## **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 populationand 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 groupliving 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

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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

conducted following the protocol described in Frentiu

et al. (2003). Fifteen unique cloned sequences were selected

for further development. Primers located in the flanking

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- $\mu$ L reactions using 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 200  $\mu$ M each dNTP, 0.3  $\mu$ M each primer and 0.2 U of *Taq* F2 polymerase (Fisher Biotech). MgCl<sub>2</sub> 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*<sub>a</sub> 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<sub>2</sub> concentration for PCR amplification; the number of alleles, size range, observed heterozygosity  $(H_0)$  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

Primer sequences (5'–3')	Repeat unit	Τ <sub>a</sub> (°C)	MgCl <sub>2</sub> (тм)	Number of alleles	Size range (bp)	$H_{O}$	$H_{\rm E}$	GenBank no.
TAGGATGGAAAGGCAGTTGG	AG	64	1.5	4	246-256	0.63	0.65	EF433779
CATGCAAGGCAGTTCAGGTA*								
GCTGCTCAGACTGTGGAACA*	GA	58	1	15	81-145	0.80	0.89	EF433780
GTGGGTCCTGCAAGTTGATT								
CAGGCTGAAATGCTGAGAAA*	CCT	60	1	7	146-170	0.69	0.80	EF433781
ACTGGTGAAACCCTTGATGC								
ACTCGAGGCAGTGCTGAAAT*	AAGG	64	1.5	17	186–316	0.60	0.93	EF433782
GGGGCATTCAGAAGACTGAG								
ACTACAGAAATGCAAAGAAACA*	AAAAG	60	1.5	39	280-640	1.00	0.98	EF433783
GGTGCCATTACTTGCCAGAC								
AAGCACGCTGTGACTCTGTG*	AAGG	60	1.25	13	196-290	0.59	0.79	EF433784
CAAATATGTTTTCCAATCAGAG								
AGTGACACCACCAGCAGTCA*	AGG	62	1	3	140-152	0.27	0.29	EF433785
CTGAAGTTCTGCTTTGACCTGA								
OT12 GGGGTAAAGCTGTGCCTTAAT*	TCCC	60	1	3	123–131	0.12	0.11	EF433786
GCAAGCTCCAGAGGCACTTA								
CCTACCACACGGTTATATTCCT*	TATC	64	1.5	24	208-281	0.71	0.95	EF433787
GCAAGAAGTCAAGGCTCTGG								
CAGTGGGATTGGGAAGAGAA*	AC	58	1	3	137-143	0.21	0.24	EF433788
TGGCAAGCAAAACAAGGAAT								
	Primer sequences (5'–3') TAGGATGGAAAGGCAGTTGG CATGCAAGGCAGTTCAGGTA* GCTGCTCAGACTGTGGAACA* GTGGGTCCTGCAAGTTGATT CAGGCTGAAATGCTGAGAAA* ACTGGTGAAACCCTTGATGC ACTCGAGGCAGTGCTGAAAT* GGGGCATTCAGAAGACTGAG ACTACAGAAATGCAAAGAAACA* GGTGCCATTACTTGCCAGAC AAGCACGCTGTGACTCTGTG* CAAATATGTTTTCCAATCAGAG AGTGACACCACCAGCAGTCA* CTGAAGTTCTGCTTTGACCTGA GGGGTAAAGCTGTGCCTTAAT* GCAAGCTCCAGAGGCACTTA CCTACCACACGGTTATATTCCCT* GCAAGAGTCAGGGCATTGG CAGTGGGATTGGGAAGAGAA* TGGCAAGCAAAACAAGGAAT	Repeat unitTAGGATGGAAAGGCAGTTGGAGCATGCAAGGCAGTTCAGGTA*GAGCTGCTCAGACTGTGGAACA*GAGTGGGTCCTGCAAGTTGATTCAGGCTGAAATGCTGAGAAA*CAGGCTGAAATGCTGAGAAA*CCTACTGGTGAAACCCTTGATGCAAGGGGGGCATTCAGAGACA*AAAGGGGGGCATTCAGAAATGCAAAGAAACA*AAAAGGGTGCCATTACTTGCCAGACAAAAAGGGTGCCATTACTTGCCAGACAAAAGGACTACAGAAATGCAAAGAAACA*AAAAGGGTGCCATTACTTGCCAGACAAGGAAGCACGCTGTGACTCTGTG*AAGGCTAACACACCACCAGCAGTCA*AGGCTGAAGTTCTGCTTTGACCTGAGGGGTAAAGCTGTGCCTTAAT*CCTACCACACGGGTATATTCCT*TATCGCAAGAGTCAAGGCTCTGGCAGTGGGAATGGGAAGAGAA*ACTGGCAAGCAAAACAAGGAAT	Repeat Primer sequences (5'-3') $T_a$ unit $T_a$ (°C)TAGGATGGAAAGGCAGTTGG CATGCAAGGCAGTTCAGGTA* GCTGCTCAGACTGTGGAACA*AG64CATGCAAGGCAGTTCAGGTA* GCTGGTCCAGACTGTGGAACA*GA58GTGGGTCCTGCAAGTTGATT CAGGCTGAAATGCTGAGAAA*CCT60ACTGGTGAAACCCTTGATGC ACTCGAGGCAGTGCTGAAAT*AAGG64GGGGCATTCAGAAAGCAAGAACA*AAAAG60GGTGCCATTACTGCCAGAC AAGCACGCTGTGACACTGTG*AAGG60CGTGACACCACCAGCAGTCA*AGG62CTGAAGTTCTGCTTGACCTGA GGGGTAAAGCTGGCCATTATCCC60GCAAGCTCCAGAGGCACTTA CCTACCACACGGGTTATATTCCT*TATC64GCAAGAGTCAAGGCACTGG CAAGAGTCAAGGCACTGG581	Repeat $T_a$ MgCl2Primer sequences (5'-3')unit(°C)(mM)TAGGATGGAAAGGCAGTTGGAG641.5CATGCAAGGCAGTTCAGGTA*GA581GCTGCTCAGACTGTGGAACA*GA581GTGGGTCCTGCAAGTTGAGATTCCT601CAGGCTGAAATGCTGAGAAA*CCT601ACTGGGGCAGTTCAGAGAA*AAGG641.5GGGGCATTCAGAAGAAGCAGAGAAAAG601.5GGGGCATTCAGAAAGAAACA*AAAAG601.5GGGGCATTACTTGCCAGACAAGG601.25AAGCACGCTGTGACTCTGTG*AAGG621CTGAAGTTCTGCTTTGACTGAGGGGTAAAGCACCACCAGCAGTCA*AGGGGGGTAAAGCTGTGCCTTAAT*TCCC601GCAAGCTCCAGAGGCACTTACCTACCACACGGGTTATATTCCT*TATC641.5GCAAGAGTCAAGGCACTGGGCAGTGGGATTGGGAAGAGAA*AC581TGGCAAGCAAAACAAGGAAATAC5811	Repeat Primer sequences (5'-3')Repeat unit $T_a$ (°C)MgCl_2 (mM)Number of allelesTAGGATGGAAAGGCAGTTGG CATGCAAGGCAGTTCAGGTA* GCTGCTCAGACTGTGGAACA* GAAG641.54GCTGCTCAGACTGTGGAACA* GAGCTGAAATGCTGAGAAA* CAGGCTGAAATGCTGAGAAA* CCTGCT6017CAGGCTGAAATGCTGAGAAA* CCTCCT6017ACTGGGGCATTCAGAAATGCTGAGAAA* CCTGAGGCAGTGCTGAAAT* CAGGCCATTCAGAAGACTGAG ACTACAGAAATGCAAAGAAACA* AAGGAAAGG641.517GGGGCATTCAGAAAAGAAACA* AAGGAAAAG601.539393933GGTGCCATTACTTGCCAGAC AAGCACCCCACCAGCAGTCA* AGGGCTAAAGCTGTGCCTTAAT* CCTACCACACGGGTATAATTGCTTTGACCTGA GGGTAAAGCTCCAGAGGCACTTA CCTACCACACGGGTTATATTCCT* CCTACCACACGGGTATAAGGAGAA* AC5813GCAAGCTCCAGAGGAAT CCTACCACACGGGTTATATTCCT* CCTACCACACGGGTATAGGAAGAGAAAAAAA AC5813	Repeat $T_a$ MgCl_2NumberSize rangePrimer sequences (5'-3')unit(°C)(mM)of alleles(bp)TAGGATGGAAAGGCAGTTCAGGTA*AG641.54246-256CATGCAAGGCAGTTGGAACA*GA5811581-145GTGGGTCCAGAATGCGAACA*GA5817146-170CAGGCTGAAATGCTGAGAAA*CCT6017146-170ACTGGTGGAAATGCTGAGAAA*CCT601.517186-316GGGGCATTCAGAAGCTGAGAAAGG641.517186-316GGGGCATTCAGAAAGAAACA*AAAAG601.539280-640GGTGCCATGACTGAGCACTAAGG601.2513196-290CAAATATGTTTTCCAATCAGAGAAGG6213140-152AGGGCACTCAGCAGCAGTCA*AGG6213123-131GCAAGCTCCAGAGGCACTTACCT641.524208-281GCAAGAGTCAAGGGAAGCA*AAC5813137-143TGGCAAGAGCAAAAGGAAAGAAAC5813137-143	Repeat Primer sequences (5'-3')Repeat unit $T_a$ (°C)MgCl_2 (mM)Number of allelesSize range (bp) $H_0$ TAGGATGGAAAGGCAGTTGG CATGCAAGGCAGTTCAGGTA* GCTGCTCAGACGTGTGGAACA* GAAG641.54246-2560.63GCTGCTCAGAGGTGGGAACA* GGCGCTCAGAAGTGGAACA* CAGGCTGAAATGCTAGAAA* ACCTGA5811581-1450.80GTGGGTCCTGCAAGTTGGAACA* CAGGCTGAAATGCTAGAAA* ACTCGAGGCAGTGCTGAAAT* CAGGCTGAAATGCAAAGAAACA* AAAGG6017146-1700.69ACTACAGAAAGCACTGAG GGGGCAATTCAGAAGACTGAG ACTACAGAAATGCAAAGAAACA* AAAGG601.539280-6401.00GGTGCCATTACTTGCCAGAC AAGGACCCCTGTGACTCTGTG* CAAATATGTTTTCCAATCAGAG AGTGACACCACCAGCGAGTCA* CCTACCACAGCGCACTTA CCTACCACACGGGCACTTA CCTACCACACGGGCACTTA CCTACCACACGGTTATATTGCTT* TATC641.524208-2810.71GCAAGCTCCAGAGGAACTAG CCAAGAGTCAAGGCTCTGG CAAGAGTCAAAGGAATAC5813137-1430.21	Repeat Primer sequences (5'-3')Repeat unit $T_a$ (°C)MgCl_2 (mm)Number of allelesSize range (bp) $H_O$ $H_E$ TAGGATGGAAAGGCAGTTGG CATGCAAGGCAGTTCAGGTA* GCTGCTCAGACTGTGGAACA* GACAGCTGGAAATGCTGGAACA* CAGGCTGAAATGCTGAGAAA* CCTGA5811581–1450.630.65GTGGGTCCTGCAAGTTGGGAACA* GAGCTGGAAATGCTGGAAAA* CCTGA5811581–1450.800.89GTGGGTCCTGCAAGTTGGAAAA* CCTGGGGACATTCAGAAA* CCTGAGGCAGTCGAAAA*CCT6017146–1700.690.80ACTGGGGCAGTCAGAAGATGCA GGGGCATTCAGAAGACTGAG ACTACAGAAAGAGAAGAACA* AAAAG601.539280–6401.000.98GGGGCATTCAGAAGAACA* CAAATATCTTTTCCCAAGACAAGG601.2513196–2900.590.79CAAATATCTTTTTCCAATCAGAG GGGGGAAGCACACACAGCAGCAGTCA* AGGGAGG6213140–1520.270.29CTGAAGTTCTGGTTAGACTTA CCTACCACAGCGGGACTTATATC641.524208–2810.710.95CCAAGAGTCAGGAAGTCAAGGAA* CCTAGAGGTCAGAGGCACTGG CCAGAGGCACTAGCAGAAGTAC5813137–1430.210.24

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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