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GENETIC VARIATION AMONG ISLAND AND CONTINENTAL POPULATIONS OF BURROWING OWL (*ATHENE CUNICULARIA*) SUBSPECIES IN NORTH AMERICA

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ABSTRACT.—Burrowing Owls (*Athene cunicularia*) have a large geographic range spanning both North and South America and resident populations occur on many islands in the eastern Pacific Ocean and the Caribbean Sea. Many owl populations are isolated and disjunct from other populations, but studies on genetic variation within and among populations are limited. We characterized DNA microsatellite variation in populations varying in size and geographic isolation in the Florida (*A. c. floridana*), the Western (*A. c. hypugaea*), and the Clarion (*A. c. rostrata*) subspecies of the Burrowing Owl. We also characterized genetic variation in a geographically isolated population of the western subspecies in central Mexico (near Texcoco Lake). Clarion Burrowing Owls had no intrapopulation variation (i.e., fixation) at 5 out of 11 microsatellite loci, a likely outcome of genetic drift in an isolated and small population. The Florida subspecies had only polymorphic loci but had reduced levels of genetic variation compared with the more-widespread western subspecies that occurs throughout western North America. Despite the extensive geographic distribution of the Western Burrowing Owl, we found genetic differentiation between the panmictic population north of the Trans-Mexican Volcanic Belt and the resident Texcoco Lake population in central Mexico.

KEY WORDS: *Burrowing Owl*; *Athene cunicularia*; *Clarion Island*; *DNA microsatellites*; *Mexico*; *subspecies*.

VARIACIÓN GENÉTICA ENTRE POBLACIONES ISLEÑAS Y CONTINENTALES DE SUBESPECIES DE *ATHENE CUNICULARIA* EN AMÉRICA DEL NORTE

RESUMEN.—*Athene cunicularia* tiene un amplio rango de distribución que cubre América del Norte y del Sur y las poblaciones residentes que se encuentran en muchas islas en el este del Océano Pacífico y en el Mar Caribe. Muchas poblaciones de búhos están aisladas y desconectadas entre sí. Sin embargo, los estudios

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realizados sobre su variación genética intra e inter poblaciones son escasos. Caracterizamos la variación microsatelital en poblaciones con tamaño variable y aislamiento geográfico de las subespecies *A. c. floridana*, *A. c. hypugaea* y *A. c. rostrata*. También caracterizamos la variación genética en una población geográficamente aislada de *A. c. hypugaea* del centro de México (cerca del Lago Texcoco). La subespecie *A. c. rostrata* no presentó variación intra-poblacional (i.e., fijación) en 5 de los 11 loci microsatelitales, probablemente como resultado de deriva génica en una población aislada y pequeña. La subespecie *A. c. floridana* presentó únicamente loci polimórficos, pero tuvo bajos niveles de variación genética en comparación con la subespecie *A. c. hypugaea*, más ampliamente distribuida a través del oeste de América del Norte. A pesar de la amplia distribución geográfica de *A. c. hypugaea*, encontramos diferenciación genética entre la población panmíctica al norte del Eje Volcánico Transversal de México y la población residente del Lago Texcoco del centro de México.

[Traducción del equipo editorial]

The study of genetic variation across subspecies and among populations that differ in population size may reveal the species' evolutionary response to current human-driven environmental changes. Therefore, managers and policy-makers need genetic studies so they can prioritize conservation efforts among populations or restore extirpated populations with birds from the appropriate genetic pool. Genetic variation is strongly correlated with population size (Frankham 1996). In addition, the maintenance of genetic variation is frequently regarded as a mechanism of adaptation and population persistence in changing environments (Lande and Shannon 1996). Subsequently, it is a tenet in conservation biology to preserve genetic variation in any at-risk population.

Burrowing Owls (*Athene cunicularia*) are widespread in North and South America, inhabiting open arid and semiarid plains from southwestern Canada to Tierra del Fuego, including islands in the Caribbean Sea and eastern Pacific Ocean (König et al. 1999). Eighteen subspecies are currently recognized. These subspecies designations are based on variation in size, weight, and plumage coloration (König et al. 1999), with three subspecies in North America: Clarion (*A. c. rostrata*), Florida (*A. c. floridana*), and Western (*A. c. hypugaea*) Burrowing Owls. Population sizes and conservation status varies among Burrowing Owl subspecies and populations. The Western Burrowing Owl is federally endangered in Canada (Committee on the Status of Endangered Wildlife in Canada [COSEWIC] 2006) and is a species of conservation concern in the United States (Klute et al. 2003), the Clarion Burrowing Owl is a federally endangered endemic subspecies in Mexico (Secretaría de Medio Ambiente y Recursos Naturales [SEMARNAT] 2010), and the Florida Burrowing Owl is a state-threatened species endemic to Florida

and the Islands of the Bahamas (Florida Fish and Wildlife Conservation Commission 2011).

The Clarion Burrowing Owl is the most isolated of the three recognized subspecies in North America (Fig. 1), occupying Clarion Island (approximately 20 km²) of the Revillagigedo Archipelago in the Pacific Ocean, about 700 km southwest of the Baja California peninsula. The Clarion Burrowing Owl was negatively affected by feral pigs (*Sus scrofa*) that were once common on Clarion Island before their removal in 2002 (Everett 1988, Brattstrom 1990, Comisión Nacional de Áreas Naturales Protegidas [CONANP] 2004). In 2014, 100–200 owls were estimated to persist on Clarion Island (G. Holroyd and H. Trefry unpubl. data). The Florida subspecies is restricted to approximately 65,000 km² of the Florida peninsula and the Bahama Islands, with an estimated population size of 1757 individuals (Bowen 2001). The Florida subspecies is genetically differentiated from the western subspecies (Korfanta et al. 2005). The Western Burrowing Owl is the most widespread subspecies, occupying a relatively contiguous breeding distribution over the western half of North America (Wellicome and Holroyd 2001, Conway 2018). However, isolated Western Burrowing Owl populations may exist on islands off the Baja California Peninsula (Palacios et al. 2000) and south of the published breeding range in central and southern Mexico (Enríquez-Rocha 1997), where Burrowing Owls have been observed during the breeding season, but their status has not been adequately studied in these areas.

We used DNA microsatellite markers to characterize genetic variation among the three North American Burrowing Owl populations (i.e., Clarion, Florida, and Western) to determine the effect of population size on within-population genetic diversity (Fig. 1). We also examined genotyped individuals from a population beyond the published

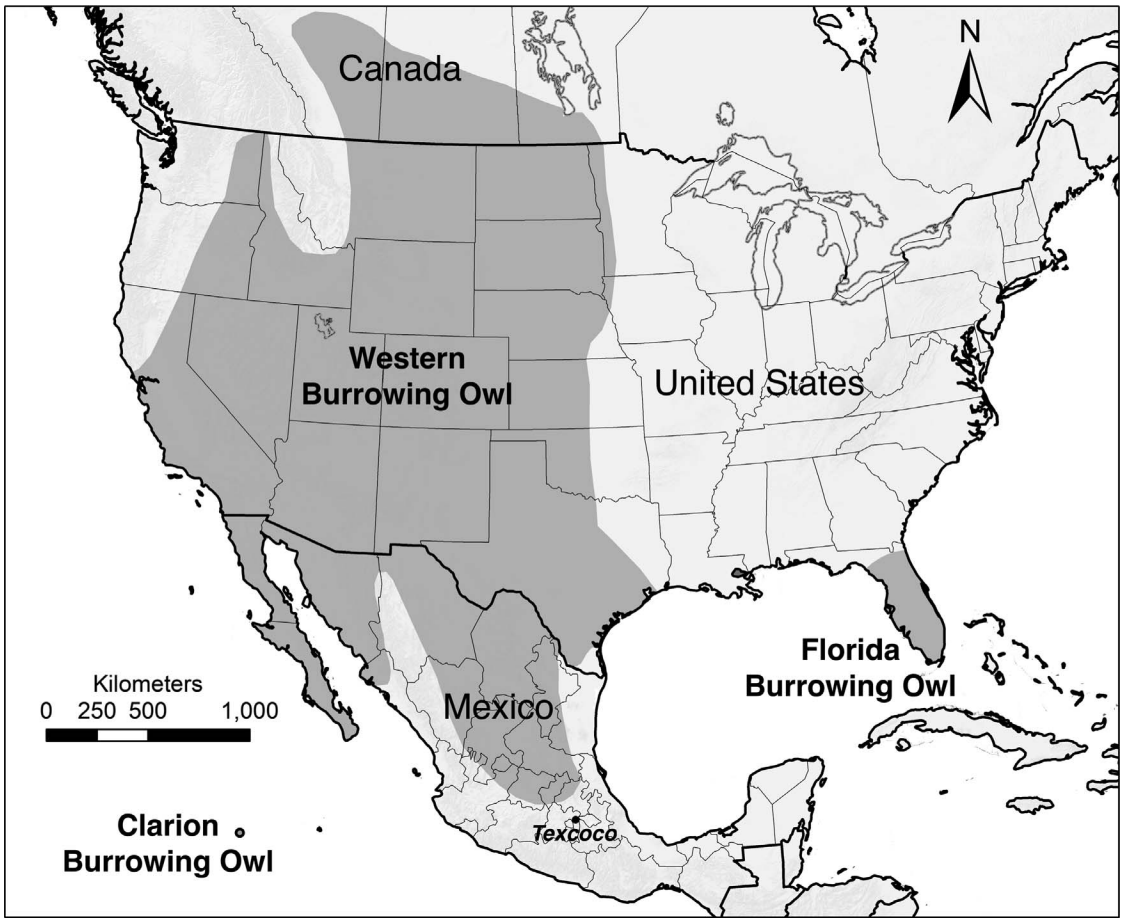


Figure 1. Insular (Clarion) and isolated mainland (Texcoco) study populations of Burrowing Owls in Mexico. The gray area denotes the breeding distribution of the western Burrowing Owl and the Florida Burrowing Owl (after Poulin et al. 2011).

southern edge of the Western Burrowing Owl's breeding range (at Texcoco Lake, Mexico) to explore the effect of geographic isolation on genetic diversity. The Texcoco population inhabits the area around the ancient Texcoco Lake, near Mexico City (Holroyd et al. 2011). This resident population appears to be geographically isolated from the rest of the subspecies' breeding distribution (the nearest known breeding population is approximately 400 km to the north).

METHODS

Study Areas. We trapped Burrowing Owls during the spring and summer of 2004–2009. Within the range of the Western Burrowing Owl (Fig. 1), we obtained DNA samples from 1671 Burrowing Owls

from 37 locations representing two provinces in Canada, 11 states in the United States, and eight states in Mexico, including 21 Burrowing Owls from Texcoco (Fig. 1). Key features of the Western Burrowing Owl breeding habitat are sparse woody vegetation, availability of burrows, and low slope within grassland, steppe, and desert biomes, and human environments such as agricultural fields and vacant urban lots (Poulin et al. 2011). The number of individuals sampled per population (location) ranged from 15–73 (\bar{x} = 43.3, SD = 17.1). See Macías-Duarte (2011) for more information on study locations and number of owls sampled per location for the Western Burrowing Owl. Birds on Clarion Island are year-round residents inhabiting different portions of the island. Lower elevations have thick

clumps of morning glory (*Ipomea pescaprae* Convolvulaceae), slopes have scattered shrubs (*Brickellia peninsularis*), and uplands are dominated by low trees (*Karwinskia humboldtiana*) (Brattstrom 1990, Mendez and Lomeí 2002). We collected samples from 19 owls on Clarion Island in November 2008. Florida Burrowing Owls are also year-round residents and they breed in altered, open grassy landscapes including cleared forests, filled wetlands, and suburban areas, airports, and industrial parks (Millsap and Bear 2000). Unlike Western Burrowing Owls, Florida Burrowing Owls dig their own burrows. We collected samples from 40 owls at Cape Coral and Marco Island in southwestern Florida. Burrowing Owls occupy urban areas throughout the city of Cape Coral (Lee County) and on Marco Island (Collier County), including landscaped yards, vacant lots, residential areas, and near the many canals that criss-cross the city (Millsap and Bear 2000). These two Florida locations are thought to support some of the highest Burrowing Owl densities in Florida.

Sample Collection. We did not include samples from any birds that were known to be closely related to other sampled owls (i.e., parents and their offspring, or juveniles from the same nest burrow). Our primary source of genomic DNA was blood. We obtained approximately 50 μ L of blood through a venipuncture of the brachial vein. We also used flight and/or body feathers occasionally (4%) as the source of genomic DNA when we could not obtain a blood sample. We handled birds, and collected and imported/exported blood and feathers in compliance with Canadian, Mexican, and US regulations, including acquisition of CITES export permits (MX 27314, MX 31960, MX 37048, MX44697 and 07CA01521/CWHQ), scientific collection permit (MX: SPGA/DGVS/08336/07), and Bird Banding Lab permits #10157 and #22524. We also complied with the University of Arizona Institutional Animal Care and Use Committee regulations under protocols #01-089 and 04-196.

Genotyping. We followed laboratory procedures for DNA extraction, polymerase chain reaction, and allele scoring for 11 microsatellite loci (ATCU04, ATCU06, ATCU08, ATCU13, ATCU20, ATCU28, ATCU36, ATCU39, ATCU41, ATCU43, and ATCU45) as described in Macías-Duarte et al. (2010). We used the DNeasy Blood & Tissue Kit (Qiagen®) to isolate genomic DNA. We performed PCR reactions in a 15- μ L volume mixture 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 μ M unlabelled M13-tailed forward primer, 0.2 μ M reverse pig-tailed primer, 0.2 μ M 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 sec, annealing at 60–52°C for 90 sec (2°C decrease every two cycles), extension at 72°C for 30 sec, followed by 30 cycles at 94°C for 30 sec, annealing at 50°C for 30 sec and 72°C for 30 sec, 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.

Data Analysis. We used Microsoft Excel macro *GENALEX* 6 (Peakall and Smouse 2006) to calculate standard measures of genetic variability within Burrowing Owl subspecies including number of alleles (N_a), observed heterozygosity (H_o), and expected heterozygosity (H_e). We used *GENEPOP* (Rousset 2008) to calculate multiloci F_{ST} (Weir and Cockerham 1984) between the Texcoco population and each of the other 36 populations of Western Burrowing Owl, as well as all F_{ST} between pairwise comparisons among the 36 populations. We used a two-sample permutation test in mean F_{ST} between these two groups (Texcoco vs. the other 36 populations, and among all other populations) using package *lmPerm* (Wheeler and Torchiano 2016) in program R (R Core Team 2015). We used a permutation test rather than a parametric test because estimates of F_{ST} are not independent.

RESULTS

The amount of genetic variation within populations varied considerably among Clarion, Florida, and Western Burrowing Owls (Table 1). The Western Burrowing Owl had high levels of genetic variation at all loci and all populations. Western Burrowing Owl populations had high observed heterozygosities ($H_o = 0.823 \pm 0.022$), similar to that of the isolated Texcoco population in central Mexico ($\bar{H}_o = 0.807 \pm 0.037$) in spite of the large difference in sample size (1650 and 21 owls, respectively). However, this large difference in sample size between the Texcoco population and the other 36 populations of the Western Burrowing Owl subspecies was evident in the mean number of alleles per loci (7.7 vs. 22.5 alleles, respectively). The

Table 1. Estimates of genetic variation per microsatellite loci in the Clarion, Florida, and Western Burrowing Owl subspecies compared to a geographically isolated population (Texcoco) of the western subspecies in central Mexico. N_a , H_o , and H_e denote number of alleles, and the observed and expected heterozygosity, respectively. Bold numbers indicate fixed loci.

LOCUS	CLARION ISLAND			FLORIDA			TEXCOCO			WESTERN		
	N_a	H_o	H_e	N_a	H_o	H_e	N_a	H_o	H_e	N_a	H_o	H_e
ATCU04	2	0.421	0.499	5	0.683	0.666	3	0.571	0.625	18	0.756	0.763
ATCU06	2	0.053	0.051	4	0.512	0.676	8	0.762	0.761	31	0.882	0.892
ATCU08	10	0.947	0.859	13	0.805	0.793	15	1.000	0.907	38	0.933	0.949
ATCU13	1	0.000	0.000	4	0.610	0.649	6	0.857	0.747	18	0.767	0.786
ATCU20	2	0.000	0.100	5	0.634	0.638	8	0.667	0.803	21	0.868	0.890
ATCU28	2	0.158	0.145	4	0.341	0.355	6	0.810	0.754	16	0.803	0.816
ATCU36	1	0.000	0.000	2	0.122	0.424	8	0.714	0.723	17	0.705	0.793
ATCU39	1	0.000	0.000	6	0.561	0.580	9	0.857	0.823	22	0.888	0.892
ATCU41	1	0.000	0.000	3	0.390	0.366	6	0.905	0.702	20	0.744	0.771
ATCU43	1	0.000	0.000	4	0.659	0.580	8	0.833	0.787	31	0.854	0.853
ATCU45	6	0.368	0.500	6	0.707	0.715	8	0.905	0.769	15	0.851	0.856
Mean	2.6	0.177	0.196	5.1	0.548	0.586	7.7	0.807	0.764	22.5	0.823	0.842
SE	0.9	0.090	0.088	0.9	0.059	0.044	0.9	0.037	0.022	2.3	0.022	0.018

Florida Burrowing Owl had lower levels of genetic variation ($\bar{H}_0 = 0.548 \pm 0.059$ and $N_a = 5.1 \pm 0.9$) compared to the Western Burrowing Owl, but had no unique alleles and no fixed loci (also see Korfanta et al. 2005). In contrast, the Clarion Burrowing Owl had even lower levels of genetic variation ($\bar{H}_0 = 0.177 \pm 0.090$ and $N_a = 2.6 \pm 0.9$) and had five fixed loci (45%; Table 1), but did not have any unique alleles.

Our results suggest that the Texcoco Burrowing Owl population is genetically different from all other populations of the Western Burrowing Owl. The mean pairwise F_{ST} for comparisons involving the Texcoco population ($F_{ST} = 0.0386 \pm 0.0011$) is significantly higher (two-sample permutation test, $P < 0.001$) than the mean pairwise F_{ST} among all other 36 Western Burrowing Owl populations ($F_{ST} = 0.0085 \pm 0.0003$).

DISCUSSION

The continental populations of the Western Burrowing Owl had higher genetic diversity and variation (mean $N_a = 22.5$ and mean $H_e = 0.842$ across 11 loci) than the Florida and Clarion Island populations, as predicted by population genetics theory. The total number of Western Burrowing Owl individuals was recently estimated as 700,000 (Partners in Flight Science Committee 2013), which is much larger than the Florida and Clarion populations by more than two orders of magnitude. In this regard, our microsatellite marker data corroborate

the results from Korfanta et al. (2005) that the Florida Burrowing Owl has lower levels of genetic variation than the Western Burrowing Owl. Korfanta et al. (2005) reported that the Florida Burrowing Owl had a 37% lower expected heterozygosity than that of Western Burrowing Owl, quite similar to the difference found in our marker set (33%). In addition, the patterns of genetic variation did match with the smaller estimates of population size for the Clarion Burrowing Owl (100–200 individuals in 2008 and 2014; G. Holroyd unpubl. data) and the Florida Burrowing Owl (1757 individuals; Bowen 2001). The estimate of 1700 owls on Clarion Island in 2003 by Wanless et al. (2009) was likely a substantial overestimate caused by extrapolation to unsuitable areas (i.e., the unlikely assumption that owl density was constant throughout the island based on a single transect).

Our results highlight the importance of monitoring population size and genetic diversity in the Clarion Burrowing Owl to help guide habitat management and predator control for this endangered subspecies and our results provide a baseline for such effort. The existence of fixed alleles in this population (in 5 of 11 loci) and lower heterozygosity suggests a population bottleneck, which may have occurred during colonization of the island or as the result of a reduced population size caused by introduced domestic mammals. In the late 1800s, Burrowing Owls were considered “common” on Clarion Island (Townsend 1890). In January of 1986,

≥10 owls were seen during several days of informal surveys on the island (Everett 1988). Therefore, active management actions to benefit owl populations on Clarion Island (e.g., installing artificial nest burrows) would likely help to halt additional loss of genetic variation (i.e., stop allele fixations via genetic drift). In addition, the removal of artificial food sources that benefit one of the owl's primary predators, the Clarion Raven (*Corvus corax clarionensis*), would likely increase survival of the Clarion Burrowing Owl. The Florida Burrowing Owl, on the other hand, may be able to buffer anthropogenic disturbances in a metapopulation fashion (*sensu* Hanski 1998) given their much larger geographic range compared to that of the Clarion Burrowing Owl.

Our microsatellite data also suggest that the Western Burrowing Owl population near Texcoco in central Mexico is genetically different from all other Western Burrowing Owl populations. This population inhabits the open areas in the former Texcoco Lake at an elevation above 2000 m. Perhaps the genetic divergence from the other Western Burrowing Owl populations reflects a recent colonization event of Texcoco Lake. This owl population is likely nonmigratory (Holroyd et al. 2011) and, hence, drift due to small population size may have caused the observed subtle divergence from other Western Burrowing Owl populations. The Western Burrowing Owl population at Texcoco Lake inhabits an area with a large human population in a highly urbanized landscape, and this differentiated population deserves the attention of conservation biologists and land managers. In addition, Burrowing Owl specimens have been collected during the breeding season in the State of Hidalgo and Veracruz (Enríquez-Rocha 1997), and more breeding populations that may also have diverged from the northern populations probably exist south of the Neovolcanic Axis. Why the Texcoco Lake populations are somehow genetically isolated (as suggested by the microsatellite data) is an interesting puzzle given the short distance (approximately 400 km) to other reported breeding populations. We recommend an extensive, well-designed survey effort to better delineate the southern limit of the Western Burrowing Owl's breeding range in Mexico. This information would help determine the species' meta-population dynamics (colonization and extinction of local populations) in central Mexico and help to assess owl population viability in Texcoco. Moreover,

detailed demographic studies are needed in both locations to determine the threats that these unique populations face, the burrowing animals that they rely on (Conway 2018), and whether they are self-sustaining.

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