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Eleven microsatellite loci isolated from the banded wren (*Thryothorus pleurostictus*)

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Abstract

We describe 11 microsatellite loci isolated from the Banded Wren (*Thryothorus pleurostictus*), a Neotropical species for which understanding the genetic mating system is important for testing questions about the species' unusual vocal behavior. Screening of these loci revealed extremely low allelic variation in a Costa Rican population. Allelic variation at these and other previously developed loci is substantially higher in two other wren species, the southern house wren (*Troglodytes aedon bonariae*) and rufous-and-white wren (*Thryothorus rufalbus*), suggesting that the low allelic diversity in the banded wren results from demographic bottlenecks rather than locus-sampling artifacts.

Many species of *Thryothorus* wrens are renowned for their highly coordinated male-female song duets; the banded wren (*T. pleurostictus*) is one of the few *Thryothorus* that does not duet, with this loss a likely derived trait (Mann et al., submitted). Freedom from the constraints of duetting is associated with vigorous immediate-variety singing by the male banded wren, in contrast to the repeat-mode singing of all duetting relatives (Molles & Vehrencamp 1999). Markers for investigating the species' genetic mating system will help distinguish among adaptive scenarios for the loss of duetting, including selection on the male to increase song versatility for the purposes of attracting extra-pair copulations, selection on the female to relinquish joint territorial defense, release from male mate-guarding, or female preference for versatile male singers (Hall 2004; Mennill & Vehrencamp 2005). Many of these scenarios predict that extra-pair paternity should be higher in the banded wren compared to duetting congeners.

Banded wren DNA was isolated from whole blood in lysis buffer (Hoelzel 1992), using DNeasy™ Tissue Kits (Qiagen Inc., Valencia, CA). A microsatellite library from genomic DNA was constructed following the enrichment procedure described in Stenzler and Fitzpatrick (2002) with the modifications described in Stenzler et al. (2004). One hundred positive plasmid clones were sequenced. PCR primers were designed and tested for the 30 microsatellite-containing sequences. Of those, 11 microsatellite loci appeared suitable for further development and were tested for variability on a panel of 11 banded wrens (Table 1).

Polymerase chain reactions (PCRs) were performed in eight-strip 0.2 mL tubes or 96-well plates using a DYAD® thermal cycler (MJ Research). The cycling profile was 1 cycle at 95° C for three min, 35 cycles of 1 min at 95° C, 1 minute at the locus-specific annealing temperature (see Table 1), and 1 min at 72° C, followed by a final extension cycle of 5 min at 72° C. Reactions (10 μL) contained 10–100 ng of genomic DNA, 0.25 units of Jumpstart™ *Taq* Polymerase (Sigma, St. Louis, MO), 10 mM Tris-HCL (pH 8.3), 50 mM KCl, $MgCl_2$ specific to each locus (Table 1), 200 μM of dNTPs (Invitrogen), and 2.0 pmol each of forward and reverse primers, one of which was modified at the 5′ end by addition of a fluorescent label (PET, 6-FAM, VIC, or NED -Applied Biosystems). Labeled PCR products were analyzed on an ABI Prism 3100 Genetic Analyser (Applied Biosystems), and allele sizes were estimated using GENEMAPPER™ version 3.0 (Applied Biosystems).

During preliminary screening of microsatellite loci isolated from the banded wren we noted that an unusually large proportion of loci were invariant, and in subsequent screening with primers developed for other wren taxa (song wren (*Cyphorhinus phaeocephalus;* Hughes and Robinson 2001) and house wren (*Troglodytes aedon*; Cabe and Marshall 2001), we similarly found little to no variation at the subset of those loci that amplified robustly in the banded wren. To explore whether this pattern of low variation was taxon-specific, we used the same protocols to test amplification and variability of the banded wren markers in panels of individuals of two additional wren taxa: the rufous-and-white wren (*T. rufalbus*), which occurs sympatrically with *Thryothorus pleurostictus* at our Costa Rica study site (Mennill & Vehrencamp 2005), and the southern house wren (*Troglodytes aedon bonariae*) from southern Buenos Aires Province, Argentina. A total of 11 banded wrens, 15 rufous-and-white wrens, and 15 house wrens were genotyped at each of the 11 banded wren-specific loci (Table 1). Of the primers that produced a PCR product, the number of variable loci in each species was: banded wren, 3 (2–3 alleles); rufous-and-white wren, 6 (2–7 alleles); and house wren, 3 (2–11 alleles). While allelic variation was present at 27% of banded wren loci in banded wrens, multiple alleles were found at 60% and 50% of loci that amplified in the rufousand-white and house wrens, respectively. Given the higher variability of these markers in related species, we suspect that the low microsatellite variability in this banded wren population stems from past demographic bottlenecks, despite the relative abundance of this species at the present time. The banded wren is a habitat specialist of lowland tropical dry-forests, whereas the rufous-and-white wren is a semi-humid and gallery forest specialist and the southern house wren is a generalist of semi-open habitat ranging from arid to humid and from lowland to montane areas. During the Last Glacial Maximum and subsequent Early Holocene, the Pacific lowlands of southern Central America experienced multiple rapid and extreme changes in climate and vegetation (Piperno and Jones 2003). During this period, the expansion of the humid lowland forest and concomitant fragmentation of dry forest habitats may have reduced and fragmented the banded wren's populations more strongly than the populations of the other two wren species.

In subsequent screening, we genotyped 40 complete banded wren families (246 individuals) with ThPl-14, ThPl-27, and ThPl-37. The numbers of alleles at these loci were 3, 6, and 3, respectively, in this larger pool of individuals. All loci were in Hardy-Weinberg equilibrium and there was no evidence of linkage among the loci (all $P > 0.05$, $n = 70$ adult males and females – offspring excluded; tests done in GENEPOP (Raymond & Rousset 1999).

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Table 1

cteristics of microsatellite loci in *Thryothororus pleurostictus* and cross-species allele size ranges and number of alleles in *Troglodytes aedon* and *horus rufalbus*

cus*	Repeat Motif	T_a (° C)	$MgCl_2$ (mM)	Primer Sequence †	*Thryothorus pleurostictus* (banded wren) Size range (bp)	N_a	*n*	*Thryothorus rufalbus* (rufous-and-white wren) Size range (bp)	N_a	*n*	*Troglodytes aedon* (house wren) Size range (bp)	N_a	*n*
Pl-01	$(GT)_8$	62	1.5	**F:**CTTTGGGGCAGTGTTGTGGAATG **R:**GGCTGGCTGGGAGGCACAG	300–302	1	11	298–299	2	14	282–299	5	15
Pl-14	$(CA)_5$ (GACATACAGA) $(CA)_7$	60	2	**F:**GTAAATTTCAGGAGTCCAGGTTGC **R:**AAGCGCCCAAAATTAGCCAGAA	237–245	3	11	245–262	5	15	243–227	11	15
Pl-15	$(GT)_2$ (GA) $(GT)_4$ (GAGTAGT)	60	3	**F:**TTGTCTTCTTCTCAGTTTGTCTCA **R:**GCGTTTGTGTTACTGAAGATTTAG	286	1	11	277	1	15	277	1	15
Pl-16	$(TG)_8$	60	3	**F:**CACTCTTTGAATTAGCTCTCCTCA **R:**GCAAAAACAAGATATCCTCAGTCC	129–131	1	11	137	1	15	N.A	-	14
Pl-17	$(GT)_8$	60	2	**F:**AGTGGCTGGGTGTTCTTTCAT **R:**CACATCCTTTCCCTCCTGGTA	157	1	11	143–160	3	15	160–162	2	15
Pl-20	$(GT)_3$ GA $(GT)_7$	60	3	**F:**CTTGCCATAGAATGCAGTTGAAT **R:**TAGTTCCAGTCCTCTTCTTTTACC	267	1	11	256	1	15	256	1	15
Pl-22	$(GT)_1(CT)_1(GT)_2(AT)_1(GT)_4(CT)_1(GT)_3$	60	3	**F:**GAGAAGAGTGCATAGGACAATCA **R:**TGGTGGCACGTTACAGGTTT	140	1	11	132–141	3	14	N.A	-	15
Pl-26	$(GT)_8$	60	3	**F:**TCAAATGTGCCACTGACTGAGT **R:**AGCCTACTTCAAACTGAGACAGA	188	1	11	182–188	3	15	182	1	15
Pl-27	$(AC)_{15}$	60	2	**F:**TCTCTGCGTCTGCTTGGTG **R:**CTTCCTGGGATAGATAATGTGAC	175–196	3	11	180	1	14	N.A	-	15
Pl-30	$(TG)_6$ TA $(TA)_3$	60	3	**F:**ATGCCAGCACTAAAGAATGACAA **R:**CTACATAGCAGGCAGCAGAGGTT	221	1	11	227–252	7	13	N.A	-	15
Pl-37	$(CAA)_7$	60	3	**F:**CCATCAGATTCCTAAGATAAACC **R:**TCCCAGCCCACCCTGTA	199–200	2	11	N.A	-	15	N.A	-	15

enBank Accession nos are DQ489538F-DQ489546 for ThPl-14 through ThPl-30 successively, DQ489536 for ThPl-01, DQ489537 for ThPl-37; †F, forward; R, reverse; T_a, annealing temperature; gCl_2, optimized concentration; N_a, number of alleles; *n*, sample size; N.A., no PCR amplification.