Molecular Ecology Notes (2007) **7**, 1067–1069 doi: 10.1111/j.1471-8286.2007.01779.x

PRIMER NOTE

Novel microsatellite loci for the study of the black-capped vireo (*Vireo atricapillus*)

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Abstract

We identified 14 novel polymorphic microsatellite loci in the black-capped vireo (*Vireo atricapillus*). We also attempted to amplify and genotype these loci in other *Vireo* species, including the white-eyed vireo (*Vireo griseus*), red-eyed vireo (*Vireo olivaceus*), and blue-headed vireo (*Vireo solitarius*). In 33 genotyped black-capped vireos from two locations, total alleles ranged from six to 20, with observed heterozygosity ranging from 0.58 to 0.91 and expected heterozygosity from 0.65 to 0.93. Two loci had detectable levels of null alleles. Many of the loci were able to be amplified in the related *Vireo* species.

Keywords: black-capped vireos, endangered species, microsatellites, population genetics, songbirds, vireos

Received 13 October 2006; revision accepted 6 March 2007

The black-capped vireo (*Vireo atricapillus*) is an endangered, migratory songbird that winters along the Pacific coast of Mexico and breeds in a range extending from northern Mexico, through central Texas, and into very limited areas in Oklahoma (Grzybowski 1995). There has been considerable emphasis on research and protection of this species (e.g. Parysow & Tazik 2002; Fazio *et al*. 2004; Cimprinch & Kostecke 2006); however, there have been no multilocus-based population studies of either the black-capped vireo or any other *Vireo* species. To this end, we have developed a library of 14 novel microsatellite loci in the black-capped vireo for the purpose of studying such vital population parameters as gene flow and effective population size. Each locus was also amplified and genotyped in samples of other *Vireo* species, including the white-eyed vireo (*Vireo griseus*), red-eyed vireo (*Vireo olivaceus*), and blue-headed vireo (*Vireo solitarius*) to explore their potential usefulness in the study of other *Vireo* species.

Black-capped vireos were captured using standard mistnetting techniques with song playback, and sampled for blood. Muscle samples from the other *Vireo* species were obtained on loan from the Louisiana Museum of Natural History. DNA was extracted from these blood and muscle samples using a PureGene DNA Isolation Kit (Gentra Systems). The library of novel markers was developed by enriching for tetranucleotide [$(GATA)_7$ $(GATC)_7$, and $(GACA)_7$] and dinucleotide [$(GT)_{12}$ and $(CT)_{12}$] repeats was and using a microsatellite cloning protocol as per Hamilton *et al*. (1999) and Hauswaldt & Glenn (2003) [and modified as reported earlier by Beheler *et al*. (2004)]. Three black-capped vireo extractions were pooled for the library development. One hundred and ninety-two colonies were screened via polymerase chain reaction (PCR), and 96 recombinant clones with products greater than 300 base pairs were sequenced. Sequence data were visualized using CHROMAS (Technelysium), and primers were developed for those sequences found to be positive for microsatellites using PRIMER 3 (Rozen & Skaletsky 2000). Sequences were submitted to GenBank (Accession nos EF363782– EF363795).

Thirty-three black-capped vireos from two putative populations (Balcones Canyonlands National Wildlife Refuge in Texas and Wichita Mountains Wildlife Refuge in

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Table 1 Primer sequences for novel loci developed for black-capped vireos. H_E is the average expected heterozygosity for the two sampled populations under Hardy–Weinberg equilibrium, and H_O is the average observed heterozygosity. T_a is the annealing temperature. Labelled primers are designated with their respective fluorescent dye

Locus	Primers	Accession no.	Repeat	Alleles	Range	T_a	H_E	H_O
BCV2-1	F(5-HEX): TTGAAGACAGGTTAGAAGAGC R: TCAGCAGGTTAGGAATAAGAG	EF363782	$(AC)_{14}(CA)_{12}$	7	224–236	55	0.70	0.64
BCV2-2	F(6-FAM): ATGGGGAAGTCAGGCAAAA R: CTTTGCCAAGCTGCAGAAAT	EF363783	$(AG)_{14}$	6	224–234	60	0.72	0.70
BCV2-3	F(6-FAM): TGCAGACACAGAATTCAGAAC R: ACCTCCTCTGTGATCTGGAC	EF363784	$(AC)_6AG(AC)_{12}$	7	151–161	60	0.79	0.76
BCV2-4	F(6-FAM): AGAAGCTCTTCATTGCTG R: AATCTCTGAATCGAGCTG	EF363785	$(AC)_{11}$	7	201–211	47.5	0.82	0.85
BCV2-5	F(5-HEX): CAGAAGCAGTTCCACTAGCA R: GGTCCTATCTTTCACACTCTTCA	EF363786	$(CA)_{24}$	16	226–260	57	0.91	0.76
BCV2-6	F(6-FAM): CTTGGATAGAGCAGCCTCTG R: CAGTCTTATCATTGCAGGATAGC	EF363787	$(GT)_{13}$	8	183–195	60	0.65	0.58
BCV2-7	F(6-FAM): GGATTGTGGTATGCCTGTTAG R: GGTTAAGCCGAGGTTATCTG	EF363788	$(GT)_8$	13	177–227	54	0.80	0.91
BCV4-1	F(5-TET): AATACATGTGTATAACTTCCTACAGTG R: GGGAATATTTGGAATATATTTGG	EF363789	$(TATC)_{16}$	13	226–274	57.5	0.89	0.67
BCV4-2	F(5-HEX): GTCACCGTATGGAGGCAAG R: GAGCTCTTCATTACAGCACTCATC	EF363790	$(GATA)_{39}$	9	178–218	68	0.82	0.94
BCV4-3	F(5-HEX): CATCTCACCTCTGTGCTC R: TCACAGCATGTGAGTGAC	EF363791	$(GATA)_{45}$	8	247–275	47.5	0.87	0.94
BCV4-4	F(5-TET): AATACATGTGTATAACTTCCTACAGTG R: TTGGAATATATTTGGAGACAGAC	EF363792	$(TATC)_{20}$	14	220–268	56	0.92	0.94
BCV4-5	F(5-HEX): TACGAGGATGTCACCGTATG R: CGGTTACTCAACACAGACCTC	EF363793	$(GATA)_{10}$	10	239–255	57	0.83	0.94
BCV4-6	F(5-HEX): GTGGGACTGCATTCCAAATA R: ATGGAGGTGGGAGGGAATA	EF363794	$(TATC)_{19}$	15	134–174	58	0.91	0.73
BCV5-1	F(5-HEX): TTCTTGTGGAACCTCACTGG R: TCATGATTACCAGGACAAGACAC	EF363795	$(AGAAG)_{30}$	20	196–286	57	0.93	0.91

Oklahoma) were genotyped, along with three individuals of each of the other *Vireo* species included in our analyses. PCRs were conducted with 10 μL reactions, including 0.5 μL of DNA template in a solution of 0.04 μM primer, 1× Ampli*Taq* Gold PCR buffer without magnesium (ABI), 200 μM dNTPs, 1 U of Ampli*Taq* Gold DNA polymerase (ABI), and 2 mM $MgCl_2$. The thermal profile for each PCR included an initial denaturing incubation at 94 °C for 2 min, then 35 cycles of three steps, including one step at 94 °C for 30 s, an annealing step of 30 s at the appropriate temperature for the locus (Table 1) and an extension step of 30 s at 72 °C, and finally, a 10-min incubation at 72 °C. Genotyping was conducted on an ABI 310 DNA Sequencer with labelled primers (6-FAM, 5-TET, and 5-HEX) and TAMRA size standard. Genotypes were analysed with MICRO-CHECKER (Oosterhout *et al*. 2004) to detect potential null alleles and peak-calling inconsistencies, and GENEPOP (Raymond & Rousset 1995) was used to estimate numbers of alleles, observed heterozygosity, expected heterozygosity, and to test for linkage disequilibrium between loci.

Several loci were monomorphic and, accordingly, excluded from this study. Homozygote excesses indicated the potential presence of null alleles in two loci: BCV2-5 (Balcones National Wildlife Refuge) and BCV4-1 (Wichita Mountains Wildlife Refuge). Otherwise, all remaining loci were in Hardy–Weinberg equilibrium in both populations. After a Bonferroni adjustment of α to account for multiple tests, two loci were found to be in significant linkage disequilibrium in both populations: BCV4-2 and BCV4-5. Many of the loci that were polymorphic in black-capped vireos were also successfully amplified and polymorphic in other vireos (Table 2). Some of the loci that appeared to have low levels of amplification success in other species might ultimately prove to be useful with species-specific optimization of PCR conditions.

These novel microsatellite loci will be useful for future studies of parentage and for estimation of genetic parameters of the black-capped vireo, addressing previous calls for such studies (Grzybowski 1991, 1995). Furthermore, this library may prove useful for similar studies in other members of the genus *Vireo*.

Table 2 Cross-amplification (per cent successfully amplified) and genotyping (total number of alleles) success with several *Vireo* species. Three individuals were amplified and genotyped from each species

Locus	White-eyed vireo (*Vireo griseus*)	Red-eyed vireo (*Vireo olivaceous*)	Blue-headed vireo (*Vireo solitarius*)
BCV2-1	0%	66.7%/3	0%
BCV2-2	100%/2	66.7%/3	100%/5
BCV2-3	100%/2	100%/3	100%/3
BCV2-4	0%	0%	100%/1
BCV2-5	0%	0%	0%
BCV2-6	100%/3	100%/3	100%/5
BCV2-7	0%	0%	0%
BCV4-1	100%/3	100%/3	66.7%/3
BCV4-2	66.7%/3	66.7%/3	33.3%/1
BCV4-3	0%	100%/4	100%/5
BCV4-4	0%	0%	0%
BCV4-5	100%/6	100%/3	100%/3
BCV4-6	100%/3	100%/5	100%/2
BCV5-1	100%/3	100%/3	100%/6

Acknowledgements

We would like to thank our hosts, the gracious members of the Rhodes and DeWoody laboratories, for their invaluable insight during the development of this library. This research was conducted in conjunction with, and funded by, the United States Army Engineer Research & Development Center Environmental Quality Basic Research Program. Finally, we would like to thank the Louisiana Museum of Natural History for providing samples.

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