	+	+	+
Thamnophilus murinus	+	−	+	+
Thamnophilus doliatus	+	+	+	+
Myrmoborus lugubris	+	+	+	+
Myrmoborus myotherinus	+	+	+	+
Myrmochanes hemileuchus	+	+	−	+

+, amplification signal and presence of corresponding microsatellite loci determined by sequencing analysis; +/−, amplification signal observed but requires further optimization; – no amplification signal.

Primer pairs were designed for 13 microsatellite sequences that contained sufficient flanking regions using GENEFISHER version 1.3 (Giegerich *et al*. 1996). An initial screening of eight to 12 individuals was carried out to test for microsatellite amplification and polymorphism. PCR volumes were of 25 μL containing 50–100 ng DNA, 1.5 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 200 μM of each dNTP, 10 pmol of each primer and 0.5 U of Ampli*Taq* DNA polymerase (ABI) at the following conditions: initial denaturation 94 °C for 3 min; 35 cycles at 94 °C 15 s, 55–61 °C 20 s, 72 °C 45 s and a final extension at 72 °C for 10 min. Forward primers of five polymorphic loci were labelled with fluorescent dye 6-FAM or HEX, and PCR was performed as described above except for a 30-min extension at 72 °C. Microsatellite alleles were sized using the ABI 3130XL Genetic Analyser with GENEMAPPER version 3.7 software (ABI). Allelic variability was assessed in 45 individuals from the Napo River, Ecuador (Table 1). Exact tests were run using TFPGA (Miller 2005) and individual populations were found in Hardy–Weinberg equilibrium for all alleles (P values from 0.086 to 0.748). Genotypic disequilibrium comparisons across microsatellite loci using the same program revealed that these loci were not in linkage disequilibrium (P values from 0.155 to 0.905). Cross-species amplification of the loci was tested in eight species of the family Thamnophilidae and all loci successfully amplified in five or more related species (Table 2), for which no other microsatellite markers had been previously published to our knowledge. The present polymorphic microsatellite loci will be useful in other applications in *Thamnophilus cryptoleucus* and other Amazonian antshrikes including characterization of population structure and migratory movements.

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References

Ciofi C, Bruford MW (1998) Isolation and characterization of microsatellite loci in the Komodo dragon *Varanus komodoensis*. *Molecular Ecology*, **7**, 133–140.

Giegerich R, Meyer F, Schlelermacher C (1996) GeneFisher-software Support for the detection of postulated genes. *Proc. Int. Conf. Intell. Syst. m.l. Biol.*, **4**, 68–77.

Irion G (1997) The large central Amazonian river floodplains near Manaus: geological, climatological, hydrological and geomorphological aspects. In: *The Central Amazon Floodplain: Ecology of a Pulsing System* (ed. Junk W). Springer-Verlag, Berlin, Germany.

Lieckfeldt D, Schmidt A, Pitra C (2001) Isolation and characterization of microsatellite loci in the Great bustard, *Otis tarda*. *Molecular Ecology Notes*, **1**, 133–134.

Longmire JL, Lewis AK, Brown NC *et al.* (1988) Isolation and molecular characterization of a highly polymorphic centromeric tandem repeat in the family Falconidae. *Genomics*, **2**, 14–24.

Miller M (2005) *TFPGA (tools for population genetic analysis)*. Available at http://www.marksgeneticsoftware.net.

Rosenberg GH (1990) Habitat specialization and foraging behavior by birds of Amazonian river islands in northeastern Peru. *Condor*, **92**, 427–443.

Salo J, Kalliola R, Hakkinen I, Makinen Y, Niemela P, Puhakka Coley PD (1986) River dynamics and the diversity of Amazon lowland forest. *Nature*, **322**, 254–258.

Sambrook J, Russell DW (2001) *Molecular Cloning*, 3rd edn, p. A1.22. Cold Spring Harbour Laboratory Press, New York.

Williams R, Dewoody JA (2004) Fluorescent dUTP helps characterize 10 novel tetranucleotide microsatellites from an enriched salamander (*Ambystoma texanum*) genomic library. *Molecular Ecology Notes*, **4**, 17–19.

Zimmer KJ, Isler ML (2003) Thamnophilidae (typical antbirds). In: *Handbook of the Birds of the World* (eds del Hoyo J, Elliott A, Christie DA), Vol. 8. Lynx Ediciones, Barcelona, Spain.