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PRIMER NOTE

Tetranucleotide and dinucleotide microsatellite loci from the northern bobwhite (*Colinus virginianus*)

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Abstract

We describe polymerase chain reaction (PCR) primers and conditions to amplify eight dinucleotide, one trinucleotide and 14 tetranucleotide microsatellite DNA loci isolated from the northern bobwhite (*Colinus virginianus*). The PCR primers were tested on 16 individuals collected from a population located within the Red Hills region of south Georgia and north Florida. The 23 primer pairs developed in this study yielded an average of 6.5 alleles per locus (range 2–11), an average observed heterozygosity of 0.47 (range 0.06–0.94) and average polymorphic information content of 0.60 (range 0.06–0.85).

Keywords: *Colinus virginianus*, Galliformes, microsatellites, PCR, primer, quail, SSR

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Bobwhites are small to medium-sized quail (140–170 g) distributed from southern Mexico north to southern New England and Canada (Stoddard 1931; Brennan 1999). Northern bobwhites are a commercially important species with estimated economic impacts in excess of $193 million in 1991 (Burger *et al*. 1999). Twenty-two subspecies of bobwhite are currently recognized throughout their range. Bobwhite populations are sedentary, year-round residents with short life spans and high mortality rates. During the nonbreeding season, they are highly social and live in groups or 'coveys' of 5–25 individuals. Females are indeterminate layers and persistent re-nesters, with females incubating and fledging up to three clutches per breeding season. Both males and females incubate nests, with males typically incubating only a single nest per season. In late summer, broods typically group together in bevies and are joined by adults in the early autumn. These groups eventually form the winter coveys (Stoddard 1931; Church & Taylor 1992; Brennan 1999).

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Similar to other Galliformes, northern bobwhites have undergone dramatic population declines across large portions of their range over the past 30 years. Drastic changes in landscape use, farming and forestry practices beginning in the late 1800s have greatly reduced bobwhite habitat, leading to a precipitous decline in the abundance of this species (Brennan 1991; Church & Taylor 1992). From 1965 to 1995, regional, statewide and local population declines ranging from 70 to 90% were recorded throughout 80% of the bobwhite's range. Fragmentation of suitable habitat within the bobwhite's range threatens to reduce gene flow among populations, potentially increasing the risk of inbreeding within isolated populations (Brennan 1999).

The development of a large panel of polymorphic microsatellite loci will enable investigations of population subdivision, gene flow among and within both isolated and panmictic populations, individual parentage, social group (covey) composition, relatedness within and among coveys across the landscape, and occurrence and degree of post-hatch brood amalgamation. Many of these questions were identified as significant barriers to current research by (Burger *et al*. 1995) and (Brennan 1999). Below we describe

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the development of markers that may be used to address these research needs.

DNA was extracted from blood obtained from a male northern bobwhite quail using a GenomicPrep Blood Isolation Kit (Amersham Biosciences). Extracted DNA was enriched for $(AC)_{12}$, $(AG)_{12}$, $(AAG)_8$, $(ATC)_8$, $(AAC)_8$, $(AAT)_{12}$, $(ACT)_{12}$, $(AAAC)_6$, $AAAG)_6$, $(AATC)_6$, $(AATG)_6$, $(ACCT)_6$, $(ACAG)_6$, $(ACTC)_6$, $(ACTG)_6$, $(AAAT)_8$, $(AACT)_8$, $(AAGT)_8$, $(ACAT)_8$ and $(AGAT)_8$ following a protocol modified from Hamilton *et al*. (1999). In brief, the DNA was digested with *Bst*UI (New England Biolabs), ligated to double-stranded SuperSNX24 linkers (forward 5′-GTTTAAGGCCTAGCTAGCAGAATC-3′, reverse 5′-GATTCTGCTAGCTAGGCCTTAAACAAAA-3′; modified from Hamilton *et al*. 1999), hybridized to biotinylated microsatellite oligonucleotides and captured on streptavidin-coated magnetic beads (Dynal). Unwanted DNA was washed away, captured DNA was recovered by polymerase chain reaction (PCR) using the SuperSNX24 forward primer. The product was ligated into PCR 2.1-TOPO vector and used to transform One Shot Top 10 Chemically Competent *Escherichia coli* cells (TOPO TA cloning kit, Invitrogen). Colonies were screened for inserts using the β-galactosidase gene and positive colonies were amplified using M13 forward and reverse primers. PCR products of 500–1000 base pairs were sequenced using Big Dye (version 3.0, Applied Biosystems) chemistry and an ABI 377–96 sequencer. Sequences from both strands were assembled and edited in SEQUENCHER 4.1 (Genecodes, Ann Arbor, MI) and exported to EPHEMERIS 1.0 (available at http://www.uga.edu/srel/DNA_Laboratory/dnacomputer_programs.htm) to automatically search sequences for microsatellites. PCR primers were developed, and an M13 reverse (5′-GGAAACAGCTATGACCAT-3′) or CAG tag (5′-CAGTCGGGCGTCATCA-3′) was added to the 5′ end of one of each primer pair using Oligo 6.67 (Molecular Biology Insights) to determine which tag would produce the fewest secondary structures. Inclusion of the 5′-tag allowed use of a third primer in the PCR (M13 reverse or CAG) that is fluorescently labelled for detection on the ABI 377 (cf. Boutin-Ganache *et al*. 2001).

Primer pairs were optimized using eight northern bobwhite DNA samples obtained from a population from the Red Hills region of Georgia and North Florida, USA. PCR amplifications were performed in a 25 μL volume using an Eppendorf Mastercycler Gradient thermal cycler. PCR final concentrations for optimizing reactions were 10 mM Tris–HCl pH 8.4, 50 mM KCl, 25.0 μg/mL bovine serum albumin, 0.4 μM unlabelled primer, 0.04 μM tag labelled primer, 0.36 μM universal dye labelled primer, 1.5 mM $MgCl_2$, 0.15 mM dNTPs, 0.5 units *Taq* DNA polymerase, and 50 ng DNA template. M13 and CAG universal primers were labelled with a FAM or HEX fluorescent dye. All primers were tested using one touchdown thermal cycling programme (Don *et al*. 1991) that encompasses a 10 °C span of annealing temperatures ranging between 60 and 50 °C. Cycling parameters were: 21 cycles of 96 °C for 20 s, the highest annealing temperature for 30 s minus 0.5 °C per annealing cycle, and 72 °C for 1 min; followed by 10 cycles of 96 °C for 30 s, 50 °C for 30 s, and 72 °C for 1 min. PCR products were initially scored for amplification on agarose gels, and successful PCR products were subsequently sized on an ABI 377–96 sequencer using a Gensize R500 fluorescent genotyping marker (Genetix). Results were analysed using GENESCAN and GENOTYPER software (PE Applied Biosystems). Following optimization an additional eight individuals were genotyped (Table 1). We calculated observed and expected heterozygosity and polymorphic information content for each locus using CERVUS 2.0 (Marshall *et al*. 1998). GENEPOP 3.3 (Raymond & Rousset 1995) was used to test for genotypic linkage disequilibrium.

Table 1 summarizes the characteristics of the 23 primer pairs developed from northern bobwhite quail. The number of alleles per locus ranges from 2 to 11, averaging 6.5. Total exclusionary power with both parents unknown exceeds 0.999. No significant linkage was detected following Bonferroni correction. Five loci (3, 9, 26, 27 and 34) deviated significantly from Hardy–Weinberg equilibrium following sequential Bonferroni correction. Three additional loci (21, 31 and 44) have P-values < 0.01 but are not significant following sequential Bonferroni correction. The observed deviation from Hardy–Weinberg probably reflects that birds were harvested from a small number of coveys potentially consisting of highly related individuals. It is also possible that the observed deviation from Hardy–Weinberg equilibrium is the result of the presence of null alleles. These loci were highly polymorphic in individuals from the Red Hills Region of north Florida and south Georgia, demonstrating their potential for helping researchers answer questions pertaining to northern bobwhite at levels spanning from parentage to population studies

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Table 1 Characterization of 23 primer pairs that amplify microsatellites from *Collinus virginianus*

Locus	Primer sequence (5′–3′)	GenBank Accession no.	Dye	Repeats in cloned allele	Clone size (bp)	*N*	*A*	Size range (bp)	H_O	H_E	PIC
Quail 3 U	GTG ATA CCC TCT GCT AGA C	AY523007	FAM	$(AC)_{13}$	120	16	9	110–136	0.44	0.89	0.844
Quail 3 L	*CAGTCGGGCGTCATCA* CCA GCA ATA CAA GAG TTT A										
Quail 9 U	TGG CTG AAC TGA TGT ATT A	AY523014	FAM	$(AC)_{23}$	234	16	10	200–238	0.19	0.77	0.727
Quail 9 L	*CAGTCGGGCGTCATCA* CCT GGA GTA TTA GCA CAC A										
Quail 10 U	*CAGTCGGGCGTCATCA* GCA GGA AAT AGA TAG GTA GAT	AY523015	FAM	$(ACAG)_4$ … $(ACCT)_4$	168	16	5	164–180	0.44	0.66	0.580
Quail 10 L	CCA CTC ACT GCC TTA CTA										
Quail 11 U	*CAGTCGGGCGTCATC* AGC TCA TTC TCA TTC ATA TT	AY523016	FAM	$(AAAC)_5$	194	16	2	194–198	0.38	0.51	0.371
Quail 11 L	ACT GAA AAC AAG CAA GTT A										
Quail 13 U	*CAGTCGGGCGTCATC* AAA CAG TTC AAG TCT TCA AAT A	AY523018	FAM	$(AC)_{35}$	170	16	10	116–170	0.94	0.84	0.787
Quail 13 L	GCA GTC TTC GCT TGA TAC										
Quail 14 U	*CAGTCGGGCGTCATCA* GCA TTT GCG TTT AAG TAA G	AY522953	FAM	$(AAAC)_6$	240	16	3	232–244	0.19	0.33	0.294
Quail 14 L	CCA AGC TCA GTG TCT AAG A										
Quail 16 U	TGA AAT GAT GTG AGG AGT AT	AY522955	FAM	$(AAC)_7$	168	16	3	162–168	0.19	0.18	0.166
Quail 16 L	*CAGTCGGGCGTCATCA* AAT CCT CAT AGT TCT TCA CAT A										
Quail 17 U	TTG TTG CCT TTC TTG TAA	AY522956	FAM	$(AC)_{13}$	160	16	11	136–172	0.88	0.88	0.840
Quail 17 L	*CAGTCGGGCGTCATC* AAT AAA ACC AAA GAT CAT TAG TA										
Quail 21 U	*GGAAACAGCTATGACCATG* CAC ACA CAA AAT GAG TAA GT	AY522965	FAM	$(AATG)_{11}$	180	16	6	168–188	0.56	0.76	0.692
Quail 21 L	TAA AAT GGG AAC AGT GTC										
Quail 22 U	TGA TCC CTA AAA CAA CAT A	AY522966	FAM	$(ACAG)_4$ … $(AGAT)_{15}$	204	16	10	156–208	0.94	0.89	0.848
Quail 22 L	*CAGTCGGGCGTCATCA* CCT TGG ACC TCT GTA AT										
Quail 23 U	*CAGTCGGGCGTCATCA* GAT TCA CTG CAT TTG TTA G	AY522967	FAM	$(AAAG)_{16}$	252	16	10	226–272	0.88	0.87	0.824
Quail 23 L	GGC AGA AAC TTA TGA CAT										
Quail 24 U	*GGAAACAGCTATGACCATG* AAA TGT GTT GAA GGA AGT AT	AY522968	HEX	$(AAAC)_6$	154	16	6	148–164	0.750	0.74	0.673
Quail 24 L	CTG TTT GCC TCC AGT C										
Quail 25 U	*CAGTCGGGCGTCATC* AGG TAT TCT ATC AAG ATT CTA TC	AY522969	HEX	$(AAAC)_5$	150	16	2	146–150	0.13	0.23	0.195
Quail 25 L	GTT TTT GTT ACG CAG TTT										
Quail 26 U	*CAGTCGGGCGTCATCA* CCT AGA ACA AAG CAC AG	AY522970	FAM	$(ACAG)_4(AGAT)_{12}$	203	16	9	172–219	0.563	0.877	0.832
Quail 26 L	TCC TTA TTT GAG AGC TTA			… $(ACCT)_6$							
Quail 27 U	TAG TCA GTG AAG CAA TAA G	AY522971	FAM	$(AAAC)_5$	240	15	4	170–244	0.133	0.678	0.592
Quail 27 L	*CAGTCGGGCGTCATCA* CCA GAA TTT CGA TAC AC										
Quail 30 U	*CAGTCGGGCGTCATC* AAT AAA ACC AGA AAT AAA CTC A	AY522978	FAM	$(AC)_{14}$	289	16	8	277–293	0.875	0.817	0.764
Quail 30 L	CGA TCC ACC TGA AAG TA										
Quail 31 U	GTG CTT GAG AAA TGA GTT A	AY522979	FAM	$(AC)_{13}$	154	16	9	152–170	0.563	0.833	0.782
Quail 31 L	*CAGTCGGGCGTCATCA* GCA GGC AAA ATC TAA AC										
Quail 32 U	*GGAAACAGCTATGACCATG* AAG ATA TGC AGG TTA GAT TA	AY522980	HEX	$(AAAC)_6$	192	16	6	182–204	0.313	0.435	0.404
Quail 32 L	TTC CCA CCA TGT TAT CTA										
Quail 34 U	TTG GTC AGG AAT CAC ATA	AY522983	FAM	$(ACAG)_5(AC)_7$	299	16	6	283–311	0.063	0.764	0.704
Quail 34 L	*GGAAACAGCTATGACCAT* GCA GGA GAC AGA CTA TGA										
Quail 41 U	*CAGTCGGGCGTCATC* ACT TGT TTT ACT TCT GGA ATA	AY522996	FAM	$(AAAT)_7$ … $(AAAC)_5$	316	16	9	290–336	0.625	0.815	0.762

Table 1 *Continued*

Locus	Primer sequence (5′–3′)	GenBank Accession no.	Dye	Repeats in cloned allele	Clone size (bp)	*N*	*A*	Size range (bp)	H_O	H_E	PIC
Quail 41 L	AAA TGT TTC TGG GAT CTT										
Quail 44 U	*CAGTCGGGCGTCATCA* TCC ACA GCA GGT CAG TA	AY523000	FAM	$(AAAC)_6$	208	16	7	192–208	0.625	0.760	0.705
Quail 44 L	CCC TCT CCA CTT GGT ATA										
Quail 46 U	CTA CTG AAC CGT GAT GTA T	AY523002	FAM	$(AAAC)_6$	136	16	2	128–136	0.063	0.063	0.059
Quail 46 L	*CAGTCGGGCGTCATCA* GTT TAA CAG TTC CAT GAA TAT										
Quail 47 U	TAT CCA TCT CTC CAT CTA TCT	AY523003	FAM	$(AG)_7$	187	16	2	187–303	0.188	0.272	0.229
Quail 47 L	*CAGTCGGGCGTCATCA* GCT CCA GGA AAA CAT AAT										

Sequences used to introduce sites for the universal primer are in italics. Clone size refers to the size of the PCR product amplified from the clone used to develop each locus which was identical to the size predicted from the DNA sequence.
N, sample size; *A*, number of alleles; size range refers to the observed distribution of alleles at each locus; H_O, observed heterozygosity; H_E, expected heterozygosity, PIC, polymorphic information content.

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