

PRIMER NOTE

Microsatellite loci for population and behavioural studies of Horsfield's bronze-cuckoo (*Chalcites basalis*: Aves)

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Abstract

Eight polymorphic microsatellite loci were identified for Horsfield's bronze-cuckoo (*Chalcites basalis*). These include seven newly isolated loci from cuckoo genomic libraries enriched for GA and GAAA repeat-containing clones. These loci have a mean expected heterozygosity of 0.71, a mean number of alleles of 13.8 and a combined exclusion probability (one parent known) of 0.9999. Two loci (Cba01 and Cba07) showed a significant deficiency of heterozygotes and may therefore have null alleles, although this effect could be the result of nonrandom population sampling.

Keywords: *Chalcites basalis*, *Chrysococcyx basalis*, enrichment, Horsfield's bronze-cuckoo, microsatellite

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The first experimental evidence for recognition and desertion of cuckoo chicks by hosts was found for superb fairy-wrens (*Malurus cyaneus*) parasitized by Horsfield's bronze-cuckoos (*Chalcites basalis*) in Australia (Langmore *et al.* 2003). These cuckoos lay a highly mimetic egg that fairy-wrens are unable to distinguish from their own in the dark interior of their dome nests (Langmore *et al.* 2003), which may have led to the evolution of cuckoo chick discrimination by hosts. Further study of this system requires greater knowledge of the poorly defined population and breeding biology of *C. basalis*. We do not know such elementary details as whether particular females prefer certain hosts for their eggs or if chicks of particular females are more susceptible to rejection by the host. Since this species is partially migratory and widespread over most of the Australian continent (Higgins 1999), there are no data on whether geographical differentiation in this species might promote regional behavioural divergence. Here, we report the isolation of polymorphic microsatellite loci to facilitate the investigation of these issues.

Cuckoo DNA was extracted using a salting-out procedure (Bruford *et al.* 1992) from frozen tissue of dead chicks or blood from chicks and adults from Campbell Park and

Black Mountain (35°19'S, 149°12'E), Canberra, Australia. General polymerase chain reaction (PCR) solutions and running conditions were reported in Adcock & Mulder (2002). Genomic libraries enriched for GAAA and GA repeat-containing fragments were created using the method of Gardner *et al.* (1999) with modifications (Adcock & Mulder 2002). Repeat-containing clones were identified with a PCR-based method and sequenced on both strands using published methods (Adcock & Mulder 2002). The 240 colonies screened in the GAAA and the 192 in the GA-enriched genomic library, yielded respectively, 26 and 39 colonies that were sequenced. Primers were manufactured (Proligo) for the 18 of these that contained eight repeats or more and flanking sequence suitable for primer design. One primer in each pair had a 5'-M13 (TGAAAACGACG-GCCAGT) tail for use in the universal dye-labelling method described by Schuelke (2000). Primer pairs that gave consistent specific products were tested for polymorphism on 30 individuals. Screening reactions (10 µL) contained an M13 primer (200 nM), 5'-labelled with an ABI dye (VIC, FAM or NED), and the locus-specific tailed (15 nM) and untailed primer (200 nM), 40–100 ng of genomic DNA and 2.5 mM MgCl₂. Amplification began with one cycle at 94 °C for 90 s, followed by 38 cycles at 94 °C for 20 s, 52 °C for 20 s and 73 °C for 90 s. PCR products were electrophoresed on an ABI 3100 automated sequencer together with a Liz-500 size standard

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Table 1 Characterization of microsatellite loci in Horsfield's bronze-cuckoo *Chalcites basalis*

Locus	Repeat motif in clone	Primer sequence (5'–3')	N_A	bp	H_O	H_E	Excl1	Excl2	SBC N_A (bp)
Cba01	(AG) ₁₂ A ₄ (GA) ₃ ((GT) ₄ TTT) ₁₁ N ₃₀ (TG) ₉ (TTT(GT) ₄ (GT) ₁₀)	AAAGTGAGGCACTAATGTTC *GTCCAAGAGATGCAAAATCC	22	147–416	0.72	0.91	0.846	0.733	3 (104–114)
Cba02	(CT) ₅ T(TC) ₈ (TTTC) ₃ (CCTT) ₃	*AGGCACCACCTTTTAAATCC AAGCGTGAATTACAGTCATGG *GCAGTGAGGTCCAATAACC CCCTGCCTTGTGTTATTC	6	261–270	0.55	0.70	0.470	0.299	4 (257–263)
Cba03	(CT) ₁₄	*TTAGAAAATAAAAAGGAAAAGTGAG AAATGAATGTGGAGGCTGTAAC	7	206–215	0.55	0.63	0.395	0.237	1 (199)
Cba04	(CT) ₂ G(TC) ₂ TT(TC) ₅ TT(TC) ₈ TT(TC) ₆ (TTTC) ₆ (CT) ₃ T(TC) ₃	*CAACAACAAGCAGCCAAC ATGAGGCATGGCAGATGTAG	20	143–435	0.92	0.90	0.799	0.667	1 (123)
Cba05	T ₁₁ (CTTT) ₂ (CTTC(CTTT) ₂) ₃ (CTTT) ₂	*GAGCCTTAAAAGGAATTTGC GTC TTTGCACACCCTACCC	8	188–212	0.72	0.66	0.470	0.286	5 (190–213)
Cba06	(CTTT) ₆ (CT) ₂ (CTTT) ₃	*GGGAAGTGTGCTAGTGCAAG TTCCPTGGATTTTCTTGTTTG	9	113–133	0.71	0.75	0.602	0.425	2 (116–120)
Cba07	(CTTT) ₂ CT(CTTT) ₃₃ ATT(CTTT) ₁₀		33	225–547	0.92	0.95	0.922	0.855	1 (347)
Tmm6	See Adcock & Mulder (2002)		10	129–166	0.68	0.80	0.658	0.486	3 (127–133)

All loci were amplified in 30 individuals using the same PCR conditions (see text). Listed for each locus is the number of alleles (N_A), allele size range in bp, observed (H_O) and expected (H_E) heterozygosities and the probability of excluding a potential parent when one is known (Excl1) or when the other is not typed (Excl2). SBC shows the number of alleles in four individuals of the shining bronze-cuckoo (*C. lucidus*). The asterisk (*) in the primer sequence denotes a 5' tail (TGTAACGACGCGCCAGT), attached to the primer sequence. Cloned sequences have been deposited in GenBank under Accession nos AY834231 to AY834237.

(ABI) according to the manufacturers instructions. Fragment sizes were estimated using the ABI GENESCAN™ software.

Seven loci were found to be polymorphic in 30 individuals ($N_A = 6–33$, $H_E = 0.63–0.95$, Table 1). Cuckoo DNA was also amplified with all 23 primers isolated for the red-capped robin (Dowling *et al.* 2003), Madagascar paradise flycatcher (Adcock & Mulder 2002) and the European cuckoo (Gibbs *et al.* 1998). Amplifications of four individuals at 50 °C annealing temperature and 3 mM MgCl₂ followed by further optimization of the seven that yielded a specific product (Pgm3, Pgm5, Tmm1, Tmm3a, Tmm6, Ccū60, Ccū100) identified one polymorphic locus, Tmm6, from the flycatcher ($N_A = 10$, $H_E = 0.80$, Table 1). Finally, all polymorphic loci were tested on four shining bronze-cuckoo (*Chalcites lucidus*) individuals collected from Campbell Park, Canberra. All were amplified successfully using the same conditions as for *C. basalis*. In this limited sample, Cba01, Cba02, Cba05 and Cba06 are polymorphic (Table 1).

We tested for deviations from Hardy–Weinberg equilibrium (HWE) and for linkage disequilibrium (LD) using the tests implemented in GENEPOP version 3.1b (Raymond & Rousset 1995) in 30 individuals. Field observations of the correspondence between cuckoo residency and the appearance of eggs in a host's nest suggest that this sample may contain many siblings. Thus, we also tested a reduced data set of 17 individuals that was unlikely to contain potential siblings. Significant LD ($P < 0.01$) was observed between the majority of loci pairs in the complete sample but among none in the reduced sample. Hence, it seems likely that the presence of related individuals in the larger

sample, rather than the physical linkage of the loci in the genome, contributed to this result.

All loci were in HWE except Cba01 and Cba07, which showed significant heterozygote deficiency ($P < 0.001$ for each locus in both data sets). This deficiency could be caused by null alleles or could have arisen if our samples did not reflect true population allele frequencies (e.g. if rare homozygotes were sampled by chance, or if chicks have related mothers). Even with null alleles present, these loci will have utility in confirming parentage. The general probability of excluding one parent when the other is known or when the other is not typed (Equations 1a and 2a in Jamieson & Taylor 1997) is shown for each locus in Table 1 and is high using all eight loci combined ($P = 0.9999$ and $P = 0.9985$, respectively).

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