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PRIMER NOTE

Characterization of polymorphic microsatellites in the logrunner, *Orthonyx temminckii* (Aves: Orthonychidae)

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Abstract

We isolated and characterized 10 polymorphic microsatellite loci in the logrunner (*Orthonyx temminckii*), a rainforest-dwelling Australian bird. The number of alleles per locus observed within two populations ranged from three to 39, with observed heterozygosities of between 0.12 and 1.00. We demonstrate that these markers are useful for both population- and individual-level analyses.

Keywords: logrunner, microsatellite, Orthonychidae, *Orthonyx*, passerine

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The logrunner (*Orthonyx temminckii*) is a medium-sized (60 g) passerine endemic to southeastern Australia. It is a ground-dwelling species, confined to rainforests and fringing wetter eucalypt forests (Higgins & Peter 2002). Previous genetic work on this species has been restricted to broad-scale analyses of mtDNA and nuclear intron variation examining phylogeographical patterns and species boundaries (Joseph *et al*. 2001; Norman *et al*. 2002). Given this species' reliance upon rainforest habitats, its status as a year-round resident and its limited dispersal ability, it is an excellent species with which to examine fine-scale patterns of genetic differentiation in response to habitat heterogeneity and fragmentation. Unlike its group-living congener, the chowchilla (*Orthonyx spaldingii*), and many other species within the ancient Australian passerine lineages (Cockburn 2003), the logrunner breeds in social pairs; however, nothing is known of its genetic mating system (Higgins & Peter 2002). In this study, we describe the development of polymorphic microsatellite markers for logrunners and test their utility for examining the population structure and mating system in this species.

Initial development of a microsatellite library and sequencing of microsatellite-containing clones was conducted following the protocol described in Frentiu *et al*. (2003). Fifteen unique cloned sequences were selected for further development. Primers located in the flanking regions around the repetitive element in each sequence were designed using the program PRIMER 3 (Rozen & Skaletsky 2000). Optimization of polymerase chain reaction (PCR) conditions and initial screening for polymorphism was conducted using seven individuals from southeastern Queensland, with unlabelled PCR product sized on a Corbett Gelscan 2000 machine. As a result of this initial screening, two monomorphic loci were dropped from further work.

The remaining 13 loci were used to screen 16 and 19 adult individuals, respectively, from two populations in southeastern Queensland (Binna Burra 28°12′S, 153°11′E and Green Mountains 28°13′S, 153°07′E) to obtain estimates of polymorphism and heterozygosity. This screening was carried out using fluorescent labelling of one primer in each pair, with PCR product being sized using an Amersham Biosciences MegaBACE 500 capillary sequencer. PCR was performed in 10-μL reactions using 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 200 μM each dNTP, 0.3 μM each primer and 0.2 U of *Taq* F2 polymerase (Fisher Biotech). $MgCl_2$ concentrations varied according to the locus being amplified (see Table 1). Thermocycling for all loci consisted of 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, T_a for 30 s (see Table 1), 72 °C for 1 min, then a final extension at 72 °C for 5 min.

Ten of the loci were polymorphic and could be scored reliably. Three loci were dropped from further investigation as two could not be scored reliably and the third primer

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Table 1 Details of 10 polymorphic microsatellite loci in *Orthonyx temminckii* including: primer sequence; repeat unit; annealing temperature (T_a) and $MgCl_2$ concentration for PCR amplification; the number of alleles, size range, observed heterozygosity (H_O) and expected heterozygosity (H_E) observed across two SE Queensland populations; and GenBank Accession no. for the cloned allele. An asterisk after the primer sequence indicates the fluorescently labelled primer

Locus	Primer sequences (5′–3′)	Repeat unit	T_a (°C)	$MgCl_2$ (mM)	Number of alleles	Size range (bp)	H_O	H_E	GenBank no.
OT2	TAGGATGGAAAGGCAGTTGG CATGCAAGGCAGTTCAGGTA*	AG	64	1.5	4	246–256	0.63	0.65	EF433779
OT3	GCTGCTCAGACTGTGGAACA* GTGGGTCCTGCAAGTTGATT	GA	58	1	15	81–145	0.80	0.89	EF433780
OT4	CAGGCTGAAATGCTGAGAAA* ACTGGTGAAACCCTTGATGC	CCT	60	1	7	146–170	0.69	0.80	EF433781
OT7	ACTCGAGGCAGTGCTGAAAT* GGGGCATTCAGAAGACTGAG	AAGG	64	1.5	17	186–316	0.60	0.93	EF433782
OT8	ACTACAGAAATGCAAAGAAACA* GGTGCCATTACTTGCCAGAC	AAAAG	60	1.5	39	280–640	1.00	0.98	EF433783
OT10	AAGCACGCTGTGACTCTGTG* CAAATATGTTTTCCAATCAGAG	AAGG	60	1.25	13	196–290	0.59	0.79	EF433784
OT11	AGTGACACCACCAGCAGTCA* CTGAAGTTCTGCTTTGACCTGA	AGG	62	1	3	140–152	0.27	0.29	EF433785
OT12	GGGGTAAAGCTGTGCCTTAAT* GCAAGCTCCAGAGGCACTTA	TCCC	60	1	3	123–131	0.12	0.11	EF433786
OT13	CCTACCACACGGTTATATTCCT* GCAAGAAGTCAAGGCTCTGG	TATC	64	1.5	24	208–281	0.71	0.95	EF433787
OT15	CAGTGGGATTGGGAAGAGAA* TGGCAAGCAAAACAAGGAAT	AC	58	1	3	137–143	0.21	0.24	EF433788

pair concurrently amplified two duplicate loci. The remaining 10 loci had between three and 39 alleles per locus. Linkage disequilibrium and Hardy–Weinberg equilibrium were examined for each locus in each population using the program GENEPOP version 3.4 (Raymond & Rousset 1995). None of the loci were linked (all $P > 0.05$). Three loci (OT7, OT10 and OT13) were out of Hardy–Weinberg equilibrium in the Green Mountains population (all $P < 0.05$ after Bonferroni correction) because of a heterozygote deficit, but all the other loci and these three loci in the Binna Burra population were in Hardy–Weinberg equilibrium. This result could indicate the presence of null alleles at these three loci, but given the nonsignificant result in the Binna Burra population, it could also indicate some substructuring within the Green Mountains population.

The utility of these loci for population genetic studies was examined using GENEPOP version 3.4. Allele frequencies differed significantly between the two populations ($P < 0.001$), despite them being only 7-km apart and separated by continuous rainforest habitat, indicating these loci will be useful for examining population differentiation even at very small geographical scales.

Microsatellite loci are also frequently used to determine parentage. We genotyped 21 nestlings, each with one genotyped parent, at the 10 loci. We conducted parentage analyses assuming no known parents and tested whether the identity of the most likely parent matched with the known social parent. Parentage analyses were conducted using the program CERVUS version 2.0 (Marshall *et al*. 1998). Simulations were run using parameters estimated from our sampling knowledge of each population and the gender of parent being assigned (Binna Burra fathers: 12 candidates, 85% of the total population sampled; Binna Burra mothers: 4 candidates, 30% sampled; Green Mountains fathers: 11 candidates, 90% sampled; Green Mountains mothers: 7 candidates, 55% sampled). The known social parent was assigned as the most likely genetic parent for 20 of the 21 nestlings. The analyses identified 14 of these parents with a confidence of > 95%, five with a confidence of > 80%, and one with a confidence of < 80%. In the remaining case, a male from an adjacent territory was assigned as the most likely father with a > 95% confidence. Given that parentage assignment is less likely with no known parent, the high success rate in identifying the likely genetic parent of most nestlings indicates the utility of these loci for further parentage analyses. The presence of a nestling whose genetic father appeared not to be its social father is the first evidence that extra-pair paternity occurs in this species, albeit at a low rate.

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References

Cockburn A (2003) Cooperative breeding in oscine passerines: does sociality inhibit speciation? *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **270**, 2207–2214.

Frentiu FD, Lange CL, Burke T, Owens IPF (2003) Isolation of microsatellite loci in the Capricorn silvereye, *Zosterops lateralis chlorocephalus* (Aves: Zosteropidae). *Molecular Ecology Notes*, **3**, 462–464.

Higgins PJ, Peter JM (2002) *Handbook of Australian, New Zealand and Antarctic Birds, Vol. 6. Pardalotes to Spangled Drongo*. Oxford University Press, Melbourne, Australia.

Joseph L, Slikas B, Alpers D, Schodde R (2001) Molecular systematics and phylogeography of New Guinean logrunners (Orthonychidae). *Emu*, **101**, 273–280.

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

Norman JA, Christidis L, Joseph L, Slikas B, Alpers D (2002) Unravelling a biogeographical knot: origin of the 'leapfrog' distribution pattern of Australo-Papuan sooty owls (Strigiformes) and logrunners (Passeriformes). *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **269**, 2127–2133.

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

Rozen S, Skaletsky HJ (2000) PRIMER 3 on the WWW for general users and for biologist programmers. In: *Bioinformatics Methods and Protocols: Methods in Molecular Biology* (eds Krawetz S, Misener S), pp. 365–386. Humana Press, Totowa, New Jersey.