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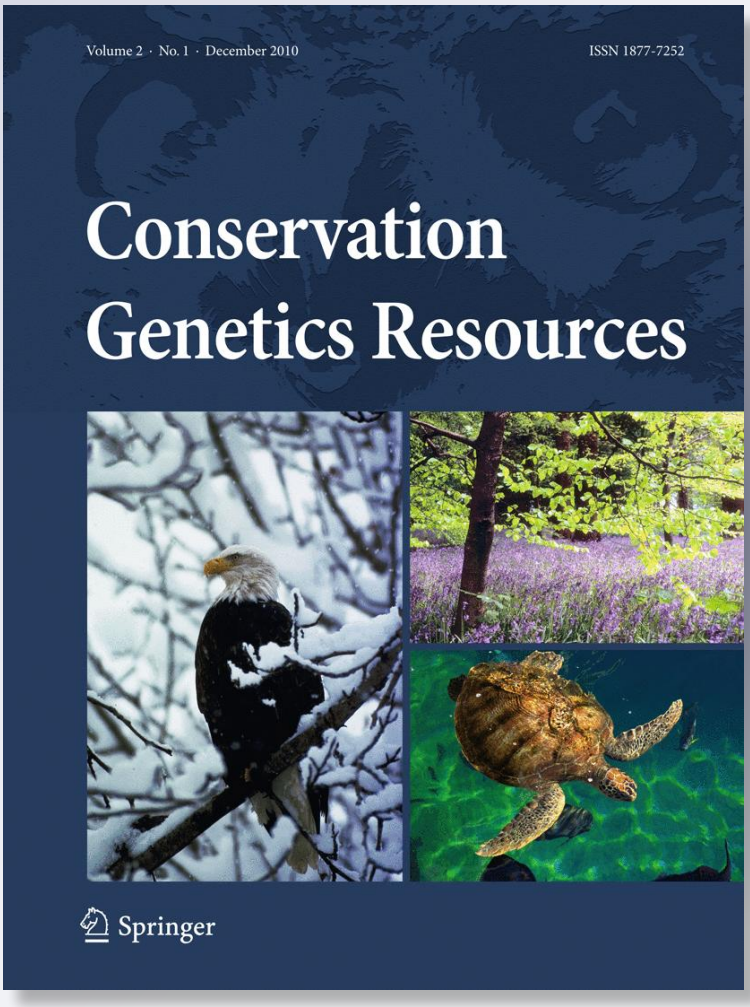

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TECHNICAL NOTE

Novel microsatellite loci for the burrowing owl *Athene cunicularia*

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Abstract The breeding distribution of western burrowing owl is experiencing an intriguing southward shift, contrary to the predictions of climate change. To determine the breeding dispersal patterns underlying this distributional change, we developed 11 novel polymorphic microsatellite loci for the species. We tested these loci in two burrowing owl breeding populations, one from central Sinaloa, Mexico, and one from the Central Valley of California, USA. All loci were at Hardy–Weinberg equilibrium, except two loci for the California population. Expected heterozygosity was relatively high ($\bar{H}_E = 0.813$, range 0.515–0.942). Average number of alleles was 11.64 (range 5–25). We found no evidence of linkage disequilibrium for any pairwise tests between loci.

Keywords Burrowing owl · *Athene cunicularia* · Microsatellites · Sinaloa · California

The western burrowing owl (*Athene cunicularia hypugaea*) has undergone an intriguing distributional change since the mid twentieth century. While many avian distributions in North America are shifting northwards in response to climate change (Hitch and Leberg 2007; La Sorte and Thompson 2007), the breeding distribution of the burrowing owl is shifting in the opposite direction. Burrowing owl populations near the northern edge of the species' breeding range in southern Canada and northern United States are declining or even disappearing (Wellicome and Holroyd 2001; Klute et al. 2003; Conway and Pardieck 2006). Because of these persistent population declines, the species has been legally protected in Canada, Mexico and the United States (Klute et al. 2003). Paradoxically, burrowing owl populations in irrigated agricultural valleys of the Sonoran desert of California and Arizona have increased during the second half of the twentieth century (Sauer et al. 2008) and may now support the highest breeding densities in the species' range (DeSante et al. 2004). The breeding distribution of burrowing owls has also expanded southwards into coastal Sonora and Sinaloa in northwestern Mexico, where recent agricultural development of coastal thornscrub has created suitable breeding habitat in an area that formerly only supported wintering owls (Enriquez-Rocha 1997). Breeding densities in the agricultural areas of coastal Sonora and Sinaloa appear to be as high as those in the Imperial Valley of California. We developed and characterized 11 new microsatellite loci to estimate migration rates among burrowing owl populations in North America and determine the breeding dispersal patterns underlying this odd distributional change. The addition of these 11 loci more than doubles the existing set of seven microsatellite loci for this species (Korfanta et al. 2002).

We constructed an enriched genomic DNA library using a modified version of a published protocol (Glenn and Schable 2005). We isolated genomic DNA using the DNeasy Blood & Tissue Kit (Qiagen®) from <25 μL of blood collected from 10 owls captured in US Army Pueblo Chemical Depot, Fort Carson Army Base, and Buckley Air Force Base, Colorado. We mixed all DNA from these 10 individuals. We digested DNA with RsaI (NEB®) and ligated fragments to double-stranded SuperSNX-24 linkers (Glenn and Schable 2005). We recovered linker-ligated

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Table 1 Eleven microsatellite loci developed for the burrowing owl

Locus (GenBank accession no.)	Cloned repeat	Primer sequences (5′–3′)	Size range (bp)	Clone size (bp)	Sinaloa ($n = 40$)			California ($n = 40$)		
					N_A	H_O	H_E	N_A	H_O	H_E
ATCU04 (GU167941)	$(CA)_3TG(CA)_{18}$	F: TTCATGGGTTTATGATCTGACTTC	349–367	335	5	0.500	0.515	10	0.800	0.764
		R: AGCCATTCCCTTCAGTCTTC								
ATCU06 (GU167942)	$(CT)_8CA(CT)_{13}$	F: GAAATGGAAGGAGGAGTGC	201–255	199	15	0.925	0.888	13	0.875	0.863
		R: GCCATCCCTAATGCTTGTG								
ATCU08 (GU167943)	$(CA)_{20}$	F: GCCCTCATATCATTAAGATCCTTC	223–293	211	25	0.925	0.942	25	0.925	0.942
		R: GGATTGTCATTTCCCCTCAG								
ATCU13 (GU167944)	$(GT)_{17}$	F: ACCCCGAGTGCTCTAGTCAG	222–258	221	10	0.725	0.821	10	0.775	0.733
		R: GTTGTGAAGCGAGGGATG								
ATCU20 (GU167945)	$(CA)_{15}$	F: GTTGCCATCATAGCAGCAG	171–197	154	11	0.900	0.881	11	0.925	0.875
		R: GCCAGATAACTACCCCAAATG								
ATCU28 (GU167946)	$(GT)_{10}AT(GT)_9$	F: CAGTGTCAGAGTCAAGACATGC	328–352	312	10	0.875	0.833	9	0.775	0.800
		R: TGGAGAGGTTTAGGGCTAGG								
ATCU36 (GU167947)	$(GT)_{13}$	F: TTGCACAGAAAATCCTGAGTC	397–413	374	8	0.725	0.812	7	0.675	0.682
		R: AACAAGAGTTACCTGAAGAGATGC								
ATCU39 (GU167948)	$(GT)_{18}$	F: GTGTGGGTTGCCTCACATC	159–189	160	13	0.800	0.851	13	0.725	**0.848**
		R: AACATCCAGGAAACAAGATGC								
ATCU41 (GU167949)	$(CA)_{12}$	F: AGAGATAGTAGTTTAGGGTAGGCTC	201–223	188	7	0.725	0.768	9	0.550	**0.728**
		R: ACGACACTTCTAGCACGTTG								
ATCU43 (GU167950)	$(CA)_{19}$	F: GATCAGCTTGCAGCAAAGG	174–212	174	14	0.825	0.843	12	0.900	0.821
		R: GGGAGATGTTGAGGAAATCG								
ATCU45 (GU167951)	$(GATA)_8GGTA$ $(GATA)_2$	F: CTACCGAGCAGTGACAGTTTG	242–282	215	9	0.775	0.824	10	0.800	0.847
		R: GGGTGGACAGTTCCTCATTC								

Number of alleles (N_A), and the observed (H_O) and expected (H_E) heterozygosities are shown for populations in central Sinaloa, Mexico and in Naval Air Station Lemoore, California. All individuals successfully amplified for all loci. The two boldface H_E values denote loci that deviated significantly from HWE

fragments ranging from 300 to 1,400 bp using the polymerase chain reaction (PCR), SuperSNX-24 forward primer, and Platinum high-fidelity Taq DNA polymerase (Invitrogen®) to create a PCR library. We hybridized these recovered fragments to 5′-biotinylated microsatellite oligonucleotide probes $(GT)_{15}$, $(CT)_{15}$, $(GATA)_{10}$, and $(GACA)_8$. We captured hybridized fragments on streptavidin-coated paramagnetic beads (Dynal®) and recovered these fragments by PCR. We immediately ligated fragments into the vector PCR4-TOPO (Invitrogen®) and transformed them into TOP10 chemically competent *Escherichia coli* cells (Invitrogen®) following the manufacturer's protocol. We directly amplified and sequenced 273 colonies in both directions using M13 primers on an Applied Biosystems 3730XL DNA Analyzer using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems®). Seventy-seven clones contained microsatellite sequences. We designed 45 primer pairs out of the 77 sequences using program Primer 3 (Rozen and Skaletsky 2000), with 11 polymorphic loci successfully amplifying (Table 1). We labeled forward primers with universal M13 primers at the 5′ end (Schuelke 2000). We designed reverse primers with a 'pig-tail' at the 5′ end to reduce variability in adenylation of amplification products (Brownstein et al. 1996). We performed PCR reactions in a 15 μL volume containing 10–50 ng genomic DNA, 1X PCR buffer (20 mM Tris–HCl pH 8.4, 50 mM KCl, Invitrogen®), 0.2 mM each dNTP, 0.02 μM unlabelled M13-tailed forward primer, 0.2 μM reverse pig-tailed primer, 0.2 μM fluorescently labeled M13 primer, 2 mM $MgCl_2$, 0.4 U Taq DNA polymerase (Invitrogen®), and 0.02% BSA. We used a unique touchdown protocol for all loci consisting of an initial denaturation at 94°C for 4 min followed by 10 cycles at 94°C for 30 s, annealing at 60–52°C for 90 s (2°C decrease every two cycles), extension at 72°C for 30 s, followed by 30 cycles at 94°C for 30 s, annealing at 50°C for 30 s and 72°C for 30 s, and a final extension of 7 min at 72°C. We analyzed PCR products on an Applied Biosystems 3730 Genetic Analyzer and scored alleles using Applied Biosystems Genotyper 3.7. We used program

Tandem (Matschiner and Salzburger 2009) to assign integers to DNA fragment sizes. We calculated observed and expected heterozygosities and deviations from Hardy–Weinberg equilibrium (HWE) using MS Excel© macro Genalex (Peakall and Smouse 2006). We calculated genotypic linkage disequilibrium with program Genepop (Raymond and Rousset 1995; Rousset 2008) using the Fisher's method. We used program Micro-Checker (Van Oosterhout et al. 2004) to detect the presence of null alleles and estimate their frequencies (Chakraborty et al. 1992). We performed statistical analyses with an $\alpha = 0.05$ adjusted for multiple comparisons through sequential Bonferroni tests (Rice 1989).

We genotyped 40 non-related owls from breeding populations in irrigated agricultural areas near Culiacán, in the Mexican State of Sinaloa, and 40 non-related owls from Naval Air Station Lemoore, in the Central Valley of California, USA. Average observed and expected heterozygosities were 0.791 and 0.816 for the Sinaloa population, and 0.793 and 0.809 for the California population, respectively (Table 1). Mean number of alleles was 11.73 (range 5–25) and 11.55 (range 7–25) for the Sinaloa and California populations, respectively. All loci were in HWE in both populations, except loci ATCU39 and ATCU41, which showed a deficit of heterozygotes in the California population (Table 1). Micro-Checker suggested the presence of null alleles at ATCU39 and ATCU41 for the California population, with frequencies of 0.0781 and 0.1396, respectively. We found no evidence of linkage disequilibrium for any pairwise tests between loci.

This set of 11 polymorphic microsatellite loci will provide a high resolution for testing different breeding dispersal patterns across North America that could explain the observed distributional changes described above. Particularly, we will test if migratory burrowing owls from declining populations near the northern edge of the species' breeding range are becoming resident breeders in the irrigated agricultural valleys of the arid southwestern United States and northwestern Mexico.

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