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TECHNICAL NOTE

Novel tetranucleotide microsatellite DNA markers for members of the *Henicorhina* Wood-wren species complex (Aves, Troglodytidae)

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Abstract We describe the isolation of eight tetranucleotide microsatellie loci from the neotropical Grey-breasted Wood-wren (Aves, Troglodytidae, *Henicorhina leucophrys*). All loci were variable, with the number of alleles ranging from 6 to 21 and observed heterozygosity ranging from 0.542 to 0.881. These loci were tested and proved to be informative in two populations of Grey-breasted Wood-wren from Colombia. Given the spatial isolation and divergence in song of many wood-wren populations we expect these loci to prove highly useful in conservation genetic studies.

Keywords Neotropics · Troglodytidae · Passeriformes · Microsatellites · Nuclear DNA markers

Four species and 31 subspecies at present comprise the *Henicorhina* Wood-wren complex (Dickinson 2003; Kroodsma and Brewer 2005). Two of the species, Grey-breasted Wood-wren (*H. leucophyrys*; 18 subspecies) and White-breasted Wood-wren (*H. leucosticta*, 12 subspecies) are geographically widespread in the Neotropics, with Grey-breasted typically replacing White-breasted Wood-wren at higher elevations where both species occur. The monotypic Bar-winged Wood-wren (*H. leucoptera*) is restricted to a few isolated mountain peaks in Ecuador and Peru, and the Munchique Wood-wren (*H. negreti*, also monotypic) is known from a handful of localities along the Pacific slope of the Western Andes of Colombia. Mitochondrial data suggest that Bar-winged Wood-wren is nested within White-breasted Wood-wren (Dingle et al. 2006) and that both White-breasted and Grey-breasted Wood-wrens exhibit considerable mitochondrial DNA structure (Dingle et al. 2006; Becker et al. 2007), often accompanied by divergence in song (e.g. Dingle et al. 2008, 2010). Thus, both widespread species likely comprise multiple lineages that warrant conservation attention, but no variable microsatellite markers with which to investigate the extent of gene flow, particularly among parapatric forms are at present available.

We developed a microsatellite library from one Grey-breasted Wood-wren (MVZ RCKB1507) using methods previously described (de Ponte Machado et al. 2009; Sellas et al. 2008). In total, 553 colonies were sequenced, of which 41 contained repetitive elements, many of which were either very short (<6 repeats) or imperfect. From these, nine tetranucleotide repeats were chosen for primer development of which eight primer pairs (Table 1) were identified as optimal based on peak morphology and variability.

PCR reactions (10 µl) consisted of 1× PCR Buffer (10 mM Tris–HCl, 1.5 mM $MgCl_2$, 50 mM KCl, pH 8.3), 0.6 µM each primer, 200 µM each dNTP, 0.6 Units Taq and approximately 5–10 ng genomic DNA. Thermal cycling profiles were as follows: initial denaturation at 94°C for 2 min, followed by 30 cycles of 94°C for 15 s, T_a for 15 s

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Table 1 Characteristics of eight microsatellite loci isolated from *H. leucophrys*

Locus	Repeat	Primer sequence 5′–3′	T_a (°C)	N	N_A	Size range	H_O	H_E	GenBank accession no.
Hle194	(GATA)8	F: GGCATGGCTTTGCTTAC R: AGCTGGCCTTGTTACGC	56	101	11	124–170	0.832	0.866	JN872330
Hle250	(TAGG)13(TAGA)12	F: AACAGAAAACACCAAGGATG R: ATGCAAAAGTTCTTCCTGG	60	101	13	171–251	0.881	0.895	JN872331
Hle258	(TATC)7	F: ATGCAACAGTCATCTGCTG R: AGTCCTACAATATCCCAATC	60	35	6	175–195	0.771	0.777	JN872332
Hle282	(TATC)13	F: GCTCCACTTTGAAGTTAGTG R: TCTGCATACTGGATTTACC	56	101	9	139–177	0.644	0.752	JN872333
Hle284[a]	(TATC)20	F: GAGATCCTGTAGTTTTGCC R: CACATTGACCTTTCAAGATC	58	101	17	146–403	0.780	0.849	JN872334
Hle286	(TCCA)14	F: TTATTTTGACTGAGAGCCAC R: TCATCGATGAAACTAGTAGC	52	101	7	83–122	0.772	0.805	JN872335
Hle315[a]	(TGGA)20	F: CTGACATTTCTGTATGGATC R: CTCAATTCACTGCACATGG	58	101	21	122–224	0.871	0.916	JN872336
Hle327	(AGAT)14	F: CATATTAATACACTGAGATGTG R: AGTTCTGATTCTGGTTGCC	56	83	10	117–159	0.542	0.828	JN872337

T_a: optimized annealing temperature; N: number of individuals genotyped; N_A: number of alleles; H_O: observed heterozygosity; H_E: expected heterozygosity

[a] Use of T4 polymerase treatment of PCR product (Ginot et al. 1996)

Table 2 Characteristics of eight microsatellite loci for *H. leucophrys* from two populations that replace each other along an elevational gradient in the Sierra Nevada de Santa Marta, Colombia

Locus	Highland population (c. 2,250–2,800 m)					Lowland population (c. 1,000–2,250)				
	N	N_A	H_O	H_E	F_{IS}	N	N_A	H_O	H_E	F_{IS}
Hle194	34	5	0.735	0.729	−0.009	67	11	0.881	0.879	−0.0023
Hle250	34	8	0.824	0.664	−0.244	67	13	0.910	0.897	−0.0162
Hle258	31	6	0.742	0.760	−0.044	4	5	1.000	0.893	0.0535
Hle282	34	2	0.529	0.507	−0.052	67	9	0.701	0.741	0.0814
Hle284[a]	33	7	0.848	0.807	−0.027	67	13	0.746	0.812	0.0075
Hle286	34	5	0.794	0.774	0.010	67	6	0.761	0.767	0.0202
Hle315[a]	34	9	0.794	0.802	0.025	67	21	0.910	0.930	−0.1429
Hle327	29	8	0.724	0. 719	−0.007	54	8	0.444	0.805*	0.4505*

N: number of individuals genotyped; N_A: number of alleles; H_O: observed heterozygosity; H_E: expected heterozygosity; F_{IS}: deviations from Hardy–Weinberg expectations

* Significant ($P < 0.01$) departure from Hardy–Weinberg expectation

(Table 1) and 72°C for 15 s. For two of the eight loci, PCR products were cleaned using a T4 DNA polymerase treatment (Ginot et al. 1996, Table 1). PCR products were mixed with the GSLIZ500 size standard (Applied Biosystems) and formamide and run on an ABI3730 automated sequencer. Fragment analysis and genotyping were performed using Genemapper version 4.0 (Applied Biosystems).

Two populations of wood-wren from the Sierra Nevada de Santa Marta of Colombia were genotyped: highland ($n = 29$–34), lowland ($n = 4$–67). All loci were highly polymorphic (Table 1) with the number of alleles ranging from 6-21 (Table 1). Observed and expected heterozygosities were calculated using ARLEQUIN (Schneider et al. 2000) and tests for linkage disequilibrium and Hardy–Weinberg equilibrium were carried out using GENEPOP (Raymond and Rousset 1995). Observed heterozygosities ranged from 0.542 to 0.881. Only one of the loci (Hle327) was found to be out of Hardy–Weinberg equilibrium ($P < 0.01$), and then only in one (lowland) of the two populations screened (Table 2), and this may be due to

possible null alleles present at this locus as suggested by the excess of homozygotes. No significant linkage disequilibrium was detected after applying Bonferroni correction for multiple comparisons (Rice 1989).

Given the considerable variation of the microsatellite loci characterized here, it is likely that these loci will prove to be suitable for many studies investigating population structure, speciation and paternity in *Henicorhina* Wood-wrens.

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