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TECHNICAL NOTE

Eighteen microsatellite loci developed from western burrowing owls (*Athene cunicularia hypugaea*)

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Abstract Western burrowing owls (*Athene cunicularia hypugaea*) are ground-dwelling owls distributed throughout western North America. Because of population declines, this species is considered endangered in Canada, and burrowing owls are listed as a species of conservation concern in states of the western USA. Korfanta et al. (2002) previously presented primers for seven microsatellite loci in burrowing owls. Parentage and relatedness studies require a larger number of markers for accuracy and precision. Here, we developed and characterized 18 additional microsatellite DNA loci, and we tested these loci in 23 individuals. The number of alleles per locus ranged from 2 to 11; two loci deviated from Hardy–Weinberg equilibrium following Bonferroni correction; we did not detect linkage disequilibrium following Bonferroni correction; and the probability of exclusion for parent pairs using all loci was >0.9999. We envision these loci will facilitate detailed analyses of the genetic mating system of burrowing owls, which is poorly understood.

Keywords Microsatellites · SSRs · Burrowing owls · *Athene cunicularia* · Strigidae

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Western burrowing owls (*Athene cunicularia hypugaea*) are small, ground-dwelling owls native to western North America. These owls nest in underground burrows typically dug by mammals and lay clutches of up to 14 eggs. Sparse and low vegetation, such as that in grasslands and steppes, characterizes burrowing owl habitat (Haug et al. 1993), but owls can be abundant in agricultural areas (Conway et al. 2006; Moulton et al. 2006; Restani et al. 2008). Adult owls frequently nest in loose colonies (Lantz et al. 2007), but they also defend the space around their nests from conspecifics (Moulton et al. 2004). Northern burrowing owl populations are generally obligate migrants, whereas those to the south are year-round residents. Many populations have declined in abundance, often in response to the eradication of ground-dwelling mammals and/or loss of habitat. Thus, numerous American states list burrowing owls as a species of concern (Klute et al. 2003). Burrowing owls are federally endangered in Canada.

Korfanta et al. (2002) previously developed primers for seven microsatellite DNA loci and used these loci to investigate genetic variation among North American western burrowing owl populations (Korfanta et al. 2005). Their analyses also included comparisons between the

disjunct western and Florida (*A. c. floridana*) subspecies of burrowing owls. Korfanta et al. (2005) found that populations within subspecies were essentially panmictic and genetic differentiation across subspecies was modest, although the western and Florida forms were easily distinguishable based on allelic absences in Florida populations, assignment tests, and well-supported branches on the inferred phylogenetic tree (Korfanta et al. 2005). Despite population declines, there was also no evidence for genetic bottlenecks (Korfanta et al. 2005).

Microsatellite loci have not been applied to studies of burrowing owl mating behavior or individual relatedness within nesting colonies, results of which interest conservation biologists and behavioral ecologists. Analysis of mating systems and relatedness is a task that requires a moderate to large number of genetic markers (Blouin 2003; Marshall et al. 1998; Milligan 2003; Selkoe and Toonen 2006). Thus, our objective was to develop a microsatellite panel sufficient to facilitate these studies that could be used alone or in combination with previously developed loci. Here we describe the isolation and characterization of 18 new microsatellite loci, building upon the panel previously developed by Korfanta et al. (2002).

We developed a double-enriched microsatellite library following Glenn and Schable (2005) and incorporating Invitrogen MyOne streptavidin beads (Invitrogen, Inc.; Faircloth et al. 2009) using DNA purified (5-Prime ArchivePure Blood Kit) from blood taken from a female burrowing owl collected in Idaho. From this library, we selected 760 positive (white) colonies using the β-galactosidase gene and bi-directionally sequenced 285 colony PCR products of 500–1,200 base pairs using 1/16th BigDye [v3.1, Applied Biosystems (ABI)] sequencing reactions and an ABI PRISM 3730*xl* sequencer. We aligned and edited sequences and assembled 184 contigs using Sequencher 4.2 (Gene Codes Corp.). We screened contigs against themselves, using BLAST (Altschul et al. 1990), to test for sequence homology, and we removed duplicate contigs. Using BLAST (Altschul et al. 1990), we also screened contigs for high-probability matches (*e*-score = 1×10^{-5}) to burrowing owl sequences (Korfanta et al. 2002) present within GenBank (Benson et al. 2008). None of the contigs screened matched burrowing owl sequences within GenBank.

Using MSATCOMMANDER (Faircloth 2008), we located microsatellite repeat arrays within 59 contigs (32%), designed 33 primers, and applied 5′-tags (CAG or M13R) to primer pairs for polymorphism testing (Boutin-Ganache et al. 2001; Glenn and Schable 2005). We manually designed primers ($N = 12$), for microsatellite-containing contigs where MSATCOMMANDER indicated primer design errors, using Oligo 6.0 (Molecular Biology Insights) and the 5′-tagging approach. We added GTTT "pigtails" to the 5′ end of all primers lacking either CAG or M13R tag to facilitate the addition of adenosine by *Taq* polymerase (Brownstein et al. 1996).

We tested 45 primer pairs for amplification using DNA collected from three burrowing owls using DNeasy kits (Qiagen Inc.). Prior to amplification, we treated DNA samples 1:1 (v/v) with 10% chelex resin (BioRad Laboratories), and we added 5 ng DNA to each PCR reaction. We performed all PCR amplifications in 10 μL volumes using ABI 9700 thermal cyclers in combination with the reaction mix and cycling parameters (60°C touchdown PCR; −0.5°C step; Don et al. 1991) presented in Faircloth et al. (2009). We labeled M13R and CAG universal primers with VIC, NED, FAM or PET fluorescent dyes (ABI). We scored amplicons using an ABI Prism 3730*xl* DNA Sequencer in combination with LIZ600 fluorescent size standard (ABI), GeneMapper 4.0 Software (ABI) and the Local Southern size calling method.

Based on the performance of primers during the initial test, we selected 28 primer pairs for subsequent optimization and polymorphism testing. We did not select primer pairs for additional testing that were monomorphic ($N = 10$, 22%) or failed to amplify cleanly ($N = 7$, 16%) during the initial test.

Using DNA collected from 23 individual burrowing owls and purified with Qiagen DNeasy kits, we optimized and screened selected primers using conditions identical to those presented above. We did not produce and analyze amplicons using multiple annealing temperatures because a majority of peak morphologies were clear and easily resolved at a starting annealing temperature of 60°C. We removed loci from the candidate set yielding ambiguous peaks or inconsistent results.

We calculated observed (H_O) and expected (H_E) heterozygosity, polymorphic information content (PIC), and exclusion probability using Cervus 3.0 (Marshall et al. 1998; Kalinowski et al. 2007), and we tested for deviations from Hardy–Weinberg equilibrium (HWE) and evaluated genotypic linkage disequilibrium (LD) using Genepop (Raymond and Rousset 1995). We conducted *a posteriori* Bonferroni correction (Rice 1989) for each analysis consisting of multiple, concurrent statistical tests (HWE and LD).

Table 1 presents the characteristics of 18 primer pairs amplifying microsatellite loci in burrowing owls. Amplification success was 99.3%, and the number of alleles ranged from two to 11, averaging 5.1. BOOB-BM4-H06 and BOOB-BM4-A01 deviated ($P < 0.01$) from HWE following Bonferroni correction, and we were unable to estimate deviation from HWE for BOOB-RM2-H08. We did not detect LD following Bonferroni correction. The exclusion probability for parent pairs was >0.9999. As indicated by the probability of exclusion, the microsatellite

Table 1 Characteristics of 18 primer pairs amplifying microsatellite DNA loci in western burrowing owls (*Athene cunicularia hypugaea*)

Locus	Sequence	Accession	T_A	N	A	Range	Repeat	H_O	H_F	PIC
BUOW-BM4-A01 U	***GGAAACAGCTATGACCAT***AGGATCTCCCAAACATTCTGGC	GQ228862	60	23	7	321–349	(ATCT)^14	0.35[a]	0.76	0.72
BUOW-BM4-A01 L	GTTTGAATCTGGACTAGATGACCTCC									
BUOW-BM4-A09 U	***CAGTCGGGCGTCATCA***GCACTTAGGGACATGGTTTAGTGG	GQ228853	60	23	5	375–391	(AGAT)^15	0.48	0.74	0.68
BUOW-BM4-A09 L	GTTTCCTATGAAGACCCTCAAGCCC									
BUOW-BM4-A10 U	***GGAAACAGCTATGACCAT***GTCCCTGAAATGTCAATACTTA	GQ228863	60	23	10	177–215	(AGAT)^9	0.74	0.82	0.77
BUOW-BM4-A10 L	GTTTGCTTGTGATAACAGTGAA									
BUOW-BM4-B06 U	GTTTCCTTATTACAAATTCACAGTG	GQ228868	60	23	11	186–227	(AG)^10	0.91	0.87	0.83
BUOW-BM4-B06 L	***CAGTCGGGCGTCAT***CAGTTCACTTTTATACATACTCCT									
BUOW-BM4-B12 U	GTTTCTCTTAGGTTTGGACTGGGACG	GQ228871	60	23	7	325–348	(ATCT)^8…(AAT)^4	0.83	0.74	0.68
BUOW-BM4-B12 L	***CAGTCGGGCGTCATCA***TGCTAGCCGTATTCCTCTACCC									
BUOW-BM4-C12 U	***CAGTCGGGCGTCATCA***TCTCTCTTGCCAGGTGTTCAGG	GQ228875	60	22	7	305–330	(ATCT)^14	0.68	0.78	0.74
BUOW-BM4-C12 L	GTTTAAGCGATTTGGGAACTGGTTGG									
BUOW-BM4-D03 U	GTTTCAGTGAGAGTGGGTTAACAGGC	GQ228876	60	23	4	407–421	(AAAT)^4	0.44	0.55	0.44
BUOW-BM4-D03 L	***CAGTCGGGCGTCATC***AGGAAGATGGGTTTCAGGAACAG									
BUOW-BM4-E11 U	***CAGTCGGGCGTCATCA***TCTGCTCAGTAACACAAAGCTGG	GQ228884	60	23	5	347–371	(AGTT)^7	0.35	0.38	0.34
BUOW-BM4-E11 L	GTTTATCTGGCTACAATGCTTCAGCG									
BUOW-BM4-F11 U	***GGAAACAGCTATGACCA***TGCTCAACAACTGCTCATGAAGG	GQ228889	60	22	6	374–392	(AAC)^11	0.64	0.78	0.72
BUOW-BM4-F11 L	GTTTACCCATCTGCCGATACGCAAG									
BUOW-BM4-H06 U	***CAGTCGGGCGTCATCA***TTTAGGAGCAAACCAGGGAGGC	GQ228854	60	23	5	374–386	(AC)^13	0.22[a]	0.68	0.63
BUOW-BM4-H06 L	GTTTGCCAGTCCAGTGAGGTGTTACG									
BUOW-RM2-B12 U	***CAGTCGGGCGTCATCA***GGCTTCCCTCTACAGCAGGTC	GQ228917	60	23	5	236–246	(AG)^12	0.57	0.68	0.61
BUOW-RM2-B12 L	GTTTGCTAAGCATTACCTCACATTGTTCC									
BUOW-RM2-C12 U	GTTTCTCATGCGGCTAATGGACAACC	GQ228927	60	23	3	166–170	(GT)^6	0.30	0.38	0.32
BUOW-RM2-C12 L	***CAGTCGGGCGTCATCA***TGGCCTGTAGGGACTTGGATAG									
BUOW-RM2-D04 U	***CAGTCGGGCGTCATCA***GCTACCAGAATTTGGGCATGGG	GQ228931	60	23	3	226–230	(AC)^7	0.65	0.50	0.42
BUOW-RM2-D04 L	GTTTACATCTGGCATTATGTTTCCCTTC									
BUOW-RM2-H07 U	GTTTGAGGACAGTGCTGAAGTAGCC	GQ228964	60	22	2	253–255	(AAC)^6	0.50	0.50	0.37
BUOW-RM2-H07 L	***GGAAACAGCTATGACCA***TTCTAGGCAGCTGATGGGAGAC									
BUOW-RM2-H08 U	***CAGTCGGGCGTCATC***ACAGCTCCAGTAGGCAACATAAAG	GQ228965	60	23	2	237–241	(CT)^6	0.04	0.04	0.04
BUOW-RM2-H08 L	GTTTGGGATGGATCACAGGCATTTC									
BUOW-RM3-1-C04 U	GTTTGCACTGGTGCCAAACCTC	GQ228990	60	23	3	300–309	(AAAC)^5	0.30	0.48	0.38
BUOW-RM3-1-C04 L	***CAGTCGGGCGTCATC***ACTCAGCTAATGCATCCAGTTTCC									
BUOW-RM3-1-C09 U	GTTTGCAGCCTTTGGCAAGTCATTC	GQ228993	60	23	2	275–279	(AGGT)^6	0.26	0.23	0.20
BUOW-RM3-1-C09 L	***CAGTCGGGCGTCATCA***TCTGGCTGCTATCACTACCTTG									

Table 1 continued

Locus	Sequence	Accession	T_A	N	A	Range	Repeat	H_O	H_F	PIC
BUOW-RM3-1-H08 U	***CAGTCGGGCGTCATCA***GCAGAGGTTGTGCAGAGTTCAG	GQ229029	60	23	4	197–211	(AAAG)^4	0.57	0.54	0.44
BUOW-RM3-1-H08 L	GTTTATAGAGAGCGCCCAGTATGTCC									

Sequences used to introduce sites for the universal, fluorescent tags are in bold italics. Underlined bases indicate sharing of nucleotides between the CAG tag (5′-CAGTCGGGCGTCATCA-3′), the M13R tag (5′-GGAAACAGCTATGACCAT-3′), or the locus-specific primer binding site and the GTTT "pigtail"

U upper primer, *L* lower primer, T_A touchdown PCR starting anneal temperature, *N* number of individuals successfully genotyped at each locus, *A* number of alleles, H_O observed heterozygosity, H_E expected heterozygosity, *PIC* polymorphic information content

[a] Loci deviating from HWE following Bonferroni correction

loci identified in Table 1 should be sufficient for future studies of burrowing owl mating behavior and relatedness when used alone or in combination with the microsatellite loci characterized by Korfanta et al. (2002).

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