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MICROSATELLITE LETTERS

Development and characterization of microsatellite loci for two species of Beringian birds, rock sandpiper (*Calidris ptilocnemis*) and Pacific wren (*Troglodytes pacificus*)

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Abstract Identification and assessment of small, endemic populations are priorities for conservation. We isolated and characterized 8 microsatellite loci from rock sandpiper (*Calidris ptilocnemis*) and 5 microsatellite loci from Pacific wren (*Troglodytes pacificus*), species with endemic populations of named subspecies that are of conservation concern. Eighteen to 20 individuals of each species from several locations in Alaska were screened for polymorphism. Loci for each species showed high polymorphism, with rock sandpiper ranging from 5 to 14 alleles per locus and 0.73–0.88 expected heterozygosity and Pacific wren ranging from 5 to 14 alleles per locus and 0.55–0.91 expected heterozygosity. Loci developed for rock sandpipers were also polymorphic in closely related taxa. These loci are the first developed for either species and will be used to identify and conserve endemic populations in the Bering Sea region.

Keywords *Troglodytes* · *Calidris* · PCR primers · Microsatellite · Aleutian Islands · Pribilof Islands

Rock sandpiper (*Calidris ptilocnemis*) and Pacific wren (*Troglodytes pacificus*) exhibit high levels of intraspecific diversity within Beringia, with endemic populations (named subspecies) primarily found on remote oceanic islands including Pribilof and Aleutian island populations that are of conservation concern (Gotthardt et al. 2012). However, little is known about the genetic diversity or connectivity among populations. Highly polymorphic genetic markers are needed to assess population structure and gene flow and to identify isolated endemic populations that should be treated as independent management units.

Total genomic DNA was extracted from tissue samples of two individuals for each species from Alaska populations. Microsatellite loci were isolated following Glenn and Schable (2005). Briefly, DNA was digested with restriction enzymes, adapters were ligated onto ends of fragments, biotinylated probes and streptavidin beads were used to physically separate microsatellite-containing DNA fragments, and PCR was done to increase the yield of fragments. DNA fragments were then run on a Roche 454 FLX with Titanium chemistry along with similarly treated libraries, each with unique adapter and Roche MID-tag combinations. We de-multiplexed with demuxipy (https://github.com/faircloth-lab/demuxipy/) and used a customized version (https://github.com/faircloth-lab/msatcommander-gs) of MSATCOMMANDER (Faircloth 2008) to find 10,247 and 15,954 sequences containing microsatellites from 15,701 to 26,433 sequence reads of sandpiper and

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Table 1 Information about 8 microsatellite loci developed for rock sandpiper (*Calidris ptilocnemis*; Capt) and 5 microsatellite loci for Pacific wren (*Troglodytes pacificus*; Trpa)

Locus	Primer sequence 5′–3′	Repeat motif	Size (bp)	N	A	H_o	H_e	TD
Capt4	F: CAGTCGGGCGTCATCATTTGTCCACCAGGACTTAGAAA	$(AGAT)^{12}$	206–226	16	6	0.81	0.78	55/45
	R: CTCCCAAGGCAGGCTGAAA							
Capt6	F: CAGTCGGGCGTCATCACATACTGGAAGGCCGCTA	$(ATCT)^{10}$	158–194	18	9	0.94	0.84	60/50
	R: CCTCGCTCCAACATCAATGC							
Capt14	F: CAGTCGGGCGTCATCACTGTCTGAAACATATTGGCAAGC	$(ATCT)^{12}$	360–396	17	11	0.65	0.88*	55/45
	R: TGAAGAGATCCAGAAGATCCGT							
Capt16	F: GGTGTGACAACATCATGGCAG	$(ATCT)^{12}$	203–227	17	5	0.47	0.73	65/55
	R: CAGTCGGGCGTCATCAAACTGGACAGGCATCCAAA							
Capt18	F: CAGTCGGGCGTCATCAAACCTGCCACCTGATGGAT	$(AGAT)^{9}$	288–312	16	7	0.75	0.81	55/45
	R: TCTTACCTATGTGTGGTCAAGC							
Capt20	F: CAGTCGGGCGTCATCATACTGGACCACTTGTGGCAT	$(AGAT)^{8}$	328–360	17	14	0.47	0.88*	55/45
	R: TCTACTGCATCCTGTTTCCCA							
Capt23	F: CAGTCGGGCGTCATCACTTCACATACTGGAAGGCCG	$(ATCT)^{10}$	163–199	16	9	1.00	0.83	60/50
	R: CCTCGCTCCAACATCAATGC							
Capt43	F: TGGTGTGGTAGCAATCTCCT	$(GGAT)^{14}$	269–305	18	9	0.56	0.81*	55/45
	R: CAGTCGGGCGTCATCACTGCCATCCAACAAGATGCC							
Trpa4	F: CAGTCGGGCGTCATCAAAGTCGTTCAGGCTTGAGT	$(AC)^{13}$	314–326	20	7	0.50	0.69	60/50
	R: CTCTACACTCTACAACCGTTCC							
Trpa12	F: CAGTCGGGCGTCATCATATCAGCACTGTTCCAGCCA	$(ACT)^{16}$	220–253	20	17	0.60	0.79*	55/45
	R: TGAAATACAGTAGGGAACCAAGTC							
Trpa21	F: CTCCCAGGCATCCCTCATTC	$(AGAT)^{14}…(AGAT)^{9}$	328–396	20	14	0.65	0.91	60/50
	R: CAGTCGGGCGTCATCACCAAGAGTCAGCTTCCACA							
Trpa22	F: CAGTCGGGCGTCATCAGCCAACAGTAGGACATGGCT	$(GT)^{10}$	210–218	19	5	0.16	0.55*	60/50
	R: CAGTGTTAAGGTTGCTACACCA							
Trpa27	F: CAGTCGGGCGTCATCACTCACTCTGGCCCAATTCCT	$(AC)^{10}$	227–241	20	5	0.50	0.60	65/55
	R: GAGAGAACTTGGCTCTTCATCA							

Size indicates range of alleles in base-pairs (bp) including CAG tag, number of individuals genotyped (N), number of alleles (A), observed heterozygosity (H_o), expected heterozygosity (H_e), and TD refers to the annealing temperatures for the touchdown protocol for each locus. Loci out of Hardy–Weinberg equilibrium ($P < 0.01$) are indicated with an asterisk next to expected heterozygosity values

wren, respectively. MSATCOMMANDER was used to design primers with the CAG primer sequence (5′-CAG-TCGGGCGTCATCA-3′) prepended to the 5′ end of one primer to enable use of a third fluorescently labeled primer identical to the CAG sequence during PCR (Hauswaldt and Glenn 2003).

Forty-five primer pairs for rock sandpiper and 28 primer pairs for Pacific wren were tested for amplification and polymorphism using genomic DNA from 5 individuals for each species. Amplifications were in 20 ul volumes (250ug/mL BSA, 10×Buffer B (Fisher Scientific), 25 mM $MgCl_2$, 5 uM unlabeled primer, 0.5 uM tag labeled primer, 5 uM universal dye-labeled primer, 2.5 mM dNTPs, 0.5 units Taq DNA polymerase (Fisher Scientific), and 20 ng DNA template) using a BioRad MyCycler or Multigene Labnet International thermal cycler. We used touchdown cycling conditions to amplify DNA and to attach the universal dye-labeled primer. Parameters consisted of an initial denaturation step of 2 min 30 s at 95° C then 20 cycles of 95° C for 20 s, 65°–50° C annealing temperature for 20 s (decreasing 0.5° C per cycle), and extension step of 72° C for 30 s followed by 15 cycles of 95° C for 20 s, 55°–45° C for 20 s, and 72° C for 30 s. Cycles were followed with a final extension step of 72° C for 10 min. Amplifications were run on an ABI3730XL sequencer (Applied Biosystems).

We examined polymorphic loci using 18 individuals for rock sandpiper from several Alaska locations, including Cold Bay, Nome, Adak Island, Shemya Island, the Pribilof Islands, and Kodiak Island, and two individuals each for three closely related species, purple sandpiper (*Calidris maritima*), dunlin (*Calidris alpina*), and sanderling (*Calidris alba*). Twenty Pacific wrens were genotyped from Juneau, Near Islands, Rat Islands, and Middleton Island in Alaska. Eight of the rock sandpiper and 5 of the Pacific wren primers amplified DNA and exhibited polymorphism (Table 1, Online Resource 1). We scored alleles using GeneMapper software (Applied Biosystems) and evaluated the number of alleles per locus (A), observed and expected heterozygosity (H_o and H_e), Hardy–Weinberg equilibrium, and linkage equilibrium using Arlequin ver 3.5 (Excoffier et al. 2005). We found that the number of alleles per locus ranged from 5 to 14 for both species. Observed heterozygosity for rock sandpiper ranged from 0.47 to 1.00 and for Pacific wren from 0.16 to 0.65, and expected heterozygosity ranged from 0.73 to 0.88 for rock sandpiper and from 0.55 to 0.91 for Pacific wren. All loci were in linkage equilibrium but several loci were out of Hardy–Weinberg equilibrium after correction for multiple tests (Table 1). Deviations from Hardy–Weinberg are expected given that samples were taken from several populations that are likely to have dissimilar histories.

All 8 loci that were polymorphic in rock sandpipers were successfully amplified in purple sandpiper, dunlin, and sanderling. Most of these were polymorphic for each species, except for Capt16 for purple sandpiper and dunlin, Capt 4 and Capt 18 for sanderling, and Capt20 for dunlin. However, these loci might be polymorphic if a larger sample size was assessed. We suggest that these loci could be successfully used for population-level assessments in these species and possibly in other members of the genus *Calidris*.

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