Molecular Ecology Notes (2005) **5**, 112–114 doi: 10.1111/j.1471-8286.2004.00856.x

PRIMER NOTE

Isolation and characterization of polymorphic microsatellite loci in the lance-tailed manakin (*Chiroxiphia lanceolata*)

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Abstract

Eight microsatellite loci were isolated from the lance-tailed manakin (*Chiroxiphia lanceolata*), a polygynous lek-breeding bird from Central America. Five of these loci were polymorphic (two to seven alleles per locus), with observed levels of heterozygosity ranging from 0.100 to 0.860 (n = 50 individuals). These variable loci provide a valuable tool for assessing patterns of parentage and relatedness within lance-tailed manakin social groups.

Keywords: *Chiroxiphia lanceolata*, cooperative courtship, manakin, microsatellite, paternity

Received 28 September 2004; revision accepted 15 October 2004

Lance-tailed manakins (*Chiroxiphia lanceolata*) are small (*ca*. 16–20 g), passerine birds in the family Pipridae that are found in Western Panama, Northern Columbia and Venezuela (Ridgely & Tudor 1994). Manakins in general are known for their elaborate lek-based courtship displays (Sick 1967). Manakins in the genus *Chiroxiphia* are unusual in that males form cooperative partnerships with other males to perform intricate courtship displays for females (Snow 1963; Foster 1977; Foster 1981). Cooperative courtship is unusual among vertebrates and hence studies of kinship and reproductive success in *Chiroxiphia* manakins provide an important opportunity to assess the adaptive bases for this type of social behaviour. Microsatellite markers were isolated and are being used to address patterns of relatedness and paternity that may affect the adaptive benefits of cooperative courtship behaviour in the lance-tailed manakin, a previously unstudied member of the *Chiroxiphia* genus.

Blood samples were collected from the brachial wing vein of 589 lance-tailed manakins captured in 1999–2004 on Isla Boca Brava, Chiriquí Province, Panamá (8°12′-N, 82°12′-W) as part of ongoing studies of the social behaviour of these animals. Blood was stored in Longmire's lysis buffer (Longmire *et al*. 1988), and genomic DNA was later extracted from tissue using the Dneasy™ Tissue Kit (QIAGEN). A genomic library was constructed using the protocol of Hamilton *et al*. (1999) with the modifications described by Nutt (2003). In short, genomic DNA was digested with restriction enzymes *Nhe*I and *Xmn*I (New England Biolabs, Inc.), ligated to universal SNX linkers, and enriched by hybridization with a 5′ biotinylated $(AC)_{15}$ repeat (Integrated DNA Technologies, Inc.) followed by isolation using streptavidin-coated magnetic beads. Polymerase chain reaction (PCR) amplification of positive bacterial colonies was conducted in a 25 μL reaction with 1.0 μL each of T7 and T3 primers (10 mM) and 2.8 mM $MgCl_2$ at an annealing temperature of 53 °C. All amplifications were performed on either a PTC-200 or PTC-100 thermal cycler (MJ Research). PCR products of approximately 400 bp or more were sequenced using the T3 and T7 primers and visualized on an ABI Prism™ 377 DNA sequencer.

Locus-specific PCR primers were designed for 20 sequences that contained a microsatellite using PRIMER 3 (Rozen & Skaletsky 2000). To genotype individuals, 5–20 ng of genomic DNA were PCR-amplified in a 10 μL reaction that included: 0.5 U *Taq* DNA polymerase (Invitrogen); 1 μL *Taq* DNA polymerase PCR reaction buffer (10 mM Tris-HCl, 1.5 mM $MgCl_2$, 50 mM KCl, pH 8.3); 0.2 μL of each primer (10 μM) and 0.2 μL dNTPs (40 mM). Samples were denatured for 5 min at 94 °C, then cycled 32 times for 30 s at 94 °C, 30 s at T_a (Table 1) and 45 s at 72 °C, followed by a final extension of 20 min at 72 °C. Eight of the isolated primer pairs produced well-resolved PCR products when visualized on 1.5% agarose gels (1× TBE) stained with ethidium bromide. Seven of the amplified loci were simple AC repeats, and one comprised two AC repeat regions separated by three nonrepeated nucleotides. Variability was

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Table 1 Microsatellite loci isolated from lance-tailed manakins (*Chiroxiphia lanceolata*). Data on allelic variability and heterozygosity are based on a sample of 50 lance-tailed manakins from Isla Boca Brava, Panamá

Locus name	Primer sequences (5′–3′)	T_a* (°C)	Repeat motif†	No. of alleles	Size range of alleles (bp)	H_E	H_O‡
Lan1	L – AGG TGC CAA CCC AGT GCT R – TGG GAG GAA CCT GAA CTC AA	54	$(CA)_{12}$	1	168	0	0
Lan10	L – GCA TGA ACA CAA TTG ATG AAG C R – GAG AAA GCT GGA GGT CAA GG	54	$(CA)_{17}$	5	195–205	0.717	0.660
Lan12	L – TTG TCA GCT GTA GTT CTG TTT TAG C R – TTT GAG GTC AGT TTC AAC TAT TGG	54	$(CA)_7$	1	160	0	0
Lan15	L – CAG AGG ACT GAA AAT GAT ATG TGG R – CAC TGC GTT ATC ACT GAA TTT AGA C	50	$(CA)_{10}$	2	204–208	0.132	0.100
Lan19	L – TGT ATT CTC ACG TAA ACC GAC TC R – CTC AAA CAC CCC AAA CAC C	52	$(CA)_{11}ATA(CA)_6$	NM	~290	NM	NM
Lan20	L – TCC CAG GAC ACC ACC AAG R – GAG CAG ATT TTG AGA CCA AGG	54	$(CA)_{25}$	5	106–124	0.748	0.740
Lan21	L – AAG GAT GTG GGC TCA GGT TA R – TTC AGT TCT TTG ATA TCT CTA AGT CAA	52	$(CA)_{14}$	5	199–215	0.751	0.860
Lan22	L – GCT TCT GGG TGC TCT GTC C R – CCT GCT TTC AGC TCT TCT GC	55	$(CA)_{18}$	7	146–167	0.526	0.520

*T_a = annealing temperature; †H_E = expected heterozygosity; ‡H_O = observed heterozygosity; NM = not measured, size estimated from 2% Metaphor gel with 100 bp ladder. GenBank accession numbers = AY752868–AY752875.

tested using 50 adult individuals. Flourescently labelled PCR products were visualized on either an ABI Prism™ 377 DNA sequencer using GeneScan® with alleles scored using Genotyper (Perkin Elmer Corp.) or on an Applied Biosystems 3730 DNA analyser with alleles scored using Genemapper (Applied Biosystems). Individual loci were analysed entirely on one type of machine to achieve consistent allele calls. One locus, Lan19, was screened using unlabelled PCR products that were separated on a 2% MetaPhor Agarose gel (Cambrex Bio Science) and visualized with ethidium bromide. This technique proved effective in identifying variable and monormophic loci for Lan1, Lan10, Lan12, Lan15, and Lan20, and revealed no apparent variability at this locus. Because definitive genotyping with fluorescent primers was not conducted, we report no data on variability for this locus.

Two of the fluorescently screened loci were monomorphic, and the remaining five loci were characterized by two to seven alleles each (Table 1). GENEPOP3.3 (Raymond & Rousset 1995) was used to calculate heterozygosities, adherence to Hardy–Weinberg equilibrium (HWE), and probability of linkage disequilibrium in variable loci for 50 randomly selected adults from the study population. None of the loci showed significant deviation from HWE or from random association (i.e. there was no evidence of linkage disequilibrium). Observed heterozygosities for the polymorphic loci ranged from 0.10 to 0.860, and all but one locus had values of observed heterozygosity (H_O) > 0.500. These are the first molecular markers isolated for *C. lanceolata*. The five polymorphic microsatellite loci reported here are currently being used in combination with loci previously isolated in other species to quantify patterns of kinship and reproductive success in lance-tailed manakins with the goal of better understanding the adaptive bases of cooperative courtship behaviour in this species.

Acknowledgements

This research was supported by grants to EHD from the National Science Foundation (DDIG #0104961); UC Berkeley Museum of Vertebrate Zoology, Department of Integrative Biology, and the Vice Chancellor for Research Fund; Animal Behaviour Society; American Ornithological Union; American Museum of Natural History; and Sigma Delta Epsilon Graduate Women in Science. Eileen Lacey and Beth Slikas provided valuable advice and assistance, as did many diligent field assistants.

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