

## PRIMER NOTE

# Characterization of microsatellites for parentage studies of white-throated magpie-jays (*Calocitta formosa*) and brown jays (*Cyanocorax morio*)

D. A. WILLIAMS,\* E. C. BERG,† A. M. HALE\* and C. R. HUGHES\*

\*Department of Biology, University of Miami, PO Box 249118, Coral Gables, FL 33124, USA, †Center for Animal Behavior, University of California, One Shields Avenue, Davis, CA 95616, USA

## Abstract

White-throated magpie-jays (*Calocitta formosa*) and brown jays (*Cyanocorax morio*) are cooperative breeders that live in complex groups composed of helpers and multiple male and female breeders. Behavioral observations and multilocus DNA fingerprinting have indicated that the social and genetic mating systems of these two species are diverse. Extra-group paternity appears to be common in both species, necessitating the use of single locus genetic methods to efficiently search for fathers. We therefore characterized 19 microsatellite loci for these two species (12 for magpie-jays and seven for brown jays) to aid in parentage studies.

**Keywords:** cooperative breeding, Corvidae, jays, microsatellites, primers

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White-throated magpie-jays (*Calocitta formosa*) and brown jays (*Cyanocorax morio*) have been the focus of long-term studies in Santa Rosa and Monteverde, Costa Rica, respectively (Lawton & Lawton 1985; Langen 1996; Berg 2004; Williams 2004). Both species live in territorial groups composed of helpers (individuals that provide care to young that are not their own) and multiple male and female breeders. Behavioral observations in both species and multilocus DNA fingerprinting in *Cyanocorax morio* have indicated that the social and genetic mating systems are diverse (Lawton & Lawton 1985; Langen 1996; Williams 2004). Extra-group paternity is common in *Cyanocorax morio* and is suspected to be common in *Calocitta formosa*. Searching for extra-group fathers is impractical using methods such as DNA fingerprinting because it is difficult to assign genotypes that can be compared consistently across gels. Single locus microsatellite loci are better suited for this task because it is possible to assign genotypes to individuals, eliminating cross-gel comparisons. We therefore developed microsatellite loci for use in genetic parentage analyses of these two jays.

Genomic DNA for library construction was extracted from blood preserved in lysis buffer (100 mM Tris pH 8.0,

100 mM EDTA, 10 mM NaCl, 0.5% sodium dodecyl sulfate, and 0.02% w/v Na azide) using a standard phenol–chloroform extraction. Microsatellite loci were isolated using the protocol of Hamilton *et al.* (1999). DNA was codigested with *AluI* and *NheI*, hybridized to biotinylated oligos [(AAAG)<sub>6</sub> (AATG)<sub>6</sub> (AAC)<sub>10</sub> (AAT)<sub>10</sub>] and captured with streptavidin Dynabeads (Dyna). The enriched fragments were cloned into the pBluescript II SK (+) vector and then transformed into XL-1 Blue MRF Supercompetent *Escherichia coli* (Stratagene). We used the three primer method of Gardner *et al.* (1999) to screen the resulting colonies for inserts containing microsatellite repeats. We screened 510 colonies for AAAG/AATG and found 144 (28%) positive clones, and 250 colonies for AAC/AAT and found 63 (25%) positive clones. We sequenced all positive clones using ABI Big Dye Terminator Cycle Sequencing version 1.0 chemistry (PE Biosystems). Sequences were analyzed on an ABI 310 Genetic Analyzer (PE Biosystems). We designed primers for 30 (19 for *Calocitta formosa* and 12 for *Cyanocorax morio*) clones that contained sufficient flanking sequence and ≥ 8 uninterrupted repeats using OLIGO® 4.04 (Rychlick 1992).

DNA for genotyping was extracted as above for library construction. PCR reactions (10 µL) contained 50 ng DNA, 50 mM KCl, 10 mM Tris-HCl pH 9.0, 1.5 mM MgCl<sub>2</sub>, 0.1% Triton X-100, 200 µM of each dNTP, 0.2 U *Taq* DNA polymerase

Correspondence: Dean Williams. Fax: +1 305 284 3039; E-mail: dwilliams642000@yahoo.com

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**Table 1** Polymorphic microsatellite loci developed for *Calocitta formosa* (Cf) and *Cyanocorax morio* (Cm).  $H_O$  is the observed proportion of heterozygotes, and  $H_E$  is the expected heterozygosity ( $n = 53$  individuals for *C. formosa* and 12 individuals for *C. morio*). Sequences of clones are in GenBank under accession numbers AY539727–AY539745

Name	Sequence (5'–3')	$T_a$ (°C)	Repeat in original clone	Size of original clone (bp)	Size range (bp)	No. of alleles	$H_O$ ( $H_E$ )
CfAAC42	F: *TTCCATGTTTCAGTTGGTTTGT R: AGCCCATCGTCAGGAGGA	55	(GTT) <sub>4</sub> (GTTT) <sub>6</sub>	102	86–113	4	0.31 (0.37)
CfAAT34	F: AGCAAACTAACTATAGGTATACTAACT R: *AGCAATAGGTAATATCACTAA	55	(ATT) <sub>2</sub> A(ATT) <sub>10</sub>	202	197–233	11	0.87 (0.83)
CfAAT47	F: GGAAGTTTGGGTTTATATACTAT R: *GCTGGCAAGGCTCAATA	55	(ATT) <sub>13</sub>	147	121–145	6	0.77 (0.73)
CfAAAG2†	F: AAAGCAACAAGTTGAAGATAGA R: CTTTGGGTTTTCATAGATT	55	(AAAG) <sub>12</sub> N <sub>2</sub> (AAAG) <sub>9</sub> N <sub>6</sub> (AAAG) <sub>10</sub>	213	160–245	6	0.18 (0.81)
CfAAAG8	F: *GGT TTTCTCCTATACTAGATTCTT R: CAACGAGACAGGAAATAAAAC	55	(CTTTTCTCTT) <sub>2</sub> CTTTTCTCTT (CTTTTCTCTT) <sub>5</sub> CTTTTCTCTT	219	182–299	12	0.85 (0.84)
CfAAAG10	F: CTGGGAAGGGGACAATCT R: *AAAGAAAATGTGTTTACAGAG	55	(AAAG) <sub>24</sub>	175	130–196	14	0.92 (0.91)
CfAAAG19	F: *TTTGCTTTT TTTTGCTTTTG R: TCCACCCTTCCTTCTCTCAC	50	(GTT) <sub>8</sub>	108	95–120	4	0.16 (0.54)
CfAAAG38	F: TGGCTGAAAATGCTTATG R: *CTGGTCTCTTCTACACACTG	55	(CTTT) <sub>19</sub>	167	108–204	16	0.76 (0.91)
CfAAAG64	F: *CCTCCTGGGTTTACTCACA R: TGCCAGACAACTTACAAAATC	55	(GAAT) <sub>8</sub>	142	136–148	4	0.64 (0.62)
CfAAAG87	F: GGGATTTTGAATCATACCAG R: *GAGGATTTGGGTGATAGTTAG	60	(AAGAAAG) <sub>15</sub>	173	130–236	11	0.77 (0.80)
CfAAAG116	F: *CCTGTTCAACTCTCTTTT R: CAGTGGTGTCAACCTAGATGT	60	(AAAG) <sub>11</sub> N <sub>2</sub> (AAAG) <sub>2</sub> N <sub>4</sub> (AAAG) <sub>2</sub>	182	170–197	8	0.87 (0.80)
CfAAAG130	F: TGGAAAGAAAGGAGAGAGA R: *AGGTGCAACTCTAAGATAAGATTAA	60	(AAAG) <sub>13</sub> N <sub>10</sub> (AAAG) <sub>5</sub> N <sub>2</sub> (AAAG) <sub>9</sub>	245	253–322	12	0.92 (0.86)
CmAAAG6	F: CTCCTGGACTGTCTTCATTC R: AGGCAGAGGCACAATTATAGAC	55	(CTTCTTTT) <sub>7</sub> N <sub>8</sub> (CCTT) <sub>13</sub>	219	190–300	10	1.0 (0.90)
CmAAAG11	F: TTGGGTTTTCATAGATTTTC R: *CCCTAAGTAATCTATAAGCAACA	60	(AAAG) <sub>6</sub> N <sub>4</sub> (AAAG) <sub>19</sub> N <sub>4</sub> (AAAG) <sub>3</sub>	212	170–200	3	0.14 (0.63)
CmAAAG14	F: ATGGGTTT TTTAGGTATATCTTCA R: GCTTGTCTCTTCAATTTTCATT	55	(AAAG) <sub>2</sub> N <sub>4</sub> (AAAG) <sub>17</sub> N <sub>4</sub> (AAAG) <sub>30</sub>	332	250–500	13	0.81 (0.94)
CmAAAG25	F: ACGCTATTTCTTGGTACTTCAT R: GCTCCCTAGACCCTGAAATAA	55	(CTTT) <sub>11</sub> N <sub>3</sub> (CTTT) <sub>17</sub>	265	250–500	13	0.83(0.93)
CmAAAG30	F: *GGATCTGTATTCATAGCATAAC R: AATGGTAGAAACAGGAATTAGTG	55	(AG) <sub>7</sub> N <sub>4</sub> (AAAG) <sub>22</sub>	228	200–300	13	0.92 (0.94)
CmAAAG34	F: GATGGAGATTTTGTAGTGTAGTTAG R: CCCATACCAGACCACCCAGAT	60	(CTTCTT) <sub>8</sub> N <sub>7</sub> (CTTCTT) <sub>2</sub>	131	100–200	10	0.83 (0.88)
CmAAAG35	F: AGCAGAGATGTATGTTTTGA R: TTCCATAATTTAGATCTGTATACT	55	(AAC) <sub>8</sub>	150	144–150	2	0.50 (0.39)

$T_a$ , annealing temperature.

†Screened with 11 individuals.

\*Primer labeled with fluorescent dye.

(Promega), and 0.5 μm of each primer. Reactions were cycled using a Thermo Hybaid PxE thermal cycler using the 'simulated tube' function. Cycling parameters were: one cycle at 92 °C for 2 min, followed by 30 cycles of 10 s at 92 °C, 10 s at annealing temperature, and 20 s at 72 °C, and then a final extension at 72 °C for 5 min. Optimal annealing temperatures and suitability of loci were initially determined

using nondenaturing 6% polyacrylamide gel electrophoresis and visualized using ethidium bromide.

Four of the primer pairs we developed did not amplify a product, and six primer pairs produced products that were either monomorphic ( $n = 3$ ) or nonspecific ( $n = 3$ ). Nineteen loci were polymorphic and gave clear amplification products (Table 1). Most (11 of 12) of the *Calocitta formosa*

**Table 2** Microsatellite loci developed for either *Calocitta formosa* or *Cyanocorax morio* that work well in the other species.  $H_O$  is the observed proportion of heterozygotes, and  $H_E$  is the expected heterozygosity ( $n = 11$  individuals for *C. formosa* and 12 individuals for *C. morio*)

Locus	Species	No. of alleles	Size range (bp)	$H_O$ ( $H_E$ )
CmAAAG6	<i>C. formosa</i>	9	190–400	0.82 (0.88)
CmAAAG11†	<i>C. formosa</i>	14	194–274	0.82 (0.83)
CmAAAG14	<i>C. formosa</i>	8	240–400	0.55 (0.81)
CmAAAG30†	<i>C. formosa</i>	11	192–240	0.81 (0.80)
CmAAAG35	<i>C. formosa</i>	2	144–150	0.46 (0.37)
CfAAAG2	<i>C. morio</i>	10	140–180	0.83 (0.91)
CfAAAG64	<i>C. morio</i>	4	130–160	0.50 (0.58)
CfAAAG65	<i>C. morio</i>	5	210–230	0.75 (0.79)
CfAAAG130	<i>C. morio</i>	8	245–300	0.83 (0.89)
CfAAT47	<i>C. morio</i>	6	130–170	0.83 (0.82)

†Screened with 53 individuals.

loci and two of the *Cyanocorax morio* loci (*CmAAAG11* and *CmAAAG30*) were screened with 53 adult *Calocitta formosa* individuals by labeling one primer of the pair with a fluorescent dye and scoring genotypes using an ABI 310 Genetic Analyzer and the software GENEMAPPER 3.0 (PE Biosystems) (Table 1). *Cyanocorax morio* loci were screened using 12 adult *Cyanocorax morio* individuals and non-denaturing 6% polyacrylamide gel electrophoresis and visualized using ethidium bromide. Ten of the loci work well in cross-species amplifications using the same annealing temperatures listed in Table 1, and in two cases loci developed for one of the species actually performed better in the other species (*CfAAAG2* and *CmAAAG11*) (Table 2). For the 13 loci that were screened with 53 individual *Calocitta formosa*, we tested for heterozygote deficits and linkage disequilibrium using GENEPOP version 3.3 (Raymond & Rousset 1995). Two of the loci had significant heterozygote deficits (*CfAAAG19* and *CfAAAG38*), suggesting the presence of null alleles. Thirteen pairs of loci out of 78 pairwise comparisons exhibited genotypic linkage disequilibrium at  $P <$

0.05, but only one pair of loci (*CfAAT34* and *CfAAAG10*) was significantly in linkage disequilibrium after a Bonferroni correction for multiple comparisons. Many of these 53 individuals were from closely related family groups, which may have resulted in significant linkage disequilibrium for these two loci. We then tested for significant linkage disequilibrium using 34 individuals that were unlikely to be closely related (primary breeders and extra-group floater males) (E. C. Berg, unpublished). Using this more restricted data set, none of the pairwise comparisons of loci exhibited significant linkage disequilibrium, suggesting that they segregate independently and can be used for parentage analyses.

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