

PRIMER NOTE

Isolation and characterization of polymorphic microsatellite markers in the white-winged chough (*Corcorax melanorhamphos*)

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Abstract

We have isolated and characterized seven polymorphic microsatellite loci in the white-winged chough (*Corcorax melanorhamphos*), a highly social, cooperatively breeding bird of Australian eucalypt woodlands. In analyses of 100 samples from 16 family groups, the number of alleles per locus ranged from four to 18, and observed heterozygosity ranged between 0.46 and 0.93. One locus appears to be sex-linked. The primers were also tested in apostlebirds (*Struthidera cinerea*), the only other species in the subfamily Corcoracinae. Five loci were successfully amplified and three were polymorphic.

Keywords: *Corcorax melanorhamphos*, microsatellite, white-winged chough

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White-winged choughs are highly social, cooperatively breeding birds that inhabit the eucalypt woodlands of southeastern Australia. They represent a monotypic genus and are one of only two species in the subfamily Corcoracinae (Sibley & Monroe 1993). Choughs are thought to be declining due to habitat loss and fragmentation (Birds Australia 2002). However, the full impact of habitat fragmentation on any species cannot be determined without an understanding of dispersal behaviour. Chough dispersal is rare and sporadic (Heinsohn *et al.* 2000). Consequently, the study of dispersal by tracking marked individuals is difficult and indirect methods such as genetic analyses must be employed. We have therefore developed microsatellite markers to assist in the study of dispersal and population genetic structure of white-winged choughs.

Blood samples were collected from white-winged choughs in Campbell Nature Park in Canberra, Australia. DNA was isolated by ammonium acetate extraction (Richardson *et al.* 2001) after digestion with proteinase K (Progen). An enriched microsatellite library constructed following the method of Fischer & Bachmann (1998) was obtained commercially from the Centre for Identification and Diagnostics,

Queensland University, Australia. One hundred recombinant clones were sequenced using the DYEnamic ET Terminator Cycle Sequencing kit (Amersham Pharmacia Biotech). Sequencing products were run on an ABI PRISM 377 DNA Sequencer and the DNA sequence edited in SEQUENCHER 3.0 (GeneCodes). Primers were designed using PRIMER3 (Rozen & Skaletsky 1997) followed by the addition of the –21M13 (5'-TGTAACCGCCAGT) sequence at the 5'-tail of the reverse primer for genotyping on an ABI Prism Sequencer following the method of Schuelke (2000).

Primers were designed for 12 loci that contained six repeats or more and had flanking sequence suitable for primer design. Ten of these primer pairs amplified successfully and were further tested for polymorphism. Polymerase chain reaction (PCR) amplifications were performed on an FTS-960 Thermal Sequencer (Corbett Research). Each reaction was carried out in a total volume of 10 µL containing 20 ng DNA template, 1 µL 10× PCR buffer [Qiagen; final 1.5 mM, but concentrations of Tris-HCl, KCl and (NH₄)₂SO₄ are proprietary], 0.2 µM dNTPs, 5 µg BSA, 200 nM forward primer, 100 nM of labelled –21M13 primer (labelled with HEX, FAM or NED; Applied Biosystems), 50 nM reverse primer, and 0.25 units of *Taq* polymerase (Qiagen).

The following touch-down thermal cycling program was used: 5 min at 94 °C; 30 s at 94 °C, 30 s at 70 °C and

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Table 1 Primer sequences and characteristics for seven *Corcorax melanorhynchus* microsatellite loci. Number of alleles and heterozygosities were calculated for 100 individuals. H_O , observed heterozygosity; H_E , expected heterozygosity. Number of alleles in apostlebirds was calculated for four individuals

Locus	Repeat motif	Primer sequence (5'–3')	No. of alleles	Allele size range (bp)	H_O	H_E	No. of alleles in apostlebirds	GenBank Accession no.
CmeA1	(CTTT) ₄₄ (ATCT) ₉	GAGCAATAAAGCAATAACAGA *CCATGGAAATGATAGATAGA	18	210–274	0.93	0.90	Did not amplify	AY330725
CmeC1	(CTTT) ₃ (CTAT) ₁₄	GTGATCGGAAATGATTTGTC *AGCTAACTCAACAGCAGGAG	8	148–176	0.69	0.59	1	AY330726
CmeG5	(TATC) ₁₀ (CCAT) ₇	AAACAGATAGGGCTCTCTAGG *ACAAAAATTCTTTTGTGTGGA	6	156–176	0.67	0.66	4	AY330727
CmeG9	(AC) ₁₂	AGAACAACCACAGAGCAGT *CCAGTTGCTTCTGATATGGT	4	236–246	0.46	0.43	4	AY330728
CmeH2	(CT) ₂₅	AAGCTTTGGGTCATATGTGT *CTGCAGCTTGAGTGAAGTT	9	130–150	0.87	0.83	3	AY330729
CmeH8†	(TATC) ₁₃ (CATC) ₉	ATCGGACGAAATAGAGATCA *TAAACGGTGCTTAAACCAIT	5	217–245	—	—	Did not amplify	AY330730
CmeH9	(GT) ₁₇	TTTCAGTTGCCTCTTCTGAT *TCAGACAACATGGCTAAAAA	7	194–210	0.85	0.83	1	AY330731

*Primers constructed with a 5' – 21M13 tail for genotyping. †CmeH8 appears to be sex-linked.

72 °C for 1 min – dropping the annealing temperature by 4 °C every cycle until it reached 50 °C – for a total of 39 cycles; 72 °C for 10 mins. PCR products were run on an ABI PRISM 377 DNA sequencer. Fragment sizes were scored using GENESCAN™ and GENOTYPER™ software (Applied Biosystems).

We assessed variability by genotyping 100 individuals from 16 family groups at each locus. In all cases, the microsatellite profiles were typical of codominant markers with at most two different alleles per locus, and with homozygous and heterozygous individuals clearly identifiable. For all loci the observed allele sizes varied in multiples of their expected repeat units.

CmeH8 appears to be sex-linked as all 52 females were homozygous at this locus compared with an observed heterozygosity of 0.71 for the 48 males in the sample. This observation is consistent with CmeH8 being on the Z avian sex-chromosome. In birds, females are the heterogametic sex, therefore possessing only one copy of the Z chromosome.

We calculated observed and expected heterozygosity (H_O and H_E) for the six putatively autosomal loci using GENALEX (Peakall & Smouse 2001), and tested for deviations from Hardy–Weinberg equilibrium using GENEPOP (Raymond & Rousset 1995). The number of alleles ranged from four to 18, and H_E ranged from 0.43 to 0.90 with a mean H_E of 0.71 (Table 1). Tests for Hardy–Weinberg equilibrium showed a significant excess of heterozygotes ($P < 0.01$) at only one locus (CmeC1), while no locus displayed a heterozygote deficiency.

The seven polymorphic loci were tested for cross-species amplification using four individuals from the only other

species in the Corcoracinae, the apostlebird (*Struthidea cinerea*). All cross-species amplifications were attempted using the above PCR conditions without modification. Five loci produced fragments within the expected size range. Of these, three were polymorphic suggesting potential for use in population genetic studies (Table 1).

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