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TECHNICAL NOTE

Ten microsatellite loci from Northern Bobwhite (*Colinus virginianus*)

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Abstract Ecological studies using microsatellite data often require the selection of an optimal marker set for use in parentage and relatedness inference. Commonly, this requires a candidate pool of microsatellite markers from which several are selected to ensure data are acquired efficiently and accurately. We developed 10 microsatellite loci for use with Northern Bobwhite (*Colinus virginianus*) and tested these loci using individuals collected from two distinct populations in GA and VA. Our new markers yielded seven alleles/locus (range: 2–16) in the Georgia population and six alleles/locus (range: 2–13) in the Virginia population. Exclusionary power of all markers in each population with both parents unknown was >0.98. These microsatellite loci should be combined with previously developed markers to select an optimal set for use in subsequent analyses of parentage and relatedness.

Keywords Microsatellites · SSRs · Galliformes · Northern Bobwhite · *Colinus virginianus*

This note has been submitted to *Conservation Genetics* and no other journal. We presented details regarding creation of the enriched library described in this manuscript in Schable et al. (2004). The primers described herein were developed in another laboratory and are independent of development efforts outlined in Schable et al. (2004), other than library construction. We have retained authors where appropriate to recognize their library development efforts. In this manuscript, we use updated sequencing and primer design techniques and different sets of individuals for polymorphism screening.

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Precision and accuracy of ecological parameters derived from microsatellite marker data are important to researchers. Ideally, loci within marker sets should: amplify cleanly and consistently across individuals and populations, have high levels of polymorphism, conform to Hardy–Weinberg equilibrium (HWE), and minimize variance in the estimated parameters (for review, see Selkoe and Toonen 2006). In certain cases, increasing the number of polymorphic loci increases both the precision and accuracy of parameter estimates (Blouin 2003; Milligan 2003; Csilléry et al. 2006). Often, this optimal set requires selection of microsatellite markers from a large candidate pool. We describe the isolation of 10 novel microsatellite loci from a commercially important, declining member of Order Galliformes, Northern Bobwhite (*Colinus virgnianus*; Brennan 1999; Burger et al. 1999). These microsatellite loci may be used in conjunction or combination with 23 previously described loci (Schable et al. 2004) to build an optimal set of genetic markers for use at the population, region, or range-wide scale.

Table 1 Characterization of 10 primer pairs amplifying microsatellite loci from Northern Bobwhite (*Colinus virginianus*) randomly sampled from a population in Grady Co., GA

Locus	Sequence	Accession	Anneal	*N*	*A*	Size range	Repeat	H_O	H_E	PIC
CV-P1A7 U	GTTTGTAGCACAGAGATGCTTG	EF687961	60	25	5	336–348	(GTT)^10	0.4*	0.8	0.7
CV-P1A7 L	***CAGTCGGGCGTCATCA***GTGCAGATGGATGTCAGCAG									
CV-P1E6 U	**CAGTCGGGCGTCATCA**TGTGCAAAGATCGTGAAAG	EF687964	60	27	7	196–217	(GTT)^7	0.4*	0.8	0.7
CV-P1E6 L	GTTTGCTTTCAGCTTGTTCCAGT									
CV-P1F2 U	***CAGTCGGGCGTCATCA***GTAAACTGCAGATGCAAAC	EF687965	60	22	8	230–248	(GT)^8	0.5	0.8	0.7
CV-P1F2 L	GTTTGTGCCATAGGTCTCCCCTG									
CV-P1F3 U	GTTTGTCACCCATCTCCCTGAATA	EF687966	60	26	9	382–404	(AC)^12	0.8	0.8	0.8
CV-P1F3 L	***CAGTCGGGCGTCATCA***CTCAGGCTGTATTGACACAAG									
CV-P2D10 U	GTTTCGGCTGAACATAAATTATCATT	EF687980	60	27	6	310–320	(GT)^8	0.3	0.8	0.7
CV-P2D10 L	***CAGTCGGGCGTCATCA***TGAAAGCTGGAGTGAGACTAAG									
CV-P2D7 U	GTTTACACTGGCTTGGTGCCTC	EF687983	60	27	2	265–269	(AAAC)^6	0.4	0.5	0.4
CV-P2D7 L	***CAGTCGGGCGTCATCA***CTGCTGCACTTGTGGTCTG									
CV-PBA4 U	***CAGTCGGGCGTCATCA***ATCAGCCCTCTGCTCC	EF687986	65	30	5	401–417	(AAAC)^5	0.6	0.7	0.7
CV-PBA4 L	GTTTACAACTTTCTGTCAACCTCATCG									
CV-PBH5 U	GTTTGCCACATTAACAGGAACGGG	EF687997	60	29	3	229–233	(AC)^7	0.1	0.1	0.1
CV-PBH5 L	***GGAAACAGCTATGACCAT***GAGTGAGGCAACATGACAGC									
CV-PCF5 U	***CAGTCGGGCGTCATCA***CCTGGCTGCTTTAGACATA	EF688003	65	30	16	188–226	(AC)^11	0.7	0.9	0.9
CV-PCF5 L	GTTTACAGGCTGAAATCATAACAG									
CV-PCG2 U	***CAGTCGGGCGTCATCA***TTTGGCCCCAGTCTCATCC	EF688004	65	25	4	295–311	(GTTT)^8	0.6	0.5	0.4
CV-PCG2 L	GTTTCAGAGGAAGGCACAAAGCC									

Sequences used to introduce sites for the universal primer are in bold italics. Underlined bases indicate sharing of nucleotides between CAG (5′-CAGTCGGGCGTCATCA-3′) tag, M13R (5′-GGAAACAGCTATGACCAT-3′) tag, or GTTT "pigtail" and the locus-specific primer-binding site. U: upper primer; L: lower primer; *N*: number of individuals genotyped at each locus; *A*: number of alleles; H_o: observed heterozygosity; H_E: expected heterozygosity; PIC: polymorphic information content; and * indicate deviation from Hardy–Weinberg equilibrium after Bonferroni correction ($P < 0.01$)

We developed a double-enriched microsatellite library as outlined in Schable et al. (2004). From this library, we selected 480 colonies containing inserts using the β-galactosidase gene and bi-directionally sequenced 95 positive (white) colony PCR products of 500–1,100 bp using BigDye (v3.1, PE Applied Biosystems) chemistry and an ABI PRISM 3730*xl* sequencer (PE Applied Biosystems). We edited sequences and assembled contigs using Sequencher 4.2 (Gene Codes Corp.). Using MSATCOMMANDER (Faircloth 2008), we located microsatellite repeat arrays within 50 contigs, designed primers, and applied 5′-tags (CAG or M13R) to 43 primer pairs for subsequent polymorphism testing (Boutin-Ganache et al. 2001; Glenn and Schable 2005). We reviewed contigs containing repeats for which the automated software could not design primers, and we attempted to manually design primers for these regions using Oligo 6.0 (Molecular Biology Insights). We added GTTT "pigtails" to the 5′-end of each primer lacking either CAG or M13-reverse tag to facilitate the addition of adenosine by *Taq* polymerase (Brownstein et al. 1996).

We optimized primer pairs using DNA extracted from feathers taken from seven Northern Bobwhite collected in Grady County, GA. We performed PCR amplifications in 10 µl volumes using MyCycler thermal cyclers (Bio-Rad Laboratories). Final concentrations for optimizing reactions were 10 mM Tris pH 8.4, 50 mM KCl, 0.5 µM "pigtailed" primer, 0.05 µM CAG or M13-reverse tagged primer (CAG or M13-reverse + primer), 0.45 µM dye-labeled tag (HEX or FAM + CAG or M13-reverse), 1.5 mM $MgCl_2$, 0.5 mM dNTPs, 0.5 U AmpliTaq Gold DNA Polymerase, and 6–10 ng DNA. M13R and CAG universal primers were labeled with FAM or HEX fluorescent dyes. We performed reactions using a touchdown thermal cycling program (Don et al. 1991), and we evaluated each primer at an initial annealing temperature of 60 and 65°C. Cycling parameters were: 1 cycle of 95°C for 5 min; followed by 20 cycles of 95°C for 20 s, either 60 or 65°C for 30 s −0.5°C per annealing cycle, and 72°C for 90 s; followed by 20 cycles of 95°C for 20 s, 50 or 60°C, respectively, for 30 s, 72°C for 90 s; and a final extension period of 10 min at 72°C.

We checked PCR products for amplification and scored each amplicon using an ABI PRISM 3730*xl* sequencer in combination with Genescan Rox500 fluorescent size standard, GeneMapper 4.0 Software (PE Applied Biosystems), and the global southern size calling method. We ran negative controls for each locus amplified. Following evaluation of the optimal annealing temperature for each locus, we genotyped 30 individuals randomly selected from two populations: Grady County, GA and Sussex and Southampton Counties, VA.

We calculated observed (H_O) and expected (H_E) heterozygosity, tested for HWE, and evaluated genotypic linkage disequilibrium using Arlequin (Schneider et al. 2000). We computed polymorphic information content for each locus and exclusionary power using Cervus 2.0 (Marshall et al. 1998; Kalinowski et al. 2007). We conducted a posteriori Bonferroni correction for each analysis consisting of multiple, concurrent statistical tests (Rice 1989).

Table 2 Characterization of 10 primer pairs amplifying microsatellite loci from Northern Bobwhite (*Colinus virginianus*) randomly sampled from a population in Sussex and Southampton Counties, VA

Locus	*N*	*A*	Size range	H_o	H_e	PIC
CV-P1A7	25	5	336–348	0.8	0.789	0.736
CV-P1E6	24	7	202–220	0.25	0.746	0.698
CV-P1F2	23	8	234–252	0.609	0.814	0.77
CV-P1F3	29	9	386–402	0.931	0.837	0.801
CV-P2D10	27	7	310–324	0.37	0.771	0.72
CV-P2D7	29	2	265–269	0.517	0.499	0.37
CV-PBA4	30	6	401–417	0.5	0.699	0.64
CV-PBH5	30	2	231–233	0.033	0.033	0.032
CV-PCF5	30	13	188–230	0.8	0.878	0.849
CV-PCG2	28	3	299–307	0.286	0.305	0.264

Table 1 summarizes the characteristics of 10 primer pairs amplifying microsatellite loci from the Northern Bobwhite and tested with individuals from Grady County, GA. The number of alleles per locus ranged from 2 to 16, averaging 6.5. Total exclusionary power with both parents unknown was >0.98. Two loci (CV-P1E6 and CV-P1A7) deviated from HWE ($P < 0.01$) after Bonferroni correction. We could not estimate an exact *P*-value for CV-P2D10 but $H_O \ll H_E$. We did not detect linkage disequilibrium after sequential Bonferroni correction. Table 2 summarizes the characteristics of primer pairs tested using individuals from Virginia. The number of alleles per locus ranged from 2 to 13, averaging 6.2. Total exclusionary power with both parents unknown was >0.98. We could not estimate exact *P*-values for CV-P1E6 or CV-P2D10, but in both cases $H_O \ll H_E$. No other loci deviated from HWE after Bonferroni correction. Deviations from HWE and loci with $H_O \ll H_E$ potentially resulted from small sample sizes. However, deviations may indicate the presence of null alleles. Issues with CV-P1E6 and CV-P2D10 in both populations suggest the results from these loci may be more than sampling artifact.

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