PRIMER NOTE

# Isolation and characterization of novel microsatellite loci for parentage assessment in the lance-tailed manakin (*Chiroxiphia lanceolata*)

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## Abstract

**The lance-tailed manakin (*Chiroxiphia lanceolata*) is a lek-breeding bird from Central America in which males court females with complex cooperative displays. To resolve detailed patterns of paternity in the wild, we isolated and characterized 12 novel microsatellite loci in this species. Eleven of these loci were polymorphic (five to 14 alleles), with observed heterozygosities ranging from 0.36 to 0.87 (*N* = 574 individuals). We tested for linkage disequilibrium using randomized subsamples of adults to control for known family structure among long-lived and sedentary individuals. These loci will be valuable in resolving paternity among many candidate fathers in this species.**

*Keywords*: *Chiroxiphia lanceolata*, cooperation, lance-tailed manakin, microsatellite, paternity, relatedness

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The lance-tailed manakin, *Chiroxiphia lanceolata*, is a small passerine bird found in the lowlands of western Panama, Columbia and Venezuela. Males of this species form long-term cooperative partnerships and display together to attract females in an exploded lek mating system (DuVal 2007b; DuVal in press). Cooperating pairs are not closely related, and previous paternity analyses demonstrated that the subordinate 'beta' partners in alliances rarely if ever sire chicks while they hold beta status (DuVal 2007a). However, the nine microsatellite loci employed in previous analyses lack the variability required to resolve the paternity of many chicks (DuVal 2007a). To investigate detailed patterns of reproductive success and female mate choice, we had 11 novel, polymorphic microsatellite markers developed for parentage analysis.

Blood samples were collected from 255 males and 319 females caught with mistnets on a long-term study site on Isla Boca Brava, Panama (8°12′N, 82°12′W; DuVal in press). Samples were stored in Longmire's or Queen's lysis buffer (0.01 M Tris, 0.01 M NaCl, 0.01 M Na-EDTA, 1% n-Lauroylsarcosine; adjusted to pH 8.0). Genomic DNA was extracted using the GFX Genomic Blood DNA Purification Kit from GE Healthcare or the DNeasy Tissue Kit (QIAGEN, Inc.). An enriched library was made by Ecogenics GmbH from size-selected genomic DNA of two individuals, ligated into *Sau*LA/*Sau*LB linker (Armour *et al*. 1994) and enriched by magnetic bead selection with biotin-labelled $(CA)_{13}$ and $(GATA)_7$ oligonucleotide repeats (Gautschi *et al*. 2000a; Gautschi *et al*. 2000b). Of 380 recombinant colonies screened, 151 gave a positive signal after hybridization. Plasmids from 68 positive clones were sequenced and primers were designed for 13 microsatellite inserts. Of these, 12 were tested for polymorphism. One tested locus was monomorphic in 10 initially screened individuals and was therefore not screened further [Chiro6: repeat motif $(TATC)_7$, 129 bp; GenBank Accession no. EF105320; 5′–3′ primer sequence, F: TTATAGGTATGTGTGTTGCCTACC, R: GCAGCCTATTACATGGGAAAC].

We optimized the polymerase chain reaction (PCR) conditions to perform multiplex PCRs using the Multiplex PCR kit from QIAGEN. Forward primers were labelled on the 5′ end with fluorescent dyes from Applied Biosystems. Each PCR was performed with a final volume of 10 μL, including 5–20 ng of genomic DNA, four primer pairs

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**Table 1** Characterization of 11 polymorphic microsatellite loci for *Chiroxiphia lanceolata* ($n$ = 574 individuals genotyped)

| Locus | Repeat motif based on sequence clone | Primer sequence (5′–3′) | No. of alleles | Size range (bp) | $H_O$ | $H_E$ | GenBank Accession no. |
|---|---|---|---|---|---|---|---|
| Chiro1 | $(GT)_{21}ATGC(AT)_4GT(AT)_5$ | F: AGGGCAGCTACTGGACAGAC<br>R: ATGGCCTCATCCTGTTCATC | 14 | 169–209 | 0.88 | 0.87 | EF105315 |
| Chiro2 | $(GATA)_3GACA(GATA)_{13}$ | F: GAAAAATAACATGAGAATCTAGC<br>R: AAAACCTCAATAAAGCCTTACC | 10 | 108–144 | 0.80 | 0.79 | EF105316 |
| Chiro3 | $(GATA)_3GAT(GATA)_6(GACA)_4$ | F: GACAGGCAGTAGACTGGGAAAC<br>R: GATTTGTGTGTCCTGACTTTCC | 8 | 154–186 | 0.57 | 0.56 | EF105317 |
| Chiro4 | $(CTAT)_{11}$ | F: ACACTGGCTACACCAGTCAATC<br>R: ATTGCATTCTTCACTGCTCAAG | 5 | 169–185 | 0.61 | 0.61 | EF105318 |
| Chiro5 | $(CTAT)_{13}$ | F: TGAGAGGCAGAAAATATTCCAG<br>R: GATTAGATCTTCATCCTGAGAGT | 7 | 146–170 | 0.74 | 0.73 | EF105319 |
| Chiro7 | $CTAT\ GTAT(CTAT)_4$ $GTAT(CTAT)_{13}$ | F: TCACCCTTTCAGTTCTTTCTCC<br>R: CACTGTCACATGCGTTTCATTAG | 13 | 231–279 | 0.83 | 0.80 | EF105321 |
| Chiro8 | $(ATCT)_{11}$ | F: TTGTACAGACAATCACATCTACCTC<br>R: TGACAATGTGGGTGTATGCAG | 7 | 139–163 | 0.70 | 0.70 | EF105322 |
| Chiro9 | $(CTAT)_{13}$ | F: AGGCCCAAAGACTTGAATATG<br>R: CAGAAATGGTCTTGCATGAGTC | 5 | 239–255 | 0.55 | 0.56 | EF105323 |
| Chiro10 | $(CTAT)_{14}$ | F: GGAGGGATAGTGGGCAAGAT<br>R: CTTTCTGGAGTTCTTTCCATGC | 7 | 203–239 | 0.57 | 0.57 | EF105324 |
| Chiro11 | $(GATA)_6\ GATC(GATA)_5$ $GAT(GATA)_3$ | F: ACAGCAAATGACTGTCAAGAGG<br>R: TGCACTAGCTGATGCTTACTGA | 5 | 162–178 | 0.35 | 0.36 | EF105325 |
| Chiro12 | $(GATA)_{12}$ | F: TGTCCCTTATTTGCCTTAGTGC<br>R: GCTTCACTTGCAATACATGTCTATCT | 10 | 116–158 | 0.71 | 0.74 | EF105326 |

F, forward primer; R, reverse primer; $H_O$, observed heterozygosity; $H_E$, expected heterozygosity. Optimal annealing temperature for all loci was 56 °C.

(0.04–0.12 μM of each primer, adjusted for differences in dye strength), and 5 μL of Multiplex PCR master mix (QIAGEN). PCRs were carried out on a GeneAmp 2700 PCR System (Applied Biosystems) with the following parameters: initial denaturation at 95 °C for 15 min followed by 30 cycles at 94 °C for 30 s, 56 °C for 90 s, and then elongation at 72 °C for 60 s. After the last cycle, elongation at 72 °C was prolonged to 30 min. PCR products were resolved on an ABI 3100 Genetic Analyser and analysed with GENEMAPPER 3.7.

We genotyped all 574 individuals at 11 variable loci (Table 1). Expected and observed heterozygosities were calculated using CERVUS 2.0 (Marshall *et al*. 1998). Exact tests for departure from Hardy–Weinberg equilibrium were performed using GENEPOP on the web (http://wbiomed. curtin.edu.au/genepop/; Raymond & Rousset 1995). None of the markers deviated significantly from Hardy–Weinberg equilibrium (Fisher's method, all $P > 0.2$). We found no evidence of null alleles, assessed by comparison of known mother–offspring pairs ($N$ = 435 chicks of 140 unique females). However, locus Chiro2 showed markedly decreased amplification of one allele in 10.7% of all genotyped individuals, suggesting mutation at the primer binding site and requiring particular care in analysis.

The 11 polymorphic microsatellites reported here have a combined probability of paternity exclusion when one parent is known of Pe (2nd parent) = 0.999236. When combined with nine additional loci previously used in this species (DuVal 2007a), Pe (2nd parent) = 0.999986. Because lance-tailed manakins are long-lived (> 11 years, E.H.D., unpublished data) and resident on the study site, we assume that the study population consists of many overlapping generations and closely related individuals. Under these conditions, calculating linkage disequilibrium using all sampled adults is very likely to overestimate associations between markers. Therefore, we randomly subsampled 100 sets of 50 adult individuals each to test for consistent linkage disequilibrium between marker pairs after correction for multiple comparisons. None of the loci pairs were identified as linked in more than 4% of randomization tests. Thus, we conclude that the markers are segregating independently. Furthermore, we performed a GenBank BLAST analysis and found that none of the loci reported here shared more than 13 alligned base pairs with sequences previously identified in this species (DuVal & Nutt 2005) or in other piprids, confirming the uniqueness of these loci. The combined set of 20 microsatellite loci are therefore highly suitable for determining paternity in the lance-tailed manakin.

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## References

Armour JA, Neumann R, Gobert S, Jeffreys AJ (1994) Isolation of human simple repeat loci by hybridization selection. *Human Molecular Genetics*, **3**, 599–605.

DuVal EH (2007a) Adaptive advantages of cooperative courtship for subordinate male lance-tailed manakins. *The American Naturalist*, **169**, 423–432.

DuVal EH (2007b) Social organization and variation in cooperative alliances among male lance-tailed manakins. *Animal Behaviour*, **73**, 391–401.

DuVal EH (in press) Cooperative display and lekking behavior of the lance-tailed manakin (*Chiroxiphia lanceolata*). *Auk*, in press.

DuVal EH, Nutt KJ (2005) Isolation and characterization of polymorphic microsatellite loci in the lance-tailed manakin (*Chiroxiphia lanceolata*). *Molecular Ecology Notes*, **5**, 112.

Gautschi B, Tenzer I, Müller JP, Schmid B (2000a) Isolation and characterization of microsatellite loci in the bearded vulture (*Gypaetus barbatus*) and cross-amplification in three Old World vulture species. *Molecular Ecology*, **9**, 2193–2195.

Gautschi B, Widmer A, Koella J (2000b) Isolation and characterization of microsatellite loci in the dice snake (*Natrix tessellata*). *Molecular Ecology*, **9**, 2191–2193.

Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology*, **7**, 639–655.

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

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