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PRIMER NOTE

Characterization of microsatellite loci developed for song wrens *Cyphorhinus phaeocephalus*

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Abstract

We developed six microsatellite loci for song wrens (*Cyphorhinus phaeocephalus*). Polymorphism ranged from four to 12 alleles with heterozygosities ranging from 21 to 90%. Three of the six loci deviated significantly from the Hardy–Weinberg equilibrium suggesting the presence of one or more null alleles. Cross-species amplification indicated that these loci will have limited utility in other wren species.

Keywords: Aves, cross-species amplification, microsatellite, polymorphic, primers, Troglodytidae, wren

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Animals that live in groups may share reproduction. How reproduction is shared among group members, or reproductive skew, affects the evolution of helping behaviour (Cockburn 1998). Multilocus microsatellite genotyping can be used to assign parentage and determine reproductive skew. This approach is useful when putative fathers are closely related (Double *et al*. 1997).

We developed and characterized six microsatellite loci in the song wren (*Cyphorhinus phaeocephalus*). The goal of this project is to assess the role of reproductive skew in the evolution of sociality by song wrens. Song wrens exhibit many of the characteristics that are thought to 'set the stage' (Brown 1987) for the evolution of cooperative breeding (e.g. year-round territoriality, delayed dispersal; Robinson 2000). Additionally, we examined the polymorphism of these loci in 16 other wren species.

DNA was extracted from blood using a proteinase K, phenol–chloroform extraction procedure (modified from Müllenbach *et al*. 1989). Genomic DNA was digested to completion with *Dpn*II. Fragmented DNA was sorted by size on a 2% agarose-in-TBE gel. Fragments of 350–700 bp in length were purified from the gel slice and cloned into Lambda Zap Express (Stratagene, La Jolla, CA) (Hughes & Moralez DeLoach 1997). We screened approximately 100 000 clones with the oligo $(AAT)_{10}$ (Hughes & Moralez DeLoach 1997), sequenced 29 positives using Thermosequenase (USB). We developed primers for six clones containing ≥ 8 repeats of the sequence AAT using Oligo® 4.04 (Rychlick 1992).

Polymerase chain reactions (PCR) (5 μL) contained approximately 5 ng of DNA, 50 mM KCl, 10 mM Tris/Cl pH 8.3, 1.5% mM $MgCl_2$, 0.1% NP40, 100 mM each dNTP, 0.25 U *Taq* DNA polymerase (Perkin Elmer), 2.5 pmol each primer, and 0.05 μL ^{35}S-dATP. Reactions were cycled using the 'tube-control' function of a Hybaid thermal cycler: once for 60 s at 92 °C, then 30 times for 5 s at 92 °C, 5 s at 55 °C, 10 s at 72 °C. Amplified fragments were resolved using 6% denaturing polyacrylamide gels. Allele length was determined by comparison to a sequencing ladder.

All loci tested were found to be polymorphic in the song wren, having between four and 12 alleles and heterozygosities ranging from 21 to 90% (Table 1). We tested all loci for heterzygote deficiency using GENEPOP (Raymond & Rousset 1995). Three of the six loci deviated significantly from the Hardy–Weinberg equilibrium (Table 1). The most likely explanation for the departure from the Hardy–Weinberg equilibrium is the presence of one or more null alleles. Null alleles are likely a population specific problem which might be rectified by redesigning primer sequences (Peatkau & Strobeck 1995). An alternative explanation for the deficiency of heterozygotes is microgeographic population structure. This seems less likely since three of the six loci fail to show an excess of homozygotes. However, future work will examine population structure in greater detail.

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Table 1 Polymorphic AAT-repeat microsatellite loci developed for the song wren. H_O is based on the proportion of heterozygotes in a population sample of 20 unrelated individuals. GenBank accession numbers: AF286726–AF286731

Locus name	Repeat motif	Primer sequences 5′–3′	H_O†	H_E	Expected length	No. of alleles
CpAAT8	ATT_{19}	GCCCAACCTGGCCTTAAAT CCCAGCAAAGGAGCAGTGT	0.40*	0.74	172	7
CpAAT18	AAT_{11}	CCAGGTTCATAGGGTTTGTA GGCACCCTAGTAGCTGATAA	0.47	0.57	131	6
CpAAT34	ATT_{14}	ACAAAGCTGGGGAAGTCTTA GAAGGCTTTCATTCAACACTAT	0.44**	0.77	215	6
CpAAT39	AAT_{15}	TCTACTTGCCTAGAGTAATTAACA GCTTTCTGAATTGCACTTATTA	0.21***	0.67	104	4
CpAAT51	AAT_{14}	GACCAGCAAGGAAGAAACAA TTCTTCAGCATTTTTCTGTTCTAT	0.80	0.85	157	12
CpAAT54	AAT_{16}	GGGCAGTTCCAGCATTAGTT ACAGGCACCATATCCAATTATC	0.90	0.87	94	10

†These loci deviated significantly from the Hardy–Weinberg equilibrium: Hardy–Weinberg exact test, GENEPOP vs. 3.1 (Raymond & Rousset 1995). *P < 0.001, **P = 0.002, ***P = 0.003.

Table 2 Cross-species amplification using the primers developed for song wrens. Successful amplification of appropriate sized product indicated as 'P' = polymorphic, 'M' = monomorphic, or '+' for successful amplification where polymorphism could not be determined. Unsuccessful amplification is indicated by a dash

			Locus					
Species	accession number*	n	Cp8	Cp18	Cp34	Cp39	Cp51	Cp54
Campylorhynchus turdinus: thrush-like wren	B6927	1	+	+	+	+	+	+
Cinnycerthia unirufa: rufous wren	B3074 B3075	2	–	–	–	–	+	–
Cistothorus platensis: sedge wren	B3037	1	–	–	–	–	+	–
Cistothorus palustris: marsh wren	B908 B907	2	+	P	–	–	P	P
Thryothorus atrogularis: black-throated wren	B23 B442	2	M	M	–	M	P	M
Thryothorus nigricapillus: bay wren	B302 B305	2	P	–	P	–	P	P
Thryothorus ludovicianus: carolina wren	—	2	P	P	M	–	M	P
Thryothorus modestus: plain wren	B5397 B5394	2	M	–	–	M	P	P
Thryothorus leucotis: buff-breasted wren	B6897	1	+	–	–	+	+	+
Troglodytes aedon: house wren	B5929 B5942	2	P	P	–	–	P	M
Troglodytes solstitialis: mountain wren	B5805 B3103	2	+	+	–	–	P	P
Henicorhina leucosticta: white-breasted wood-wren	B5088 B5074	2	+	+	+	+	P	P
Henicorhina leucophrys: grey-breasted wood-wren	B1527 B1480	2	+	P	+	P	P	P
Microcerculus marginatus: nightingale wren	B1426 B1406	2	P	–	–	M	M	M
Microcerculus luscinia: whistling wren	B5306 B5312	2	P	–	P	–	M	M
Cyphorhinus aradus: musician wren	B5029 B5009	2	M	P	+	P	P	P

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The six loci were tested on 16 other wren species from North, South, and Central America (Table 2). PCR conditions were those described above. Our primers had less utility in these species. However, all loci appear useful in the congeneric musician wren, the thrush-like wren, and both wood-wren species.

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