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PRIMER NOTE

Isolation and characterization of microsatellite loci in the blue manakin, *Chiroxiphia caudata* (Aves, Pipridae)

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Abstract

We designed primers for amplifying 11 polymorphic microsatellite loci in the blue manakin, *Chiroxiphia caudata*, a neotropical passerine bird which inhabits a critically endangered tropical ecosystem, the Brazilian Atlantic forest. Based on genotypes from 24 individuals from a single population, we detected between four and 22 alleles per locus with observed heterozygosities ranging from 0.54 to 0.92. These highly variable loci will be useful for determining levels of population differentiation and assessing the impact of habitat fragmentation on levels of genetic variation in isolated populations of these birds.

Keywords: Brazilian Atlantic coast forest, *Chiroxiphia caudata*, manakins, microsatellites, Pipridae

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Determining the genetic structure of populations is essential for testing hypotheses about factors promoting diversification and speciation (McDonald 2003) as well as for finding appropriate scales for conservation and management (Cegelski *et al*. 2003). Neotropical passerine birds that inhabit forest understorey habitats are thought to be highly sedentary, which may result in greater genetic differentiation among populations than in temperate birds (Bates 2000). However, studies addressing the genetic structure of neotropical birds are limited (Bates 2000; Höglund & Shorey 2003; McDonald 2003) and little is known about whether genetic diversity has been lost in specific populations due to the effects of habitat fragmentation. The manakins are small highly phylopatric frugivorous birds which are common in the understorey habitats of neotropical forests making them excellent model species for studies of the conservation and population biology of tropical birds (Blake & Loiselle 2002). While microsatellite loci have been isolated for a few manakin species (McDonald & Potts 1994; Piertney *et al*. 2002), the availability of loci for other species in this diverse group is limited. Here we characterize 11 polymorphic microsatellite loci for the blue manakin, *Chiroxiphia caudata*, a species widely distributed in a critically endangered South American ecosystem, the Brazilian Atlantic forest.

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To obtain microsatellite loci for this bird, we generated a plasmid library containing genomic inserts that had been enriched for eight tetranucleotide repeats via a hybridization capture procedure developed by T. Glenn (Savanna River Ecology Laboratory, University of Georgia, USA), which is a modification of the protocol presented by Hamilton *et al*. (1999). Clones containing inserts were sequenced, with no additional screening, using a BigDye sequencing kit and products resolved on an ABI 3100 automated sequencer. Primers were then designed based on the sequence flanking the microsatellite repeat in each clone using the software PRIMER3 (Rozen & Skaletsky 2000).

Variation at these loci was then analysed by PCR for up to 24 individuals from a single population located in Parque Estadual da Serra do Mar, southeastern Brazil (23°32′ S, 45°20′ W). DNA extractions from blood samples followed the method of Debomoy & Nurnberger (1991). PCR reactions were performed using a PTC-200 (MJ Research) thermal cycler in a 10 μL volume containing 150 ng of DNA, 0.2 mM of dNTPs, 1 × PCR buffer (200 mM Tris-HCl, pH 8.4, and 500 mM KCl; Invitrogen Life Technologies), 0.2 μM of each primer, 3 mM $MgCl_2$ and 1 U *Taq* polymerase. The thermal cycler was programmed for an initial denaturing step at 94 °C for 5 min followed by 35 cycles consisting of 30 s at 94 °C, 30 s at the annealing temperature specified in Table 1 and 30 s at 72 °C followed by a 10 min final extension at 72 °C. Amplification products

Table 1 Primer sequences, repeat motif, optimal annealing temperature (T_a), clone size, number of alleles and number of individuals analysed (n) and observed (H_O) and expected (H_E) heterozygosities for 11 microsatellite loci isolated from the blue manakin, *Chiroxiphia caudata* (Aves, Pipridae)

Locus	Primer sequence (5′–3′)	Motif	T_a (°C)	Clone size (bp)	No. of alleles (n)	H_O	H_E
CHIR1-6	ATACTAAATGCGTTACCAAGC ATCTTGGGTTAACATAACCTG	$(CTTT)_3TT(CTTT)_{12}$ $CTGT(CTTT)_2CTGT$ $(CTTT)_2(CTGT)_{12}$	57.8	261	22 (22)	0.90	0.96
CHIR1-16	AGAGCCACCTAGTTAAACTGC TTAGATCACCTGGTGTATGTTC	$(GATA)_2AATA(GATA)_{11}$ $GACA(GATA)_2$	50.0	161	10 (24)	0.75	0.89
CHIR1-18	TTACCTAGAAGCAGAGGTAG ACTCTGATGAAATGGTATGC	$(CA)_{14}$	45.0	164	15 (24)	0.92	0.89
CHIR2-9	CACTCTTACTACTCAACATATTC ACTGCTAAGAGAGAATACCAG	$(CA)_6CG(CA)_{14}$	51.0	141	15 (24)	0.79	0.93
CHIR3-2	TTTCACCTTGGGAACAGCTC AATCACAGGCAAGCCTTCAG	$(AC)_{10}$	55.0	159	4 (24)	0.62	0.70
CHIR3-15	AGTAAGATAGTAGTGGGAATCG TACTGCTACATGCTACAGACC	$(CAA)_{10}$	49.3	156	10 (24)	0.83	0.88
CHIR3-22	TGAAGTCCAGAGACAACAG GAACTAATGCAACTTCTGAG	$(GT)_3GCAC(GT)_{16}$	52.4	167	7 (24)	0.58	0.76
CHIR3-27	TGCATGAATGGTGAAAGATGTC GCAGAATCACCCATAAGAGACTG	$(CA)_{13}$	51.0	226	11 (24)	0.75	0.75
CHIR4-21	TCATCTGAACAGTAGAGCTTTCTC CTTATCAACATTCTTCTCCCATAG	$(CAAA)_7$	49.3	186	4 (24)	0.62	0.58
CHIR4-33	AAAGCTGTGAACACGTAACTGC AATCTCCACTTTGTGGGACAG	$(CT)_7(CA)_{14}(TA)_3(CA)_4$	49.3	153	10 (24)	0.92	0.86
CHIR4-34	TCTCATCACACACTCCCTTGAG TGTTCTTTCTTCCAGCTTCTGAC	$(CA)_{12}GACG(CA)_7$	49.3	217	8 (24)	0.54	0.83

were resolved on 7.0% polyacrylamide gels and visualized by silver staining (Comincini *et al*. 1995). Alleles were sized by comparison with a known size clone and 10-bp ladder (Invitrogen Life Technologies).

Of 106 clones analysed, 23 (22%) contained microsatellite motifs consisting of at least six repeats. Eleven of these loci (48%) were successfully amplified using the primers given in Table 1 and all showed high levels of polymorphism. The number of alleles detected per locus ranged from four to 22 while observed heterozygosities ranged from 0.54 to 0.92 (Table 1). Analyses using GENEPOP (version 3.4; Raymond & Rousset 1995) revealed no evidence for linkage disequilibrium between any two loci but significant ($P < 0.001$) heterozygote deficiencies at loci *CHIR2-9* and *CHIR4-34*, indicating the possible presence of null alleles.

We evaluated the applicability of these loci to other taxa in an attempt to amplify similar sized products in two other manakin species, *Manacus manacus* and *Ilicura militaris*, and one antbird, *Pyriglena leucoptera*, using the same DNA extraction protocol and PCR conditions. Amplification products of two individuals of each species were scored in 1% agarose gel and sized by comparison with a 100-bp ladder (Invitrogen Life Technologies). All loci amplified a band of approximately the same size as that present in *C. caudata*.

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