

PRIMER NOTE

Characterization of polymorphic microsatellite loci in a neotropical wood-quail, *Odontophorus leucolaemus*

AMANDA M. HALE and COLIN R. HUGHES

Department of Biology, University of Miami, PO Box 249118, Coral Gables, FL 33124, USA

Abstract

We developed a set of nine polymorphic microsatellite loci for black-breasted wood-quail, *Odontophorus leucolaemus*. We screened 50 individuals from Monteverde, Puntarenas Province, Costa Rica and found that locus-specific allelic diversity ranges from two to 15 alleles (mean 10.2) and observed heterozygosity ranges from 0.24 to 0.96 (mean 0.78). These markers appear to be useful in other members of the *Odontophorus* genus.

Keywords: cross-amplification, microsatellites, New World quail, Odontophoridae, primers

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We developed nine polymorphic microsatellite loci for black-breasted wood-quail (*Odontophorus leucolaemus*), a little known species restricted to the remaining highland forest of Costa Rica and western Panama. This species is a cloud forest specialist that lives in groups of two to 15 defending year-round territories. Deforestation has reduced species-wide population size and fragmented the remnant populations. Indeed, six of the 15 species of New World wood-quail are classified as 'threatened' by the IUCN – World Conservation Union because of habitat loss and hunting (Carroll 1994; Fuller *et al.* 2000). The aim of this study was to isolate microsatellite markers that could be used to describe the social structure and genetic mating system, two behavioural attributes that have been directly linked to reduced persistence of populations in fragmented landscapes (reviewed in Caro 1999; Reed 1999). We also tested the potential utility of these primers in six additional species representing five genera of New World quail.

DNA was extracted from blood using a standard phenol–chloroform extraction. Genomic DNA was digested with *DpnII* and fragments 350–700 bp in length were cloned into Lambda Zap Express (Stratagene) (Hughes & Moralez DeLoach 1997). We screened 150 000 clones with the oligo AAT₁₀ and sequenced 56 positives using ABI PRISM Big Dye™ Terminator Cycle Sequencing chemistry

v. 3.0 (PE Biosystems). Sequences were analysed on an ABI 310 Genetic Analyser (PE Biosystems). We designed primers for 11 clones that contained ≥ 8 uninterrupted microsatellite repeats using OLIGO® 4.04 (Rychlick 1992).

Polymerase chain reactions (10 µL) contained ~50 ng template DNA, 50 mM KCl, 10 mM Tris/Cl, pH 9.0, 1.5 mM MgCl₂, 0.1% Triton X-100, 200 µM of each dNTP, 0.125 U *Taq* DNA polymerase (Promega) and 0.5 µM of each primer. One primer of each pair was labelled with 5'-fluorescent tags for genotyping. Reactions were cycled using a Hybaid Touch Down thermal cycler using the 'simulated tube' function. Cycling parameters were 60 s at 92 °C, 30 cycles of 5 s at 92 °C, 5 s at annealing temperature and 10 s at 72 °C and finally 2 min at 72 °C. Allele size data were collected by an ABI 310 Genetic Analyser (PE Biosystems) and analysed by the GENESCAN® and GENOTYPER® software programs.

This procedure produced nine polymorphic microsatellite loci (Table 1). The mean observed heterozygosity (H_O), determined by a direct count of genotypes in 50 individuals, was 0.78 and the mean number of alleles per locus was 10.2. All loci were tested for Hardy–Weinberg equilibrium using GENEPOP version 3.3 (Raymond & Rousset 1995). After sequential Bonferroni correction, only one locus, OIAAT37, showed an excess of homozygotes ($P < 0.006$). The combined exclusionary power of the eight polymorphic loci (excluding OIAAT37) was high (nonrelative = 0.999, calculated by CERVUS 2.0, Marshall *et al.* 1998; single first-order relative = 0.934, Double *et al.* 1997).

We tested the utility of the primers in six other species of New World quail representing five additional genera

Table 1 Polymorphic AAT-repeat microsatellite loci developed for black-breasted wood-quail

Locus (GenBank Accession no.)	Primer sequences (5'–3')	Predicted length (bp)	Repeat in original clone	Annealing temp. (°C)	No. of alleles	Size range (bp)	H_O	H_E
OIAAT2 (AY180203)	F: ATGGGAAGGGGTAAATTAGTAA R*: CCCACAGTATTAATTTGATTATA	91	(AAT) ₁₆	50	8	67–90	0.82	0.82
OIAAT20 (AY180204)	F: GTTCCACACGTTAGCAGTTATGT R*: GAGCGTGTPTTAATGTTGGTAGT	142	(ATT) ₁₁ (TTA) ₉	55	6	112–141	0.78	0.78
OIAAT34 (AY180205)	F*: GATGCCCTGCTGCTCACACT R: GGGGGTGTTCCTGGAGAGA	153	(TTTTTA) ₇ (TTTTA) ₃	55	15	116–196	0.96	0.92
OIAAT37 (AY180206)	F*: CTTTCGCTTTTTTATTATTATTG R: AGCATCCCTAACCCCTCAAG	110	(AAT) ₉	50	2	104–110	0.24	0.42
OIAAT74 (AY180207)	F*: TTTCTTTTTCTACTGTTTACTC R: GATCCTGATTTGTTATCGTTAC	166	(TTTTA) ₁₄	50	12	144–201	0.80	0.81
OIAAT85 (AY180208)	F*: CCTGCTGCTCACACTGACTG R: GAGCTGGGGTTGGTATGTT	173	(AAAAA) ₉	60	13	157–216	0.84	0.90
OIAAT96 (AY180209)	F*: AAGTCGTAACATTTTGGTAAGTAGT R: GCCAGTAAAAAAGGACTCTGT	174	(TAAAA) ₁₆	60	15	155–218	0.84	0.91
OIAAT108 (AY180210)	F*: AGGGGAAGAGAGAAGGAGGT R: AGCTTGAAAAACAAGGAGATC	175	(TAAAA) ₁₆	55	11	146–200	0.90	0.86
OIAAT128 (AY180211)	F*: AAATGAGCTGGAAGATTGAC R: TACCGACAGCATGTCTGTAG	201	(AAT) ₂₃	55	10	148–202	0.86	0.80

*Labelled primer.

The predicted length is based on the sequence of the original clone. H_O , proportion of heterozygotes in a sample of 50 presumed unrelated individuals; H_E , expected heterozygosity (calculated with GENEPOP version 3.3; Raymond & Rousset 1995).

Table 2 Cross-species amplification using the primers developed for black-breasted wood-quail

Locus	Species (Accession nos*)					
	<i>Odontophorus erythropus</i> (1412, 7868)	<i>O. stellatus</i> (9314, 11128)	<i>Callipepla californica</i> (29626, 30848)	<i>Oreortyx pictus</i> (6421, 6422)	<i>Colinus virginianus</i> (3283, 3713)	<i>Cyrtonyx montezumae</i> (21821, 23462)
OIAAT2	P	P	—	—	—	—
OIAAT20	M	M	—	M	—	M
OIAAT34	?	—	—	?	?	?
OIAAT37	M	M	—	M	—	M
OIAAT74	P	P	—	—	—	—
OIAAT85	?	—	?	?	?	?
OIAAT96	—	—	—	—	—	—
OIAAT108	P	P	—	P	—	P
OIAAT128	P	P	—	?	—	?

*Louisiana State University Museum of Natural Science Collection of Genetic Resources.

P, polymorphic in the two individuals tested; M, monomorphic; ?, amplification of products outside the size range; —, unsuccessful amplification.

(Table 2). Two individuals from each species were tested at an annealing temperature of 50 °C. Four loci were polymorphic in the other *Odontophorus* species and one locus was polymorphic in two additional genera (Table 2). These cross-species amplifications indicate that the primers will be useful for other studies within this genus of threatened birds. Furthermore, this genus seems distinct from other New World quail, which suggests that trying to adapt micro-

satellite markers developed in other genera (e.g. *Gallus* and *Tetrao*) for population-level studies would be inefficient.

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