CHANGE IN MIGRATORY BEHAVIOR AS A POSSIBLE EXPLANATION FOR BURROWING OWL POPULATION DECLINES IN NORTHERN LATITUDES

By

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As members of the Dissertation Committee, we certify that we have read the dissertation prepared by Alberto Macias-Duarte entitled "Change in Migratory Behavior as a Possible Explanation for Burrowing Owl Population Declines in Northern Latitudes", and we recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy

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Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copies of the dissertation to the Graduate College.
I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

Dissertation Director: Courtney J. Conway Date: 04/01/11

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SIGNED: Alberto Macias-Duarte

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DEDICATION

This dissertation is dedicated with all my love to my wife Maria Eumelia, my son Alberto and my daughter Mariana.

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ABSTRACT

Recent observed changes in bird distributions provide an unprecedented opportunity to gain a deeper understanding of the processes that influence species' persistence. By modelling presence-absence data from the North American Breeding Bird Survey, we found evidence that the breeding range of the western burrowing owl has contracted at its northern, western, and eastern boundaries since 1967. We suggest that the species' breeding distribution is also expanding southwards to former wintering grounds into northern Mexico, facilitated by the appearance of new breeding habitat created by irrigated agriculture in the arid areas of southwestern United States and northwestern Mexico. This dissertation explores the hypothesis that burrowing owls from northern migratory populations have become resident breeders in areas of northwestern Mexico that were formerly used only by migratory owls during winter, contributing to both population declines near the northern extent of the species' breeding range and population increases in the southern half of the species' range. We used novel DNA microsatellite markers to test patterns of gene flow predicted by this migration-mediated range-shift hypothesis. We genotyped 1,560 owls from 36 study locations in Canada, Mexico, and the United States. Analyses of molecular variance provided evidence that burrowing owl populations in both northwestern Mexico and Canada are genetically different from the rest of the populations in the breeding range, lending some support to the migration-mediated range-shift hypothesis. We found evidence of subtle genetic differentiation associated with subtropical irrigated agricultural areas in southern Sonora and Sinaloa, demonstrating that land use can produce location-specific population

dynamics leading to genetic structure even in the absence of dispersal barriers. We also used stable isotopes ^{2}H, ^{13}C, and ^{15}N in feathers to test philopatry and breeding dispersal patterns predicted by this migration-mediated range-shift hypothesis. Burrowing owl populations near the northern edge of the species' breeding range had a high proportion of immigrants compared to interior populations, while other populations had high levels of philopatry. Stable isotopes also provided evidence of breeding dispersal events from Canadian populations to northwestern Mexico in support of the migration-mediated range-shift hypothesis, but similar isotope signatures in nestling feathers between these 2 regions prevent stronger inferences.

CHAPTER I. DISTRIBUTIONAL CHANGES IN THE WESTERN BURROWING OWL (*ATHENE CUNICULARIA HYPUGAEA*) IN NORTH AMERICA FROM 1967-2008

1. Abstract

Quantifying the extent to which bird distributions shift in response to recent changes in climate provide an unprecedented opportunity to gain a deeper understanding of the processes that influence species' persistence. We used data from the North American Breeding Bird Survey (BBS) to document changes in the distributional limits of the western burrowing owl from 1967 to 2008. We used logistic regression to model presence probability (p) as a function of longitude, latitude, and year. We modeled a linear trend in logit(p) through time with slope and intercept modeled as a double Fourier series of longitude and latitude. We found that the western burrowing owl has experienced an intriguing southward shift in the northern half of its breeding range, contrary to what is predicted by most species' niche models and what has been observed for many other species in North America. The burrowing owl's breeding range has been shrinking in its northern, western, and eastern edges. Our model detected population declines observed in California and eastern Washington where maps based on route-specific estimating equations predict significant population increases in those locations. We suggest that the northern boundary of the burrowing owl's breeding distribution has contracted southward and the southern boundary of the species' breeding distribution has

expanded southward into areas of northern Mexico that were formerly used only by wintering migrants.

2. Introduction

Understanding the factors that constrain species' distributions may provide important insights into the processes that limit population growth and species persistence. Indeed, several well-known textbooks have suggested that the central question in ecology is: *What determines the abundance and distribution of organisms?* (Andrewartha 1961, Krebs 2009). Understanding the answer to this "central question in ecology" is of particular interest for species of high conservation concern and for migratory birds because understanding the causes underlying changes in both the breeding and wintering distributions can help to understand the factors that led to the evolution of, and currently maintain, migratory behavior. In this regard, the rapid changes in climate during the past 50 years (Solomon et al. 2007) are providing a natural experiment by which breeding and wintering distributions of many species are moving polewards as atmospheric temperatures continue to rise (Thomas and Lennon 1999, Hitch and Leberg 2007, La Sorte and Thompson 2007). This response may be driven by several interacting ecological factors, including species' physiological tolerance to temperature and changes in the distribution of food and other resources. Therefore, identifying bird species that fail to follow this poleward distributional shift is critical because these species may have either intrinsic or extrinsic limitations to adapt to further changes in climate. In this regard, special attention must be paid to species that are declining, not increasing, at the northern edge of their breeding and wintering ranges. The western burrowing owl

(*Athene cunicularia hypugaea*), a migratory bird of conservation concern, appears to be one such species. Burrowing owl populations near the northern edge of the species' breeding range have declined (Desmond et al. 2000, Wellicome and Holroyd 2001, Klute et al. 2003, Conway and Pardieck 2006). Hypotheses proposed to explain population declines at the northern and eastern limits of the species' breeding range include extirpation of black-tailed prairie dogs (*Cynomis ludovicianus*), the reduction in quality of breeding habitat caused by conversion of grassland to agriculture, pesticides, and collisions with vehicles (Haug et al. 1993, Clayton and Schmutz 1999, Klute et al. 2003). However, all of the hypotheses proposed in the scientific literature have failed to fully explain the extent and the location of observed population declines (Holroyd et al. 2001). These documented changes in burrowing owl abundance have apparently led to changes in the species' breeding range. The most recent range map depicting the current and historical distribution of the burrowing owl in North America (Fig. 1 in Wellicome and Holroyd 2001) suggests a contraction at the northern and eastern edges of the species' breeding distribution in southern Canada and through the eastern Great Plains. The current and historical distributional limits were drawn based on numerous sources of information: published literature (Zarn 1974, Wedgwood 1978, Haug et al. 1993, Sauer et al. 2001), papers from the Second International Burrowing Owl Symposium, and surveys and opinions of burrowing owl experts. In this chapter, we provide a quantitative, model-based approach to document the changes in burrowing owl breeding range limits based on presence-absence data generated by the North American Breeding Bird Survey (BBS)

from 1967 to 2008. We also compare the performance of our approach to the population trend maps in Sauer et al. (2008) and the range map of Wellicome and Holrord (2001).

3. Methods

Range limits of a species are difficult to define (Gaston 2003). Several analytical approaches have been suggested to allow investigators to draw range limits on maps based on presence-absence data (Fortin et al. 2005). We followed an approach that intended to model the change in the range limits of burrowing owls as a dynamic process that involves time without partitioning the dataset into discrete subsets of space and time. We used BBS data (USGS Patuxent Wildlife Research Center 2009) from 1967 to 2008 to fit a logistic regression model to predict the probability of burrowing owl presence as a function of longitude, latitude, and year. Logistic regression is a generalized linear model whose link function is:

$$\text{logit}(p) = \log\left(\frac{p}{1-p}\right),$$

where p is the probability of presence on a BBS route, given that ≥1 burrowing owl was detected from 1967-2008. We modeled logit(p) to be a linear function of year (t):

$$\text{logit}(p) = \beta_0(x,y) + \beta_1(x,y)t \qquad (1)$$

where x and y are longitude and latitude, respectively. We modeled the spatial variation of the linear temporal trend in logit(p) by making the intercept (β_0) and the slope (β_1) a function of longitude and latitude. By following this procedure, we avoided partitioning the dataset into discrete subsets for each BBS route to obtain local estimates of temporal trends. Partitioning the data into subsets creates as many models as the number of BBS

routes with burrowing owls (i.e., 588 BBS routes), each with 2 parameters (β_0 and β_1, and hence 1176 regression parameters total). Hence, our approach is a more parsimonious way to examine temporal changes in the breeding distribution of the burrowing owl. We also avoided the problem of complete or quasi-complete separation by BBS route (when a year *t* exists such us that only absences are recorded before *t* and only presences are recorded after *t*, or vice versa) in the maximum likelihood estimation procedure (Hosmer and Lemeshow 1999) which can lead to numerical errors. We used a double Fourier series to model for $\beta_0(x,y)$ and $\beta_1(x,y)$ in equation (1). This approach assumes that $\beta_0(x,y)$ and $\beta_1(x,y)$ can be modeled as a sum of two-dimensional wavelets of different frequencies. In this regard, each $\beta_j(x,y)$ (j = 0,1) in equation (1) is a linear combination of sine and cosine functions given by

$$\beta_j(x,y) = \sum_{m=0}^{h}\sum_{n=0}^{h}\beta_{1\,jmn}\cos\left(\frac{n\pi x}{L_x}\right)\cos\left(\frac{m\pi y}{L_y}\right) + \sum_{m=1}^{h}\sum_{n=0}^{h}\beta_{2\,jmn}\cos\left(\frac{n\pi x}{L_x}\right)\sin\left(\frac{m\pi y}{L_y}\right) + \sum_{m=0}^{h}\sum_{n=1}^{h}\beta_{3\,jmn}\sin\left(\frac{n\pi x}{L_x}\right)\cos\left(\frac{m\pi y}{L_y}\right) + \sum_{m=1}^{h}\sum_{n=1}^{h}\beta_{4\,jmn}\sin\left(\frac{n\pi x}{L_x}\right)\sin\left(\frac{m\pi y}{L_y}\right) \quad (2)$$

where each β_{ijmn} (for sub-indices i = 1,…, 4; j = 0, 1; m = 0,…, h; n = 0,…, h) is a logistic regression coefficient, and L_x and L_y are the basic harmonic wavelengths equal to a half of the longitudinal range and latitudinal range of BBS routes, respectively. We used the deviance (-2log$\mathcal{L}$, where $\mathcal{L}$ is the maximized likelihood) and the number of regression parameters (β_{ijmn}) in the models to determine the harmonic (h) for which the model performed better in describing large-scale patterns in the probability of species' presence. Akaike's Information Criterion (AIC; Anderson 2007) was not a suitable tool for

selecting the number of harmonics to use in the double Fourier series in equation (2) because AIC decreased as the number of harmonics increased, but increasing the fit of the model by means of AIC did not provide visually stable contours of burrowing owl presence probabilities and generated numerous "bull's eyes" surrounding individual (or clusters of) BBS routes. Instead, we used 3 harmonics in the double Fourier series which provided the best balance between deviance (model fit) and the number of model parameters (model complexity) (Fig. 1). We added a low-degree polynomial in x and y to $\beta_j(x,y)$ ($j = 0,1$) in equation (1) to lessen the effect of periodic extension produced by the Fourier series at the limits of the data range (i.e., the tendency of the Fourier series to fit the same values in the response at the extreme of the dataset range in both x and y directions, Eubank and Speckman 1990). Specifically, we added $\beta_1 x + \beta_2 y + \beta_3 xy$, where each β_k is also a logistic regression coefficient. From equation (1), the probability of burrowing owls being present (i.e., a location being occupied with longitude x and latitude y at year t) is given by:

$$p(x,y,t) = \frac{e^{\beta_0(x,y)+\beta_1(x,y)t}}{1+e^{\beta_0(x,y)+\beta_1(x,y)t}} \qquad (3)$$

Based on equation (3), each observed datapoint (x_k, y_k, t_k) (for $k = 1, 2, \ldots, 11350$) was then classified as "burrowing owls present" if $p(x_k, y_k, t_k) \geq p_0$ for a cutoff value p_0, or "burrowing owls absent" otherwise. We defined the burrowing owl range limit as the surface $p(x,y,t) = p_0$, where p_0 is the cutoff value where 90% of the predicted absences outside of the predicted breeding range were actually observed absences. In this way, the area outside of the predicted breeding range contained few instances where burrowing

owls were present. We plotted the curves $p(x,y,1967) = p_0$ and $p(x,y,2008) = p_0$ to visualize the extent of change in the distributional limits of burrowing owls over the past quarter-century (from 1967 to 2008). We used the *glm* command in the *stats* package of program *R* version 2.10.1 for Mac® (R Development Core Team 2009) to fit the BBS data to our logistic regression model.

Sampling effort has changed since the initial implementation of the BBS and may hinder our ability to accurately model the probability of burrowing owl's presence in space and time. The number of BBS routes surveyed (and hence the number of routes with ≥1 burrowing owl detection) has steadily increased since the initial implementation of the BBS in 1967 (Fig. 2), which creates an unbalanced sampling design in the *year* variable. Balanced designs reduce bias (i.e. regression coefficients shifting away from zero, Firth 1993) in maximum likelihood estimates for logistic regression in discrete variables (Dietrich 2005). We used simulations to determine if the increase in sampling intensity of BBS biased our results and was exclusively responsible for the inferred spatio-temporal patterns in the probability of presence by the original dataset. We ran 10 simulations by randomly assigning a presence or absence value to each BBS route sampled through 1967-2008 using a Bernoulli distribution. We used the average yearly proportion of BBS routes with presence of burrowing owls estimated from our logistic regression analysis as the Bernoulli parameter.

4. Results

Our model suggests temporal and spatial changes in the likelihood of detecting burrowing owls on BBS routes throughout North America. The overall proportion of BBS routes at

which surveyors detected burrowing owls has decreased in several areas near the northern and eastern edge of the burrowing owl distribution, especially in southern Canada, in eastern North and South Dakota, in eastern Nebraska, and in southern Texas since the first half of the 1970s (Fig. 3). In this regard, our logistic regression model suggests a contraction of the burrowing owl's breeding distribution, primarily at the edges of its range (Fig. 4). Overall, the burrowing owl's breeding range evidently retreated from 1967 to 2008 in southern and northern California, Washington, southern Canada, eastern North and South Dakota, eastern Nebraska, eastern Kansas, and southern Texas. Our model also suggests an expansion towards unoccupied areas in southern Montana, eastern Oregon, central Nevada, and the Four Corners region (Fig. 4).

All 10 simulations (not shown) failed to reproduce the range contraction observed when we modeled the original dataset. Our simulations produced inconsistent, random contractions and expansions throughout the eastern and northern edges of the burrowing owl's breeding distribution. The results of these simulations suggest that our inferred contraction in the species' distribution is not an artifact of the sampling scheme in the BBS.

5. Discussion

The breeding range of burrowing owls in North America has contracted over the past 40 years. The map of burrowing owl population trends on the BBS website (Sauer et al. 2008) also reveals population declines near the eastern and northern limits of the species' breeding range based on trend estimates at each BBS route and inverse distancing (Cressie 1992) to contour these trends over a map. However, the inverse distancing

approach fails to detect observed population declines and extirpations in northern California (DeSante et al. 1997) and eastern Washington (Conway and Pardieck 2006), instead suggesting annual population increases >1.5% in these 2 regions. Our analyses demonstrate the utility of using presence-absence data to examine changes in species distributions especially when the precision of abundance data is low (i.e., species that are rare in both space and time).

The current breeding distribution of the burrowing owl inferred by our model also differs from that in Wellicome and Holroyd (2001), which extends the northern limit of the species' distribution further north in Canada. However, some areas outside the limits of our inferred distribution still have breeding owls, but they occur below a specified threshold and/or dramatic population declines have occurred in those areas. Still, the BBS (and any systematic survey) will fail to identify every hectare of occupied burrowing owl habitat near the periphery of the species' distribution, contributing to the inference of a more retracted distribution.

Our analyses (and any analysis based on BBS data) can only reveal changes in the distribution of burrowing owls in Canada and the United States. No BBS data exist for the breeding range of burrowing owls in Mexico, which includes the Baja California peninsula, the coastal plain of the Gulf of California in Sonora and Sinaloa, and the Mexican Highlands (Haug et al. 1993, Wellicome and Holroyd 2001). However, BBS routes in the southwestern United States and some anecdotal observations may provide some insight about distributional changes in Mexico. Burrowing owl populations dramatically increased in irrigated agricultural valleys of the Sonoran Desert of

California and Arizona during the second half of the 20th century, particularly in the Imperial Valley of California (Sauer et al. 2008). The Imperial Valley may currently support the highest density of breeding burrowing owls within the species range (DeSante et al. 2004, Rosenberg and Haley 2004), although high densities in Imperial Valley have declined somewhat over the past 5 years (Manning 2009). Densities of burrowing owls are similarly high in northwestern Mexico even in areas that were formerly outside their published breeding range (A. Macias-Duarte, personal obs.). These past population increases in the arid southwestern United States and northwestern Mexico are as intriguing as population declines in the north. These large breeding populations in the southern half of the species' breeding range are completely associated with irrigated agriculture and suburban areas in hot deserts, with few burrowing owls breeding in the surrounding native desert vegetation. Chapter II explores a possible link between population declines in the northern half of the species' range and the increases in the southern half of their range.

CHAPTER II. CHANGE IN MIGRATORY BEHAVIOR AS A POSSIBLE EXPLANATION FOR BURROWING OWL POPULATION DECLINES IN NORTHERN LATITUDES

1. Abstract

The breeding range of the western burrowing owl (*Athene cunicularia hypugaea*) has contracted along its northern and eastern edges. In this paper, we explored the possibility of further changes in the species' breeding distribution on the southern edge of its current breeding distribution. We suggest that the burrowing owl's breeding distribution has recently expanded southwards into areas that formerly supported only wintering owls, and that this expansion was facilitated by the appearance of new breeding habitat created by irrigated agriculture in the arid areas of southwestern United States and northwestern Mexico. Agricultural areas in the Imperial Valley of southeastern California and similar valleys in northwestern Mexico now harbor the highest breeding densities of burrowing owls in North America. In this paper, we explore the hypothesis that burrowing owls from northern migratory populations have become resident breeders in areas of northwestern Mexico formerly used only by wintering migrants, contributing to both population declines near the northern extent of the species' breeding range and population increases in the southern half of the species' range.

2. Introduction

The breeding distribution of the western burrowing owl (*Athene cunicularia hypugaea*) historically extended from southern British Columbia, Alberta, Saskatchewan, and Manitoba in Canada throughout the western United States to northern Sinaloa, the central Baja California Peninsula, and the Mexican Highlands (Wellicome and Holroyd 2001). Burrowing owl populations near the northern edge of the species' breeding range in southern Canada and northern United States have declined or even disappeared (Desmond et al. 2000, Wellicome and Holroyd 2001, Klute et al. 2003, Conway and Pardieck 2006). These population declines may have led to a contraction in the eastern and northern edges of the species' distribution, as shown by North American Breeding Bird Survey (BBS) data (Chapter I). Past authors have proposed numerous hypotheses to try to explain the cause of the observed population declines in the northern portion of their range, and these include the reduction in quality of breeding habitat caused by conversion of grassland to dryland farming, extirpation of prairie dogs (*Cynomis* spp.), toxicological effects of pesticide use in agricultural areas, and collisions with vehicles (Haug et al. 1993, Clayton and Schmutz 1999, Klute et al. 2003). Indeed, only 20% of the original extent of grasslands, the primary habitat of the species, remains in Canada. The remaining grasslands in Canada are highly fragmented (World Wildlife Fund Canada 1989, Gauthier and Wiken 2003), negatively affecting habitat suitability because burrowing owls tend to avoid agricultural fields in fragmented grasslands (Clayton and Schmutz 1999).

Changes in the southern edge of the species' breeding distribution are less evident because no BBS data are available for Mexico. However, burrowing owl populations have increased over the past 40 years in the southern portion of the species' breeding distribution in the United States. Burrowing owl populations in irrigated agricultural valleys of the Sonoran desert of California and Arizona have steadily increased during the second half of the 20th century, particularly in the Imperial Valley of California (Sauer et al. 2008). Imperial Valley is thought to support the highest density of breeding burrowing owls within the species' range (DeSante et al. 2004, Rosenberg and Haley 2004). Population increases in the arid southwestern United States are as intriguing as population declines in the north. These large breeding populations are associated with irrigated agriculture and suburban areas in hot deserts, with few burrowing owls breeding in the surrounding native desert vegetation (Palacios et al. 2000, A. Macias-Duarte, personal observ.). Several mechanisms may be responsible for the high densities of burrowing owls within agricultural areas of the arid deserts, such as high food supply, high burrow availability, and reduced predation (Moulton et al. 2006).

Irrigated agriculture in hot arid areas is even more prominent in coastal Sonora and Sinaloa. The post-Mexican revolutionary era and the so-called Green Revolution (Evenson and Gollin 2003) created large irrigation districts in northwestern Mexico since the 1950s. The irrigated area in the states of Baja California, Sinaloa, and Sonora was only about 2,110 km^2 in 1950 (Rodriguez-Cisneros et. al 1983) but increased to 13,138 km^2 by 1970 and to >15,000 km^2 by 1990 (Instituto Nacional de Estadística Geografía e Informática 1994). These irrigated agricultural areas are 6 times larger than those in the

Imperial Valley in southeastern California (2,810 km^2, DeSante 2004). Based on the association between irrigated agriculture and burrowing owls observed in the Imperial Valley, burrowing owls may breed in high densities in agricultural areas throughout the coastal plains of Baja California, Sonora, and further south than the currently-accepted southern limit of the species' breeding distribution near the Sonora-Sinaloa border (Wellicome and Holroyd 2001) including northern Nayarit (eBird 2011). Published information provides little insight on the abundance of burrowing owls in agricultural areas in this region. Breeding areas of burrowing owls in Mexico are not well documented (Klute et al. 2003). Burrowing owls are considered rare in this region, aside from high concentrations in the Mexicali valley which is merely the southern extension (the Mexican half) of the Imperial Valley (Palacios et al. 2000, Itubarria Rojas 2002). A summary of 279 specimens from 27 major Mexican and foreign museums (Enriquez-Rocha 1997) did not contain any specimens of owls collected during the breeding season in Sonora or Sinaloa. Moreover, a recent atlas of Sonoran avifauna states that "burrowing owls are uncommon to local in Sonora" and the authors did not confirm any breeding record for the species in the state (Russell and Monson 1998). To fill this gap of information in the breeding distribution of burrowing owls in Mexico, we conducted preliminary surveys for breeding burrowing owls in irrigation districts in the states of Baja California, Sonora, and Sinaloa to provide rough estimates of density of burrowing owls.

3. Methods

A. Study area

We searched for burrowing owls during the breeding season (April-July) of 2005 and 2006 in irrigation districts in Baja California, Sonora, and Sinaloa in northwestern Mexico (Table 1). Climate varies from north to south from hot and arid in the Mexicali Valley of Baja California (mean annual temperature, 22.3°C; mean annual rainfall, 76 mm) to tropical dry in the Culiacan Valley of Sinaloa (25.3°C; 614 mm). Native vegetation in the region includes Sonoran desertscrub, Sinaloan thornscrub, and Sinaloan deciduous forest (Brown 1994). Without human disturbance (such as that provided by agricultural development), these vegetation types do not provide open habitats required by burrowing owls.

B. Burrowing owl surveys

We searched for burrowing owls from 1300 until dusk in areas without previous knowledge of the presence of burrowing owls. We occasionally asked local residents about burrowing owl sightings, but their information rarely lead to the location of burrowing owls. In areas where we located burrowing owls, we began trapping, banding and collecting blood and feather samples from owls at ~1600, although we kept detecting owls until 20:00. Actual search time varied among survey dates and was influenced by the time required to obtain permission for land access and for trapping owls. For calculation of density, we excluded days when search times deviated substantially from 1300–2000. We used our encounter rate (pairs·day^{-1}) during 3 search days and previous

estimates of owl density throughout the Mexicali Valley (Itubarria Rojas 2002) to estimate a proportionality constant *k* (units: day·km^{-2}) that applied to our daily encounter rate (pairs·day^{-1}) in other agricultural areas in Sonora and Sinaloa which allowed us to obtain rough estimates of owl density (pairs·km^{-2}). Our assumption of direct proportionality between daily encounter rate and density has not been tested and, therefore, our estimates of density should be taken with caution.

4. Results

We estimated a proportionality constant of $k = 0.56$ day·km^{-2} for 3 days of surveys in Mexicali. We mutiplied our encounter rates by *k* to estimate a mean density of breeding pairs in southern Sonora (Valle del Yaqui-Mayo) of 3.2 pairs·km^{-2} (number of search days, $n = 8$, 46 pairs), in northern Sinaloa (Valle del Fuerte) of 4.5 pairs·km^{-2} ($n = 4$, 32 pairs), and in central Sinaloa (Valle de Culiacan) of 4.7 pairs·km^{-2} ($n = 7$, 58 pairs). We did not apply this technique for central and northwestern agricultural areas in Sonora because these areas differ from those in Mexicali by the lack of extensive irrigation and drainage canals. These estimates suggest that densities of breeding burrowing owls in these irrigated agricultural valleys are likely similar to those in the Imperial Valley of California (2.0 pairs·km^{-2}, DeSante et al. 2004; 8.3 pairs·km^{-2}, Rosenberg and Haley 2004).

5. Discussion

The presence of these large burrowing owl breeding populations in Sonora and Sinaloa is puzzling. Burrowing owls are in low densities in native Sonoran desert of the

Baja California peninsula and the lower Colorado river valley but only abundant in agricultural areas (Palacios et al. 2000, DeSante et al. 2004). Likewise, we suggest that burrowing owls were very scarce or even absent in the Sonoran desert and in subtropical Sinaloa before the expansion of irrigated agriculture in these states. Burrowing owls breed in open, treeless plains (Haug et al. 1993) and do not breed in areas with high densities of woody plants. Natural vegetative communities in northwestern Mexico, such as Sonoran desertscrub, Sinaloan thornscrub, and Sinaloan deciduous forest do not provide the openness preferred by breeding burrowing owls. Currently, burrowing owls in Sonora and Sinaloa are almost exclusively associated with agriculture. We did not find any burrowing owls breeding outside the influence of agricultural or urban areas. Indeed, no burrowing owls were detected during an extensive bird monitoring effort within native vegetative communities throughout northwestern Sonora (Flesch 2008). Therefore, we suggest that burrowing owls expanded their breeding distribution into these new areas along the coastal plains of Sonora (currently regarded as year-round distribution) and Sinaloa (Haug et al. 1993).

Founder individuals for these large burrowing owl populations in agricultural lands of northwestern Mexico may have come from several sources. Burrowing owls from local or nearby populations, if any, may have colonized the newly available habitat created by agriculture. Another possible source of founder individuals are migratory burrowing owl populations wintering in the area or passing through in route to their wintering grounds further south in central Mexico. Burrowing owls are exclusively migratory in the northern part of their breeding distribution (Haug et al. 1993) and they

are thought to winter from central Sinaloa to central Mexico and southern Texas (Haug et al. 1993, Enriquez-Rocha 1997, Duxbury 2004, Holroyd et al. 2010, Holroyd and Trefry 2011). When migratory burrowing owls encountered the newly created agricultural areas in Sonora and Sinaloa during migration, high food abundance may have promoted residency. If formerly migratory burrowing owls have become resident breeders in northwestern Mexico because of the expansion of irrigated agriculture, this process may be contributing to the population declines observed in the northern half of the species' distribution.

We suggest that the creation of irrigated agricultural areas in northwestern Mexico may have attracted burrowing owls that once migrated annually between the northern United States and southern Canada to wintering grounds in central Mexico, contributing to population declines in the northern portion of their range (migration-mediated range-shift hypothesis). We propose a mechanism for this hypothesis with 3 components.

The first component is a difference in habitat suitability between the south and the north. Suitability and extent of breeding habitat in the north may have drastically decreased due to habitat loss and fragmentation leading to lowered demographic parameters such as survival (Clayton and Schmutz 1999), and ultimately to low site fidelity (Duxbury 2004, Wellicome 2005, Chapter IV). However, the latitudinal effects of agriculture on burrowing owl habitat may play the most important role and requires further clarification. Burrowing owls avoid nesting in cultivated fields in Canada (Clayton and Schmutz 1999, but see, Restani et al. 2008), they nest in both cultivated

areas and native vegetation at intermediate latitudes (Klute et al. 2003), and they breed at much higher densities in agricultural areas in the southern part of their range. This pattern suggests that conversion of desert and tropical vegetation to agriculture enhances habitat suitability for burrowing owls in the southern portions of their range, but conversion of temperate grasslands to dryland farming reduces habitat availability in the northern portions of their range without much gain in plant productivity and therefore overall prey supply for burrowing owls. Cultivation of native grasslands in the Great Plains increased net primary productivity (NPP) of the region by only ~10%, with a pre-cultivation NPP of 125-360 g C m^{-2} yr^{-1} (Bradford et al. 2005), whereas cultivation of Sonoran desert shrublands has potentially increased NPP by an order of magnitude, from ~50 g C m^{-2} yr^{-1} (Mueller and Diamond 2001) to ~800 g C m^{-2} yr^{-1} (Hicke et al. 2004). Therefore, the creation of open areas in the southern portion of the owls' breeding range may support high densities of breeding owls because primary productivity is higher (and less seasonal) in the southern portions of their distribution than in the northern portion. Open habitats are abundant in the northern portions of their range but absent or scarce in the coastal Sonora and Sinaloa with abundant woody plants. For example, the Sinaloan thornscrub, that historically covered the area of the Yaqui-Mayo Valley, contained shrubs and trees from 2-7.5 m high at densities up to 2,000 plants ha^{-1} providing up to 90% overhead canopy cover (Brown 1994). Therefore, conversion of native vegetation to agriculture creates additional habitat in the southern portion of the species' range but does not create (or even eliminates) habitat in the northern portion of its range (Clayton and Schmutz 1999). Analogous to the range expansion of burrowing owls in subtropical northwestern

Mexico due to agriculture, Florida burrowing owl (*A. cunicularia floridana*) populations show a similar pattern of range expansion towards human-altered landscapes which opened dense subtropical native vegetation to burrowing owls (Ligon 1963). The conversion to agriculture enhances breeding habitat by increasing the primary productivity in the form of crops much more in the south than in the north. An additive factor that may make southern agricultural areas more suitable for burrowing owls is the presence of irrigation and drainage canals (Table 1), intensively used by breeding burrowing owls in the lower Colorado River valley (Palacios et al. 2000, Itubarria Rojas 2002, Rosenberg and Haley 2004, Bartok and Conway 2010) and in southern Sonora and Sinaloa (this study). Irrigation infrastructure provides substrate for round-tailed ground squirrel (*Spermophilus tereticaudus*) and rock squirrel (*S. variegatus*) burrows and the possibility of foraging near cropland and increased visibility to avoid terrestrial predators (coyotes, domestic dogs, cats, etc.).

The second component is the ability of individual burrowing owls to change migratory behavior in response to the difference in habitat suitability suggested above. Migratory behavior may be amenable to rapid evolution. Recent changes in many aspects of avian migratory behavior have been extensively documented (for reviews in the topic see Fiedler 2003, Newton 2007). Migratory tendency in burrowing owls is not genetically fixed, but rather is influenced by extrinsic, environmental factors (Ogonowski and Conway 2009). We hypothesize that agricultural areas provide the environmental stimulus to behavioral components that leads to year-round residency in burrowing owls. For example, benign winters in northwestern Mexico (Fig. 5) allow continuous

agricultural production and consequently an abundant and continuous food supply allowing for extended breeding seasons (Rosenberg and Haley 2004). These factors can promote residency in migratory burrowing owls by triggering double brooding in females (Gervais and Rosenberg 1999, C. Conway, unpubl. data). Moreover, anecdotal evidence suggests that a burrowing owl with northern origins attempted to breed (i.e., become a resident breeder) in southern Arizona. A female burrowing owl laid and hatched a clutch in southern Arizona in spring of 2003 and then travelled to Saskatchewan and laid another clutch that same season following her breeding attempt in Arizona (Holroyd et al. 2011). The stable isotope ratios of the bird's feathers suggested that she hatched in near the U.S.-Canada border in 2002 (G. Holroyd, unpubl. data). Birds breeding in areas that were formerly only used by wintering individuals has been documented in several other species (Sutherland 1998).

The third component involves individual owls from the northern-most breeding populations altering their migratory behavior more so than individuals from populations in other portions of their range (i.e., in mid latitudes). Burrowing owls may exhibit a leap-frog migration pattern (James 1992). Leap-frog migration occurs when populations occupying the northernmost part of the breeding range winter in the southernmost part of the wintering grounds, whereas those breeding further south in the breeding range winter further north in the wintering grounds (Boulet and Norris 2006). Under this migration pattern, burrowing owls from northern migratory populations would have been more likely to locate new habitat in agricultural areas in northwestern Mexico whereas owls from other mid-latitude populations may not, possibly explaining why northern

burrowing owl populations would decline more than other populations. Many of the burrowing owls that breed in prairie Canada appear to winter from south Texas to central Mexico (Duxbury 2004).

We also searched for burrowing owls in the Mexican Highlands, east of the Sierra Madre Occidental. We found large burrowing owl populations breeding in 2 major agricultural areas, Delicias in central Chihuahua and Comarca Lagunera in southwestern Coahuila. We hypothesize that these agricultural populations were more likely to originate from pre-existing local burrowing owl populations inhabiting the surrounding native Chihuahuan desert grasslands and shrublands (Rodriguez-Estrella and Ortega-Rubio 1993). Competition with local burrowing owl populations may have deterred migratory burrowing owls from establishing in these areas. In addition, irrigated agricultural areas in northern Tamaulipas and Guanajuato (areas we failed to visit) deserve further attention. Both districts have ~8,300 km of irrigation and drainage canals and almost 4,000 km^2 of irrigated land (Direccion General de Distritos de Riego 1973). These 2 agricultural areas are not currently considered part of the burrowing owl breeding distribution either (Wellicome and Holroyd 2001). Therefore, the migration-mediated range-shift proposed here may also apply to these agricultural areas in Guanajuato and Tamaulipas if burrowing owls are present there.

CHAPTER III. IRRIGATED AGRICULTURE IN NORTHWESTERN MEXICO CREATES SUBTLE POPULATION GENETIC STRUCTURE IN THE PANMICTIC WESTERN BURROWING OWL (*ATHENE CUNICULARIA HYPUGAEA*)

1. Abstract

Many burrowing owl populations have declined or become extirpated near the northern edge of the species' breeding distribution during the second half of the 20th century. In the same period, large extensions of thornscrub were converted to irrigated agriculture in northwestern Mexico. These irrigated areas may now support the highest densities of burrowing owls in North America. We tested the hypothesis that burrowing owls that colonized this recently created habitat originated from declining migratory populations from the northern portion of the species' range (migration-driven breeding dispersal whereby long-distance migrants became year-round residents in the newly created habitat). We used 10 novel microsatellite markers to genotype 1,560 owls from 36 study locations in Canada, Mexico, and the United States. We found that burrowing owl populations are practically panmictic throughout the entire breeding range. However, an analysis of molecular variance provided some evidence that burrowing owl populations in northwestern Mexico and Canada together are more genetically differentiated from the rest of the populations in the breeding range, lending some support to the migration-mediated range-shift hypothesis. We found evidence of subtle genetic differentiation associated with subtropical irrigated agricultural areas in southern Sonora and Sinaloa. Further research will be necessary to determine with more certainty whether there is a

link between population increases in northwestern Mexico and population declines in the northern portions of the species' breeding range. Our results demonstrate that land-use can produce location-specific population dynamics leading to subtle genetic structure even in the absence of dispersal barriers.

2. Introduction

Understanding ecological and evolutionary dynamics of a species at the edges of its distribution can help unveil the mechanisms that limit abundance throughout a species' entire geographic range (Holt and Keitt 2005). In this regard, ecological theory and empirical evidence support the idea that species tend to be less abundant and more prone to local population extinctions at the periphery of their geographic ranges (Gaston 2003). Populations at the edge of a species' distribution may be maintained by dispersal and re-colonization from interior populations (Curnutt et al. 1996). This scenario whereby populations on the periphery are repeatedly "rescued" (Brown and Kodric-Brown 1977) by interior populations may be particularly important for species of conservation concern. Understanding the processes by which peripheral populations are maintained in those species is important for designing effective recovery efforts. For example, burrowing owl populations have been extirpated from some areas and are rare and declining in other areas near the northern edge of their breeding distribution (Clayton and Schmutz 1999, Skeel et al. 2001, Wellicome and Holroyd 2001). The species' breeding range historically comprised semiarid grasslands from southern Canada to central Mexico (Haug et al. 1993). Hypotheses to explain population declines in the northern portion of their range include local mechanisms such as conversion of grassland to dryland farming in the

northern Great Plains, extirpation of black-tailed prairie dogs, toxicological effects of pesticides, collisions with vehicles, and annual dispersal (Haug et al. 1993, Clayton and Schmutz 1999, Desmond et al. 2000, Klute et al. 2003, Duxbury 2004). All these hypotheses seem insufficient to explain the extent of burrowing owl population declines observed in the northern portion of their breeding range because much seemingly suitable habitat remains unoccupied. Nevertheless, the highest breeding densities occur in the southern portion of the burrowing owl's breeding range (in Imperial Valley, California; DeSante et al. 2004, Rosenberg and Haley 2004, Sauer et al. 2008). In addition, we documented densities of breeding burrowing owls in the coastal plains of Sonora and Sinaloa that were similar to those in southeastern California. These high densities of burrowing owls in the southern portions of the species' range are all in areas associated with irrigated agriculture (Chapter II). High densities of breeding burrowing owls in this portion of their range is a recent phenomenon; more than 1.5 millions hectares of coastal thornscrub and tropical dry forest in Sonora and Sinaloa were converted to irrigated farmland in the last 50 years (Instituto Nacional de Estadística Geografía e Informática 1994). This re-distribution of burrowing owls (the breeding range contracting in the north and expanding in the south) poses interesting questions about the mechanisms that shape and maintain the geographic range of the species. In this paper, we test the hypothesis that the contraction at the northern periphery of the species' range and their expansion in the southern portion of their range are directly related.

Most breeding populations of burrowing owls include at least some migrants and northern populations in the Great Plains are 100% migratory (Haug et al. 1993).

Burrowing owls were speculated to have a leap-frog migration pattern (James 1992), and most owls that breed in the northern portion of the breeding range appear to spend their winters in southern Texas and central Mexico (Duxbury 2004, Holroyd et al. 2010). We tested the hypothesis that burrowing owls that once migrated annually from northern portions of their breeding range to central Mexico became resident breeders in these agricultural areas, contributing to both population declines in the north and population increases in the south. Birds breeding within what was formerly their wintering grounds (migrants becoming year-round residents) has been documented in other species (Sutherland 1998).

Because we cannot directly estimate the past patterns of breeding dispersal, we used genetic markers to infer the extent of past breeding dispersal (i.e., gene flow) by measuring genetic differentiation among populations. Permanent breeding dispersal leaves its fingerprint in the gene pools of populations. We tested 3 predictions of our hypothesis that infer patterns of genetic variation produced by gene flow from northern migratory (declining) populations to southern agricultural populations. First, our hypothesis predicts that genetic differentiation between a northern migratory population and a southern agricultural population will be lower than the expected genetic differentiation predicted by the geographic distance between the 2 populations. This prediction assumes an isolation-by-distance pattern (Wright 1943), where populations further apart geographically are more genetically differentiated than populations closer to each other due to limited dispersal. Second, our hypothesis predicts that all northern migratory populations and all southern agricultural populations together are genetically

differentiated from the rest of the breeding populations within the burrowing owl breeding range. This prediction can be tested via a significance test of the two-group classification of burrowing owl populations mentioned above to explain overall genetic variation. We can state a third prediction in terms of an assignment test. Assignment tests use individual genotypes to estimate the probability of membership of each individual genotype to predefined clusters of individuals. In this regard, our hypothesis predicts southern agricultural populations will have more individual owls with probabilities of membership similar to those found in individuals from northern migratory declining populations compared to the non-agricultural populations in the southern part of the species' range. We used DNA samples from owls throughout their North American breeding range to test these 3 predictions.

3. Methods

A. Study area

We obtained DNA samples of breeding burrowing owls from 36 locations ('study locations' hereafter) in Canada, Mexico, and the United States (Fig. 6, Table 2). To test our predictions, we grouped the 36 study locations into 3 categories: agricultural areas in the southern portion of the species' range, areas in the northern portion of the species' range where migratory populations are declining, and all other study locations. Seven of our study locations were located in irrigated agricultural areas of northwestern Mexico and southern Arizona ('southern agricultural study locations' hereafter). These study locations were Casa Grande (CAG), Mexicali Valley (MEX), Caborca (CAB), Hermosillo (HER), Yaqui-Mayo Valley (YAQ), Rio Fuerte Valley (FUE), and Culiacan

(CUL) (Fig. 6, Table 2). Some population declines have been documented throughout the breeding range of the burrowing owl, but systematic regional declines have been most evident in Alberta, Saskachewan, North Dakota, and South Dakota, where the species is close to extirpation (owls have been extirpated from Manitoba and British Columbia). Therefore, we only defined Alberta (ALB), Saskatchewan (SAK) and Grand River-Little Missouri National Grasslands (GRL) as northern study locations with declining migratory breeding populations ('northern study locations' hereafter, Fig. 6 and Table 2).

B. Sample collection

We trapped burrowing owls during the summers of 2004-2009. We did not include in our analyses any birds that were closely related (i.e., a parent and its offspring, or >1 juvenile from the same nest burrow). Our primary source of genomic DNA was blood. We obtained ~50 μL of blood through a venipuncture of the brachial vein. We also used flight and/or body feathers occasionally as a source of genomic DNA when we could not withdraw a blood sample. We performed bird handling, and blood and feather collection, as well as the import-export through international boundaries, under the compliance of Canadian, Mexican, and U. S. regulations. We also complied with the University of Arizona Institutional Animal Care and Use Committee regulations under protocols #01-089 and 04-196 (Appendix B).

C. Genotyping

We used 10 microsatellite markers developed specifically for this study (Appendix A) to obtain genotypic data from our 36 study locations. We followed the manufacturer's

protocols in the DNeasy Blood & Tissue Kit (Qiagen®) to isolate genomic DNA from < 25 μL of blood. 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 primer pig-tailed with GTGTCTT, 0.2 μM fluorescently labeled M13 primer, 2 mM $MgCl_2$, 0.4 U Taq DNA polymerase (Invitrogen®), and 0.02% BSA. We used 1 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 2 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 used an Applied Biosystems Genotyper 3.7 to score alleles. We used program Tandem (Matschiner and Salzburger 2009) to assign integers to DNA fragment sizes. We used program Micro-Checker (Van Oosterhout et al. 2004) to identify null alleles (Chakraborty et al. 1992).

D. Data analysis

We used MS Excel© macro *GENALEX* 3.6 (Peakall and Smouse 2006) to calculate standard descriptive statistics of genetic diversity of burrowing owls in our study locations, including observed heterozygosity, expected heterozygosity, and fixation index *F*. We also used program *ARLEQUIN 3.1.1* (Excoffier 2006) to estimate the Weir and Cockerham's F_{ST} (θ, Weir and Cockerham 1984) for all populations.

We computed actual differentiation *D* (Jost 2008) to test our prediction that gene flow between declining migratory populations in the north and populations in southern

agricultural areas would disrupt an otherwise apparent isolation-by-distance relationship. We used the web-based platform GMSOD 1.2.5 (*http://www.ngcrawford.com/django/jost/*) to compute actual differentiation *D*. We used *D* as our measure of population-pairwise genetic differentiation because F_{ST} does not adequately measure genetic differentiation when within-population allelic diversity is high (Jost 2008). *D* ranges from 0 to 1, corresponding to complete similarity to complete differentiation. We performed a Mantel test (Mantel 1967) to test our assumption of the existence of an isolation-by-distance pattern (i.e., that the genetic differentiation between 2 populations is positively correlated to the geographic distance that separates those populations). If our hypothesis is true, we expected that pairwise comparisons between northern locations and southern agricultural locations would fall below the predicted Mantel regression line in the scatterplot of genetic *vs.* geographic distances.

We performed an Analysis of Molecular Variance *AMOVA* (Weir and Cockerham 1984) using *ARLEQUIN* 3.1.1 to test our prediction that all declining migratory populations in the north and all populations in agricultural areas in the south, pooled together, would be genetically differentiated from the remainder of the breeding populations within the species' range (pooled together). The *AMOVA* is analogous to a nested Analysis of Variance and uses a permutational approach to test the statistical significance of any given classification of study locations in explaining the overall genotypic variation. We performed 2 *AMOVA*s, one based on allele sizes (R_{ST}) and the other based on the number of different alleles (F_{ST}) (Michalakis and Excoffier 1996). The former measure assumes the stepwise mutation model (Ohta and Kimura 1973), which is

appropriate for microsatellite loci. We used the *AMOVAs* to test for evidence of 2 distinct genetic groups: Group 1 with southern agricultural locations (CAG, CAB, CUL, FUE, HER, and YAQ) together with northern locations (ALB, SAK, and GRL), and Group 2 including all other locations. Our large sample size (1,560 individuals) may confer enough statistical power to reject the null hypothesis for any grouping of study locations. To explore this possibility, we conducted 7 additional AMOVAs using 2-group classifications by replacing northern study locations (ALB, SAK, and GRL) from Group 1 with other study locations and moving them to Group 2 (Table 5).

We conducted an assignment test as implemented by program *STRUCTURE* (Pritchard et al. 2000, Hubisz et al. 2009) to test our prediction that southern agricultural study locations will have more individual owls with probabilities of membership similar to those found in individuals from declining populations in the north compared to the non-agricultural study locations in the southern part of the species range. *STRUCTURE* 2.3.3 implements an algorithm suited to infer weak population structure (Hubisz et al. 2009). *STRUCTURE* estimates the posterior probability of the data ($\mathcal{L}(K)$=Prob[*Data* |*K*]) given existence of *K* burrowing owl populations under Hardy-Weinberg equilibrium and estimates the posterior probability of membership of each individual owl to each of *K* populations. We used study locations as prior information to assist the inference of population structure (Hubisz et al. 2009) by setting LOCPRIOR=1 in *STRUCTURE*. We performed 10 runs for each $K = 1, 2, \ldots 10$. Each run consisted of a burn-in period of 50,000 Markov Chain Monte Carlo repetitions followed by 50,000 repetitions to sample from the posterior distribution of *K*. We estimated $\mathcal{L}(K)$ for each *K* from correlated allele

frequencies and an admixture model. This approach is superior when population differentiation is low at detecting subtle genetic structure compared to the use of uncorrelated allele frequencies and a non-admixture model (Falush et al. 2003). We used the outputs of the web-based platform *STRUCTURE HARVESTER* 0.56.3 (*http://taylor0.biology. ucla.edu/struct_harvest/*) to assess the number of inferred populations. *STRUCTURE HARVESTER* estimates the statistic ΔK at each value of K. ΔK performs better in detecting population genetic structure than $\mathcal{L}(K)$ (Evanno et al. 2005). Therefore actual number of populations is revealed by the value of K with the highest value of ΔK. We used program *CLUMPP* (Jakobsson and Rosenberg 2007) to calculate the posterior probabilities of membership of each individual owl to each of the K populations from our multiple runs in *STRUCTURE*.

4. Results

Burrowing owls exhibited high levels of genetic diversity (Table 3) with relatively low variation among study locations. Per-locus average of number of effective alleles (range 5.70–7.82), expected heterozygosity (range 0.78–0.84), observed heterozygosity (range 0.78–0.87), and fixation index (range -0.06–0.04) were similar among the 36 study locations (Table 3) in spite of the relatively large differences in sample size (range 21-73; Table 2), per-locus average number of alleles (range 9.40–15.70), and number of private alleles (alleles present at only 1 population, range 0.00–0.50; Table 3). We detected the possible occurrence of null alleles for locus ATCU13 in BUC and CUL study locations,

for locus ATCU20 in LAG and SAK study locations, for locus ATCU39 in NTS study location, and for locus ATCU45 in MEX study location.

Burrowing owls had low levels of genetic differentiation among study locations as shown by relatively low overall F_{ST} (θ = 0.008) and low pairwise F_{ST} statistics ($\overline{F}_{ST}$ = 0.0113±0.0002, *n*=630). Low levels of genetic differentiation were also evident in our estimates of actual differentiation *D*, ranging from 0.00 to 0.11. In this regard, we found no apparent relationship between genetic distance and geographic distance among our study locations (Fig. 7). Lack of isolation-by-distance is also supported by a non-significant Mantel's test (r = 0.015, P = 0.43 based on 1000 permutations). Our prediction of a disrupted isolation-by-distance pattern cannot therefore be fully supported. Nevertheless, pairwise comparisons of genetic and geographic distances among northern study locations and southern agricultural locations fall below the Mantel regression line (Fig. 14) in agreement with the prediction of the migration-mediated range-shift hypothesis.

Low levels of genetic differentiation among populations were also highlighted by our *AMOVA*s based on the R_{ST} and F_{ST} statistics. Genetic variation within study locations explained 99% of the total genetic variation, whereas between-study locations and between two-group classifications of study locations explained the remaining 1%. Despite the low levels of genetic differentiation described above, our *AMOVA* based on the F_{ST} statistic provided support the range-shift hypothesis. Both a standard *AMOVA* and a weighted-averaged *AMOVA* over all loci provided suggestive evidence that northern study locations (ALB, SAK, and GRL) and southern agricultural study locations (CAG,

CAB, CUL, FUE, HER, MEX, YAQ) together are genetically differentiated from the rest of the study locations (P = 0.03 and P = 0.01, respectively) although this result did not hold true for the 2 *AMOVA*s based on R_{ST} (P=0.38 and P=0.34, respectively). In addition, only 1 of the 7 additional *AMOVA*s based on F_{ST} was significant for both the standard *AMOVA* and the weighted-averaged *AMOVA* over all loci (Table 4), which is precisely the *AMOVA* that included the nearest 3 study locations (CHI, JAN, and TUC) within Group 1.

STRUCTURE revealed a genetic structure consisting of 3 populations in the western burrowing owl in spite of the low levels of genetic differentiation among study locations shown by F_{ST} and D statistics. Mean log-likelihood of the observed genotypic data and ΔK was highest at K=3 (indicating 3 distinct populations; Fig. 8). The posterior probabilities of membership of each of our 1,560 individual owls assigned to these putative populations had a noticeable geographic pattern (Figs. 9 and 10). Almost all burrowing owls in southern agricultural study locations in southern Sonora (YAQ) and Sinaloa (FUE and CUL) had a higher probability of membership to one inferred population (Sinaloan population). This genetic structure was corroborated by a standard *AMOVA* (based on the F_{ST}) which differentiates this Sinaloan population (CUL, FUE, and YAQ) from the rest of the study locations (P = 0.005). This Sinaloan fingerprint is relatively common within nearby populations in Sonora, southern Arizona, and as far as Chihuahua (CHI), northern Texas (TXP) and the Central Valley of California (DIX) (green color in pie charts in Fig. 10). Similarly, burrowing owls from Nellis Air Force Base in southern Nevada (NEL) define a distinctive population (Mohave population),

whose fingerprint also appears in burrowing owl populations in the western portion of the breeding range in Washington, California, and Utah (blue color in pie charts in Fig. 10). Finally, the great majority of the individuals in the remainder of the study locations, including northern study locations, had the fingerprint of a third inferred population (North American population) where northern study locations and the northern half of the southern agricultural study locations (HER, CAB, MEX, and CAG) are included. Under this scenario, our hypothesis is not supported. Individual owls from 4 southern agricultural study locations (CAG, MEX, CAB, HER) had similar probabilities of membership to those found in owls from northern locations but also similar to those found in owls from non-agricultural study locations in the southern part of the range (e.g., JAN, GAL). In addition, probabilities of membership were remarkably different in owls from the 3 southernmost agricultural locations (CUL, FUE, and YAQ), compared to those found in owls from northern locations (ALB, SAK, and GRL).

5. Discussion

Western burrowing owl populations in North America have low levels of differentiation as shown by F_{ST} and D statistics. Low levels of genetic differentiation were previously reported for the western burrowing owl in the United States. Korfanta et al. (2005) estimated $F_{ST} = 0.01$ (95% CI: 0.007-0.02) and concluded that western burrowing owl populations were practically panmictic. Our estimation of $F_{ST} = 0.008$ is slightly lower but still within the 95% confidence interval of their F_{ST} estimate. Our study was more comprehensive than Korfanta et al. (2005) because it represents a 10-fold increase in the number of individuals (155 *vs.* 1560), and 4-fold increase in the number of study

locations (9 *vs*. 36), and we included populations in Mexico and Canada. Our study also represents a 43% increase in the number of microsatellite loci used (7 *vs*. 10). In addition, markers used in this study were more variable, with an average of 11.6 alleles per locus (range 5-25, Appendix A) *vs*. 8.3 alleles per locus (range 3-19, Korfanta et al. 2002). Therefore, our study confirms, with increased statistical power, that this low genetic differentiation extends throughout the entire breeding range of western burrowing owl in North America (including populations in Canada and Mexico). However, a major assumption for our 3 predictions is that burrowing owl populations were genetically structured before the development of the agricultural valleys in southwestern United States and northwestern Mexico. Therefore, this low genetic population differentiation throughout the burrowing owl breeding range therefore hindered our ability adequately test the range-shift hypothesis.

Genetic diversity in DNA microsatellite loci among our sampling locations is higher than that found in other owl species of wide distribution, and in other owl species of conservation concern. Average expected heterozygosity per locus across study locations ranged from 0.77–0.86 for burrowing owls (36 locations, this study), from 0.54–0.62 in the ferruginous pygmy-owl *Glaucidium brasilianum* (8 locations, Proudfoot et al. 2006), from 0.48–0.56 in the boreal owl *Aegolius funereus* (6 locations, Koopman et al. 2007), from 0.47–0.63 in great gray owls (5 locations, Hull et al. 2010), and from 0.72–0.77 in the spotted owl *Strix occidentalis* (6 locations, Funk et al. 2010). Similarly, low genetic differentiation also been documented in the boreal owl (F_{ST} = 0.004 using microsatellite loci; Koopman et al. 2007), and the flammulated owl *Otus flammeolus* (F_{ST}

< 0.04 using DNA fingerprinting; Arsenault et al. 2005), as well as the endangered northern spotted owl *Strix occidentalis caurina* (F_{ST} = 0.024 using microsatellite owls; Funk et al. 2010), although strong genetic structure has been documented for the great gray owl *Strix nebulosa* (F_{ST} < 0.17 from microsatellite loci; Hull et al. 2010). Low levels of genetic differentiation in the burrowing owl is highly relevant for burrowing owl conservation and restoration programs everywhere in North America. Low genetic differentiation among study locations suggests that burrowing owls are a large panmictic population across the species' breeding range. Reintroduction programs may be able to use individuals from populations throughout western North America without substantially compromising genetic variation for local adaptation. Low genetic differentiation, presumably caused by continent-wide breeding dispersal, also means that population trends in a given location may be caused by changes in demographic processes (e.g, fecundity, mortality, emigration) in other portions of the species' range. Therefore, population declines in the northern edge of the species' breeding distribution may be alternatively explained by declines in immigration from more interior populations or low local recruitment.

The measures of genetic differentiation discussed above are based on summarized genotypic information across 36 study locations over a broad geographic area and they may fail to detect subtle genetic structure. Hence, the use of our genetic markers to detect past and current patterns of breeding dispersal is imperfect. However, the use of several analytical methods and algorithms that make full use of the individuals' genotypic data (e.g., program *STRUCTURE*) can unveil subtle patterns of genetic differentiation.

Estimates of ΔK revealed a subtle genetic structure and identified 3 populations. However, ΔK cannot be computed for K=1 (Evanno et al. 2005) and therefore the scenario of 1 single population in Hardy-Weinberg equilibrium is still possible given our low values for F_{ST} and D. However, consistent geographic patterns in probabilities of membership suggest the validity of our results. *STRUCTURE* is a spatially-blind analysis since geographic coordinates are not an input in the analysis. Therefore, the fact that the 3 southern-most agricultural populations (CUL, FUE, and YAQ in northwestern Mexico; Fig. 10) all had higher probability of membership to a single population suggests to us that the inferred population structure is real. This genetic structure suggests that irrigated agriculture in Sonora and Sinaloa has influenced population dynamics of burrowing owls and has created populations that are subtlety distinct from the rest of the populations within the breeding range, distinct even from the neighboring agricultural populations in central Sonora and those in the Colorado River delta. Although *STRUCTURE* did not support a direct link between southern agricultural locations and the northern-most locations, our *AMOVA* did provide evidence of such a link. Our *AMOVA* based on F_{ST} provided support for the predicted pattern of breeding dispersal from northern locations to southern agricultural locations, differentiating this group from other burrowing owl locations. In contrast, our *AMOVA* based on allele sizes (R_{ST}) did not provide support of the hypothesis. However, measures of allele size have been criticized for having large sampling errors and low efficiency in reconstructing simulated phylogenies (Takezaki and Nei 1996). In addition, the lack of statistical significance in 6 of the 7 additional *AMOVA*s (Table 4) suggests that the genetic connectivity inferred between southern

agricultural locations and northern locations is not an artifact of a large sample size (1,560 individuals and 10 loci). In fact, the only other significant *AMOVA* included southern agricultural locations and neighbouring Tucson, Janos and Delicias locations in Group 1, which makes sense because of regional gene flow.

In summary, our study provides suggestive evidence that declines near the northern edge of the breeding range of burrowing owls may be at least partially caused by migration-driven dispersal to subtropical agricultural areas in northwestern Mexico. However, low levels of genetic differentiation among populations hindered the resolution of our analysis. Increasing the statistical power of this study by adding more individuals and additional microsatellite markers may help clarify the subtle genetic structure we detected within the western burrowing owl. Our results demonstrate the influence of the land-use mosaic on the distribution and movement of animals, which can produce location-specific population dynamics leading to subtle genetic structure even in the absence of dispersal barriers or isolation by distance. Evidence of genetic connectivity among populations in areas with similar land uses suggests to us that the processes which constrain the continental breeding distribution of the burrowing owl likely includes food limitation and tolerance of vegetation density. Therefore, the long-term conservation value of agro-ecosystems in Sonora and Sinaloa should be evaluated because these ecosystems harbor dense breeding populations and may support a surprisingly high proportion of the burrowing owls that breed in North America.

CHAPTER IV. CONTINENTAL PATTERNS OF PHILOPATRY AND BREEDING DISPERSAL AMONG BURROWING OWL POPULATIONS IN NORTH AMERICA AS REVEALED BY STABLE ISOTOPE ANALYSIS OF FEATHERS

1. Abstract

The breeding range of the western burrowing owl has shifted southward, opposite of the northward shifts (attributed to climate change) documented in many other bird species in North America. We used stable isotopes ^{2}H, ^{13}C, and ^{15}N in burrowing owl feathers to determine the breeding dispersal patterns underlying this southward contraction of the species' breeding distribution. In particular, we tested the hypothesis that burrowing owls from declining migratory populations near the northern edge of the species' range are becoming resident breeders in recently cultivated irrigated agricultural areas in southwestern United States and northwestern Mexico through a migration-mediated breeding dispersal. We used nestling feathers collected in 36 study locations in Canada, Mexico, and the United States to infer local isotope signatures throughout the burrowing owl breeding range. We compared $\delta^{13}C$ and $\delta^{15}N$ isotope ratios of adult feathers to the local isotope signatures of nestlings to estimate the proportion of philopatric *vs.* immigrant owls at each of 27 study locations. We also used a subset of our sampled owls for which we also had $\delta^{2}H$ to build a more refined map of local isotope signatures, and we used this refined map to infer geographic origin of adult feathers collected at each of the 27 locations (i.e., breeding dispersal vectors). Burrowing owl populations near the northern edge of the species' breeding range in Canada and those in the Baja California

Peninsula had a high proportion of immigrants (>90%) compared to interior populations ($\bar{x}$ =30% immigrants). Most other populations had high levels of philopatry, and central Sonora had the highest levels of philopatry providing some support to the migration-mediated range-shift hypothesis. We found geographic gradients of $\delta^{2}H$, $\delta^{13}C$, and $\delta^{15}N$ values in nestling feathers across North America, allowing us to infer the frequency of burrowing owl dispersal events at locations throughout North America. In general, burrowing owl dispersal is apparently unconstrained, with high rates of breeding dispersal among mid-latitude populations. Northern populations receive immigrants from southern populations, but southern populations rely mostly on local recruitment and immigration from neighboring populations. Stable isotopes also provided evidence of breeding dispersal from Canadian populations to northwestern Mexico in support of the migration-mediated range-shift hypothesis, but similar isotope signatures in nestling feathers between these 2 regions prevent stronger inferences.

2. Introduction

Breeding dispersal, the movement of an individual between 2 consecutive breeding attempts, can influence the size and shape of a species' geographic range. The edge of most species' ranges are thought to include: 1) an outer submarginal zone within which the species occurs but often fails to produce local recruits (Emlen et al. 1986), or 2) a zone with periodic extirpation where immigrants dispersing from core areas during productive years recolonize and persist for ≥1 generations (Gaston 2003). Therefore, the edge of a species range may advance or retreat depending on demographic processes that affect the production of dispersers within source populations across the distribution.

Thus, changes in distributional limits may signal generalized changes in environmental conditions throughout the species' range, which may be particularly relevant to the conservation of widespread but uncommon species.

The western burrowing owl (*Athene cunicularia hypugaea*) is a widespread but uncommon species that has shifted its breeding range southward, opposite of the prevalent trend of northward range shifts in many North American birds (Thomas and Lennon 1999, Hitch and Leberg 2007, La Sorte and Thompson 2007). The northern edge of the burrowing owl's breeding range has recently moved southward (Chapter I) with a southern expansion of its breeding range into areas that were previously occupied only by wintering migrants (Chapter II). Understanding the cause(s) of these distributional changes is important because burrowing owls are a species of conservation concern throughout North America (Klute et al. 2003, Holroyd 2005, U.S. Fish and Wildlife Service 2008) and endangered in Canada (COSEWIC 2006).

In this paper, we tested the hypothesis that the creation of irrigated agriculture within desert and subtropical ecosystems in Sinaloa, Sonora, Baja California, California, and Arizona (which previously supported fewer breeding owls) has created new breeding habitat thereby causing population declines in areas at the northern extent of the species range. This hypothesis seems plausible given that: 1) burrowing owl prey is more abundant in agricultural areas compared to native vegetation (Garcia 2005, Moulton et al. 2005), 2) breeding density of burrowing owls is often higher in agricultural areas compared to native vegetation (DeSante et al. 2004), 3) burrowing owls were not known to occur in native Sonoran desertscrub and subtropical coastal areas of Sinaloa that have

been recently converted to irrigated agriculture and these areas now support dense breeding populations (Chapters II and III), and 4) many burrowing owls from northern populations appear to migrate south to central Mexico during the winter and cross the continental divide (Duxbury 2004, Holroyd et al. 2011) where they would encounter these agricultural areas along their migratory journey. Burrowing owls that once bred in more northern latitudes and spent the winter in Mexico may have skipped spring migration and instead colonized (and became year-round residents) in these agricultural areas. If true, these continental-scale breeding dispersal events from the northern United States and Canada to northwestern Mexico were therefore mediated through migratory behavior (Chapter II).

Direct evidence for this hypothesis requires detecting individuals of northern origin (owls that formerly bred in Canada or the northern United States) breeding in Sinaloa and Sonora. If this migration-mediated range-shift hypothesis were true, we would expect to see adult owls that were banded in Canada and northern United States breeding in northwestern Mexico. However, given the relatively small number of owls banded, and the extensive unmonitored agricultural valleys in northwestern Mexico, alternative approaches may be more efficient to document the presence of once migratory burrowing owls breeding in northwestern Mexico. One such approach is the use of stable isotopes of hydrogen (H), carbon (C), and nitrogen (N).

The use of stable isotopes in ornithological research has expanded tremendously since the seminal work by Chamberlain et al. (1997) and Hobson and Wassenaar (1997) who used deuterium (^{2}H) to track the summer origin of migratory songbirds on their

wintering grounds. In fact, ^{2}H, ^{13}C, and ^{15}N have recently been used to track the summer origin of burrowing owl feathers collected in the winter in Texas and central Mexico and to determine the origin of breeding owls in the northern Great Plains (Duxbury 2004, Holroyd et al. 2010, Holroyd and Trefry 2011). The use of stable isotopes relies on the existence of a functional relationship between isotope signatures and geographic coordinates, recently referred to as an isoscape (West et al. 2008), which can be inferred through statistical methods to build "base maps" of local isotope signatures. Development of these isoscapes are possible because deuterium ratios in atmospheric water follow a continental gradient in North America (Dansgaard 1964), and stable carbon isotopic fractionation in plants differs between the 3 photosynthetic pathways: C_3, C_4, and CAM (Crassulacean acid metabolism; Peterson and Fry 1987). Therefore, geographic variation in floristic composition and plant life forms, such as the latitudinal gradient in C_3 versus C_4 grasses (Teeri and Stowe 1976), creates geographic gradients in carbon isotopic ratios. Nitrogen isotope fractionation in soils and plants varies with climate, N residence time, topography, and soil characteristics (Amundson et al. 2003). Animal tissues, including feathers, capture the isotopic signature of the local food chain where those tissues are grown. North American migratory birds are particularly suitable to study both natal and breeding dispersal because many species undergo pre-formative and pre-basic molts on the natal and breeding grounds (respectively) prior to fall migration (Pyle 1997). Therefore, the collection of feathers during the breeding season (prior to the pre-basic molt) can provide information about the location where adult birds hatched (for first-year breeders) or molted (for birds >1 year old) the previous breeding season, providing a

breeding dispersal distance vector (magnitude and direction) for each sampled bird. One assumption of this technique is that the molt for birds >1 year occurs on or near the breeding grounds, since the stable isotope signature reflects the birds diet where it grows the feather.

In this regard, stable isotope analysis of feathers allows us to test 2 predictions of our migration-mediated range-shift hypothesis. First, if irrigated agricultural areas in northern Mexico are perceived as optimal breeding habitat by burrowing owls and breeding owl populations are near carrying capacity, agricultural populations will have a higher proportion of returning breeders (i.e., lower natal and breeding dispersal) compared to non-migratory populations in non-agricultural areas (measured as the proportion of adult burrowing owls with isotopic signatures similar to that of the local nestlings). Second, some breeding burrowing owls in Sinaloa and Sonora will have feathers with isotope signatures similar to the local isotopic signatures in the northern Great Plains of Canada and the United States as predicted by our inferred isoscape (because they spent the prior breeding season in more northern latitudes). We tested these 2 predictions by sampling H, C, and N isotopic signatures of burrowing owl feathers throughout North America.

3. Methods

A. Study areas and sample collection

Adult burrowing owls undergo a complete pre-basic molt in late summer (Pyle 1997), and basic plumage is therefore assumed to have the stable isotope signature that

corresponds to the site where they bred the previous breeding season. Under this assumption, we collected feathers from young and adult burrowing owls during the breeding seasons of 2004-2009 at 36 study locations throughout the species' breeding range in Canada, Mexico, and the United States (Fig. 11 and Table 5). We defined populations in Alberta (ALB) and Saskatchewan (SAK) as declining migratory populations on the northern edge of the species' breeding distribution based on survey data in those locations (Sauer et al. 2008, Chapter I). We defined populations in Casa Grande (CAG), Salton Sea National Wildlife Refuge (SSW), Mexicali Valley (MEX), Caborca Valley (CAB), Hermosillo (HER), Yaqui-Mayo Valley (YAQ), Rio Fuerte Valley (FUE), and Culiacan Valley (CUL) as southern resident populations within irrigated agricultural areas. We pulled breast, back, and head feathers from nestlings that were 10-40 days-old, and we pulled the third right rectrix from adult burrowing owls. We did not use natal down feathers from nestlings in our study, which may have the isotope signature of the mother's diet during spring migration (Duxbury et al. 2003). We performed bird handling, feather collection, and the import and export of feathers through international boundaries under the compliance of Canadian, Mexican, and U.S. regulations. We also complied with the University of Arizona Institutional Animal Care and Use Committee regulations under protocols #01-089 and 04-296 (Appendix B).

B. Sample analysis

We initially used a chloroform:methanol solution for cleaning feathers in 45.6% of our samples to remove oils from feathers. We subsequently changed our cleaning protocol to a two-step cleaning procedure that included both a detergent solution and

chloroform:methanol solution after a paper was published by Paritte and Kelly (2009). We processed all our samples in the Environmental Isotope Laboratory at the University of Arizona. We used a Finnegan MAT TC/EA connected to Finnegan Delta Plus mass spectrometer through a Finnegan MAT CONFLO III Interface to measure δ^2H in feather samples. Our analytical precision for δ^2H based on the repeated analysis of a benzoic acid lab standard was better than 1.8‰ (parts per mil). We used sheep wool and whooper swan (*Cygnus cygnus*) feather tracer standards to calculate non-exchangeable δ^2H in owl feather samples. We equilibrated samples and tracer standards with ambient water vapor in the laboratory for at least 4 days. After equilibration, samples were dried with P_2O_5 dessicant to remove any adsorbed water. Three tracer standards were included with each batch of owl feather samples analyzed to monitor the effects of lab water vapor on measured δ^2H values. Tracer standards were calibrated based on room temperature equilibration with 3 water vapors with a wide range of δ^2H values and an estimated fractionation of α =1.12 between water vapor and exchangeable hydrogen in feather keratin. Using the calibrated non-exchangeable δ^2H value of the tracer standards and a mass balance equation, the δ^2H value of the exchangeable hydrogen in all feather samples can be calculated. We then calculated the δ^2H value of the non-exchangeable hydrogen in the owl feather samples using a mass balance equation based on the proportion exchangeable hydrogen and their total measured δ^2H value. At room temperature, the percent exchangeable hydrogen in feathers is 9.0% (determined based on the swan feather tracer calibration). We measured $\delta^{13}C$ and $\delta^{15}N$ on a continuous-flow gas-ratio mass spectrometer (Finnigan Delta PlusXL). Samples were combusted with added

oxygen in an elemental analyzer (Costech) coupled to the mass spectrometer. Standardization was based on acetanalide for elemental concentration, NBS-22 and USGS-24 for $\delta^{13}C$, and IAEA-N-1 and IAEA-N-2 for $\delta^{15}N$. Precision based on repeated internal standards was better than 0.08‰ for $\delta^{13}C$ and better than 0.2‰ for $\delta^{15}N$. Values of $\delta^{2}H$, $\delta^{13}C$, and $\delta^{15}N$ are computed for the Vienna Standard Mean Ocean Water standard, PeeDee Belemite standard, and atmospheric N_2, respectively. Precision for $\delta^{2}H$, $\delta^{13}C$, and $\delta^{15}N$ based on replicate subsamples from the same feather were ±4.78‰ (1,263 feathers), ±0.36‰ (221 feathers), and ±0.32‰ (222 feathers), respectively, measured as square root of the mean square error from an analysis of variance with feather sample (for $\delta^{2}H$,$\delta^{13}C$, and $\delta^{15}N$) and date of measurement (for $\delta^{2}H$) as fixed effects. We included date of measurement in our estimates of precision for $\delta^{2}H$ (and not for $\delta^{13}C$ and $\delta^{15}N$) to account for variability in $\delta^{2}H$ measurements caused by the uncontrolled exchange of ^{2}H atoms between ambient water (vapor) and the keratin in our feather samples (Wassenaar and Hobson 2003). The magnitude of this interchange can vary from date to date with temporal changes of $\delta^{2}H$ in ambient water and humidity, and can considerably affect measurements of $\delta^{2}H$ in feather samples.

C. Data analysis

Variability of $\delta^{2}H$ measurements on the same feather among laboratories (Smith et al. 2009) and across time within the same laboratory (Lott and Smith 2006) create challenges for using deuterium to track animal movements. We attempted to address these sources of measurement error by measuring $\delta^{2}H$ twice in almost all feather samples

in the same laboratory (within 2 different batches analyzed $\bar{x} = 30$ days apart, range = 0–479 days). We replicated samples within and among dates of analysis. We used a generalized linear mixed model (Bolker et al. 2008) in the *R* package *nlme* to generate δ^2H for individual owls (fixed effect) accounting for date of measurement (random effect).

Our dataset is not complete for ^{2}H, ^{13}C, and δ^{15}N. We have measured δ^2H in 76% of the owls for which we have measured both δ^{13}C and δ^{15}N. Therefore, we used our larger dataset (δ^{13}C and δ^{15}N from 1,592 owls) to test our first prediction (that local recruitment would be higher in agricultural areas), since a larger sample size produces a better estimate of a proportion (law of large numbers; Poisson 1835). We used our smaller dataset (all 3 isotopes from 1,213 owls) to test our second prediction (concerning breeding dispersal). By following this approach, we could include 5 more locations in our study (LEM, SDO, MOS, TXP, FBL) that either lacked of δ^2H or lacked of δ^{13}C and δ^{15}N measurements in nestling feathers (nestling feathers are not necessary for every location in our second prediction). First, we assumed that adult burrowing owls with stable isotope signatures outside of the 95th-percentile ellipses defined by nestling signatures were not in the location the previous breeding season. The purpose of this approach was not to predict the origin of owls classified as migrant, but rather to estimate the proportion of adults in each population that was immigrant. We used package *ellipse* in program *R* (R Development Core Team 2009) to generate and plot the 95th-percentile ellipses from a bivariate normal distribution for δ^{13}C or δ^{15}N. Second, we used a local regression analysis (LOESS, Cleveland et al. 1992) for spatial interpolation to build base

maps of $\delta^{2}H$, $\delta^{13}C$, and $\delta^{15}N$ across North America based on the stable isotope signatures of nestling feathers. ^{2}H base maps for feathers are available for other bird species for portions of our study area (Lott and Smith 2006, Hobson et al. 2009). The use of $\delta^{13}C$ and $\delta^{15}N$ base maps to track animal movements remains largely unexplored (Bowen and West 2008). Therefore, feather base maps for $\delta^{13}C$ and $\delta^{15}N$ are not currently available, although surrogate base maps exists for ^{15}C in terrestrial vegetation (Suits et al. 2005) and for ^{15}N in soil and plants (Amundson et al. 2003). We decided to build our own base maps specific to burrowing owl feathers given: 1) the lack of information regarding interspecific variation in fractionation processes, and 2) our exhaustive sampling of nestling feathers throughout the species' breeding range (we typically caught juveniles while attempting to catch adults). We are not aware of the availability of software to conduct geographic assignment of individuals with known isotopic signature and unknown origin. Therefore, we wrote our own script in program *R*. We created a 100×100 grid of points for the region encompassed by our study populations, with 0.23°×0.31° grid cells. We trimmed this grid by a maximum convex polygon with vertices defined by our study populations to avoid assignment of adult owls to locations out of our range of inference. We then used the command *predict.loess* to predict $\delta^{2}H$, $\delta^{13}C$, and $\delta^{15}N$ values for each location in the final grid based on the *R* object generated with command *loess* on nestling data. We computed the standardized Euclid distance from the isotopic signature of the i-th adult burrowing owl ($i = 1, 2, \ldots, 894$) to the isotopic signature predicted by the 3 base maps at each point on the geographic grid ($j = 1, 2, \ldots, 5129$). We computed the standardized Euclid distance (d) as:

$$d(i,j)=\left[\frac{(\delta^{2}\mathrm{H}_{i}-\delta^{2}\mathrm{H}_{j})^{2}}{\sigma^{2}_{\delta^{2}\mathrm{H}}}+\frac{(\delta^{13}\mathrm{C}_{i}-\delta^{13}\mathrm{C}_{j})^{2}}{\sigma^{2}_{\delta^{13}\mathrm{C}}}+\frac{(\delta^{15}\mathrm{N}_{i}-\delta^{15}\mathrm{N}_{j})^{2}}{\sigma^{2}_{\delta^{15}\mathrm{N}}}\right]^{\frac{1}{2}}$$

where each $\sigma^{2}_{\delta^{n}\mathrm{X}}$ is the estimate of variance for each isotope computed as the variance of LOESS residuals. We assigned each adult burrowing owl (*i*) to the location on the grid that produced the lowest *d*(*i*, *j*). That is, we assigned each adult burrowing owl to the location on our grid that had the most similar stable isotope signature to that of the adult owl. However, several locations on the grid may be equally close in *d* (rounded up to 2 decimals). In situations where >1 location had similar *d* values, we assumed that the location closest to the collection site was more likely to represent the true origin of an owl. Therefore, we assigned each adult burrowing owl's origin to the closest geographic location on the grid among competing locations with the same *d*. We used this as a conservative approach intended to prevent the detection of spurious long-distance dispersal events. A basic assumption in this analysis is that stable isotope signatures of juvenile body feathers are comparable to those of rectrices in adult feathers grown in the same location (Langin et al. 2007, Smith et al. 2008). Meehan et al. (2003) showed a large, unexplained difference in δ^{2}H values between juvenile and adult Cooper's hawks (*Accipiter cooperii*). Burrowing owls molt their retricies simultaneously or nearly so, towards the end of the nestling stage. Thus, we do not expect substantive differences such as those found in Cooper's hawk primaries that are molted over a longer time period during the post-breeding season. We decided to use juvenile body feathers (rather than rectrices of fledglings) because nestlings are considerably easier to trap and estimate age than fledglings (which can be mistaken as after-hatching-year birds) and we could obtain

a large sample allowing for more precise estimates. Moreover, we did collect developing rectrices from juveniles to avoid any harm to the growing birds. We used program *R* v. 2.9.2 for Mac to perform all statistical analyses.

4. Results

A. Philopatry

The distribution of isotopic signatures of nestling feathers varied among study populations in the $\delta^{13}C$-$\delta^{15}N$ space (Fig. 12). Although we found a general pattern of increased enrichment in both $\delta^{13}C$ and $\delta^{15}N$ with decreasing latitude, we also found similar ^{13}C–^{15}N signatures in nestling feathers from distant locations. For example, the northwestern Sonora ellipse (CAB) overlaps with that of distant populations in central Colorado (BRM; Fig. 12). In addition, the ellipse from northern Baja California (MEX) overlaps with those for Alberta and Saskatchewan. The use of 95^{th}-percentile ellipses allows us to minimize the error of classifying a local burrowing owl as an immigrant, although this procedure may classify some immigrants as locals. The area of the 95^{th}-percentile ellipses was not correlated ($r = 0.25$) with the number of data points used to generate them, suggesting that the sample sizes we used to generate the ellipses (3-36 nestlings) did not bias our results.

In most locations, the signatures of feathers collected from adults (open circles and triangles in Fig. 12) had higher intra-population variation than those of the nestlings in that same location (filled circles in Fig. 12), which is expected given the likely existence of immigrants within the adult population. We found a remarkable geographic

pattern of philopatry-immigration among burrowing owl populations. Northern populations in Alberta (ALB), Saskatchewan (SAK), and southwestern Idaho (MNH) had the highest proportion of immigrants among all burrowing owl populations, with 95%, 92%, and 67% of their breeding populations originating elsewhere, respectively (Fig. 13). We also observed a high proportion of immigrants in the peripheral populations in central and southern Baja California (MUL and SDO), as well as in southwestern Utah (SGE), although our sample sizes for both nestlings and adults in those locations were low. We observed the highest proportion of philopatic birds in an isolated population in central Mexico (TEX) where 100% of the adult owls shared the stable isotope signature of the local nestlings. This result suggests that our estimates of philopatry rate are meaningful because a genetic survey indicates that this population in central Mexico is genetically differentiated from the remainder of the continental population (Chapter V). The proportion of return birds in the remaining populations, including agricultural populations in northwestern Mexico, averaged 70.0±2.7% (range 41.7-89.5%). The highest proportion of philopatric birds among these populations (89.5%) was within an agricultural area in central Sonora (Hermosillo, HER; Figs. 12 and 13), providing some support to the first prediction of the migration-mediated range-shift hypothesis: that irrigated agricultural areas in the southern portion of the species' range have higher site fidelity than southern populations in non-agricultural areas. However, agricultural areas in Imperial Valley (SSW) had a relatively low proportion of philopatric owls (41.7%) compared to the rest of the interior populations. Populations in eastern Washington (TCY) at the northwestern edge of the species' breeding distribution also had relatively

high levels of philopatry compared to that of populations in Canada.

B. Breeding dispersal

LOESS regression allowed us to find geographic gradients in δ^2H, $\delta^{13}C$, and $\delta^{15}N$ values in nestling feathers across North America (Fig. 14), and use these isoscapes to document general patterns of burrowing owl breeding dispersal throughout the North American continent. Deuterium showed a latitudinal gradient consistent with the well-known geographical pattern documented for precipitation, with more enriched deuterium in southern latitudes. However, we found a noticeable disruption in the general latitudinal pattern in δ^2H in southwestern United States and northwestern Mexico (Fig. 14). This disruption originated from extraordinarily low δ^2H values in burrowing owl nestling feathers at Salton Sea National Wildlife Refuge (SSW) and the Mexicali valley (MEX) along the lower Colorado River (Fig. 15). We also observed a latitudinal pattern in $\delta^{15}N$ without major disruptions, with more ^{15}N -enriched nestling feathers in southern latitudes (Fig. 14). $\delta^{13}C$ showed a longitudinal pattern with the less ^{13}C-enriched values towards the Rocky Mountains (Fig. 14). Despite these latitudinal and longitudinal patterns in our 3 isotopes, we found large variation within study populations that limited our precision in predicting the origin of adult burrowing owls based solely on their isotopic signatures. The difference between the maximum and minimum predicted values (range) of δ^2H, $\delta^{13}C$, and $\delta^{15}N$ in basemaps was 76.5, 9.7, and 5.6‰, respectively (Fig. 14). This overall geographic variation approaches the within-population variation in δ^2H, $\delta^{13}C$, and $\delta^{15}N$ (Fig. 16). Therefore, our results should be interpreted with caution.

Breeding dispersal in burrowing owls seemed unconstrained throughout the North

American continent, although some latitudinal patterns in dispersion distances were evident. Northern populations (e.g., ALB, SAK, MOS, TCY and WYO) received immigrants from more southern populations. Populations in Canada received immigrants from locations as far south as central California, southern Nevada, and western Arizona. However, adult burrowing owls captured in Canada that were assigned to southern locations irrigated with water from the Colorado River may be an artifact of similar isotopic signatures in these 2 regions (Fig. 15). Burrowing owl breeding populations at intermediate latitudes, such as eastern Colorado (BRM) received immigrant owls from an extensive region spanning from Canada to northwestern Mexico, as well as central Mexico (Fig. 17). Southern populations relied more on local recruitment and immigration from neighbouring populations than northern populations. Populations in the California Central Valley (DIX) seemed to recruit breeding burrowing owls exclusively within the valley. Populations in the Mohave Desert (EDW) apparently received immigrants from populations in agricultural areas in southern California and northwestern Mexico, as well as from populations in Canada and central Mexico.

Burrowing owl populations breeding in agricultural areas in southwestern United States and northwestern Mexico differed in the geographic origin of their immigrants. Our study populations at Casa Grande (CAG) and Mexicali Valley (MEX) showed high levels of local recruitment and immigration from neighboring populations. However, the burrowing owl population in southern California (SSW), adjacent to the Mexicali Valley (MEX), received immigrants from a much larger segment of the species' breeding range, including central Sinaloa, eastern Washington, and Canada. Both Sinaloa populations

(FUE and CUL) had high local recruitment in addition to immigrants from areas east of the Sierra Madre Occidental, but received no migrants from Canada. FUE and CUL study populations appeared to receive fewer long-distance immigrants compared to other populations. Owl populations breeding in agricultural areas of southern Sonora (YAQ) relied exclusively on local recruitment and immigration from the Sonoran desert populations, including those in southern California. Our results suggest a relatively high proportion of local recruits in agricultural populations in central Sonora (HER), with some immigrants for central Mexico. Isotopic signatures of adults breeding in agricultural areas in northwestern Sonora (CAB) suggest immigration from neighbouring populations and from as far north as Canada and as far south as central Mexico. Therefore, we found evidence of burrowing owls from northern latitudes (where only migratory populations breed) becoming resident breeders in agricultural areas in both southern California and northwestern Sonora. This pattern is what was predicted by the migratory-mediated range-shift hypothesis.

Populations in the Mexican Highlands in Chihuahua (JAN, CHI, and DEL), Coahuila (LAG), and Nuevo Leon (GAL) suggest primarily breeding dispersal at a regional level (within the Mexican Highlands and the Great Plains) plus immigrants from elsewhere: eastern Washington for JAN and CHI, Sonora and Sinaloa for LAG and GAL, and central Mexico for all 5 populations. Burrowing owls in central Mexico (TEX) relied mostly in local recruitment with only few immigrants from Sinaloa and Sonora.

5. Discussion

We found regional variation in the extent of philopatry and breeding dispersal of

burrowing owls throughout North America. However, these results should be regarded as patterns that require further verification given the high variation in $\delta^{2}H$, $\delta^{13}C$, and $\delta^{15}N$ in nestling feathers within study locations (Figs. 12, 15, and 16). Overlap in local ^{13}C–^{15}N stable isotope signatures throughout the species' breeding range may hinder our ability to accurately estimate rates of philopatry (Fig. 12). In addition, high intra-population variation in $\delta^{15}N$ in nestling feathers observed in this study may pose concerns of the validity of ^{15}N to produce continental-scale basemaps. The mapping of $\delta^{15}N$ bird feathers at the continental scale has no precendent in spite of the potential of ^{15}N to track large-scale landbird movements (Chamberlain et al. 2000). Although ^{15}N in plants and soils has a distinct geographic pattern (Amundson et al. 2003), ^{15}N deposition due to agricultural production may disrupt this geographic pattern, artificially increasing $\delta^{15}N$ in bird feathers (Hebert and Wassenaar 2001). We did not have nestling feathers from an agricultural study site and a non-agricultural study site adjacent to each other and with similar climate conditions (since $\delta^{15}N$ varies with temperature and precipitation; Admundson et al. 2003) to examine the extent to which $\delta^{15}N$ in bird feathers is elevated in agricultural areas. Nevertheless, $\delta^{15}N$ of nestling feathers decreased with latitude (Fig. 18), a pattern consistent with both the positive correlation between mean annual temperature and plant $\delta^{15}N$ and the latitudinal gradient in temperature, supporting the validity of our ^{15}N basemap.

Canadian burrowing owl populations (ALB and SAK) stand out for their low rates of philopatry compared to other sampled populations. Our results with ^{13}C and ^{15}N suggest that more than 90% of burrowing owls breeding near the northern edge of the

species' distribution in Canada originated further south (i.e., very low local recruitment). High rates of breeding dispersal in Canada has been reported from field studies; 137 banded burrowing owls did not breed more than once (0% return rate of breeders) in a 4-year period at a study location in Saskatchewan (Wellicome 2005), a pattern which corroborates the conclusions from our isotope analysis. However, other estimates of adult return rates in Canada are higher (29-58%, Haug et al. 1993). Moreover, a previous stable isotope study estimated that 43% and 46% of breeding burrowing owls were non-immigrants in Alberta and Saskatchewan, respectively (Duxbury 2004). If reproductive success and juvenile survival in Canadian populations is also low (Clayton and Schmutz 1999), the northern edge of the species' distribution could be a population sink (Pulliam and Danielson 1991), where mortality and emigration (breeding dispersal) exceed local recruitment and populations are maintained via dispersal and re-colonization by individuals from interior populations. This pattern of immigration from interior populations compensating for low recruitment has been referred to as the "rescue effect" (Brown and Kodric-Brown 1977) and forms the basis of metapopulation dynamics (Hanski 1998). The northern edge of the species' distribution could also be a population source if juveniles produced there successfully disperse to other locations and breed. Indeed, we found evidence suggesting that burrowing owls with Canadian isotope signatures had dispersed to other study locations (Fig. 14). However, low philopatry in Canadian populations and theory on geographic ranges (Gaston 2003) suggests the existence of suboptimal conditions for reproduction and survival at species' range edges. Estimation of survival and reproductive rates for first-year birds produced in Canada

would help unveil the population dynamics at the northern edge of the species' distribution. An alternative explanation for the low philopatry in Canadian populations is that migratory burrowing owls from the northern edge of the species' range may molt their feathers after leaving the breeding grounds during migration (Duxbury 2004). Initiation date of fall migration might be under strong selection at the northern edge of the owl's breeding range and, hence, owls may migrate even before molting (and timing of molt may not be flexible due to high phylogenetic inertia, Svensson and Hedenstrom 1999). However, collection of molted feathers on the Canadian breeding grounds suggest that most burrowing owls do molt at the end of the nestling stage prior to fall migration (G. Holroyd, R. Poulin, H. Trefry, T. Wellicome, pers. comm.). Better information on molting schedules in burrowing owls that breed in Canada (and elsewhere) would aid future studies of breeding dispersal based on stable isotope analyses of feathers in migratory burrowing owl populations.

We also found a relatively high proportion of immigrants in the burrowing owl population in southwestern Idaho (MNH), where owl populations appear to be increasing (Sauer et al. 2008), and in 2 populations in southern Baja California peninsula (which is also on the periphery of the species' breeding range (Fig. 13). The apparent lack of philopatry in the agricultural area near Santo Domingo (SDO in Fig. 12) does not support the idea of newly-created agricultural areas in the southern part of the species' range being more attractive for burrowing owls than natural habitats. In addition, the low philopatry observed in the burrowing owl population in Imperial Valley (Fig. 13) does not support the idea that burrowing owls perceive agricultural areas as high-quality

habitats that promote residency (high philopatry), in spite of the possible high intra-specific competition for breeding territories due to a high population density there (DeSante et al. 2004, Rosenberg and Haley 2004).

The proportion of return birds inferred from our stable isotope results for most of the populations in the interior portion of their breeding range (including those in agricultural áreas in the southern portion of the species' range) are comparable to estimates of annual return rates for a non-migratory population in Florida (68% in adult males, Haug et al. 1993). High rates of philopatry in burrowing owl populations in North America may reflect overwintering birds seeking to retain high-quality territories. However, the proportion of return birds in eastern Washington (64%) was higher than estimates of annual return rates from the same study area based on band returns (<44%, Conway et al. 2006). We would expect estimates from stable isotopes to be higher than those from band returns because the former is merely the percentage of breeders that have the local isotopic signature (rather than the percentage of banded birds that return to a particular study site and are detected).

Our inferred geographic patterns in $\delta^{2}H$, $\delta^{13}C$, and $\delta^{15}N$ in nestling feathers show some agreement with published model-based isoscapes. Isotopic signatures from feathers of our burrowing owl nestlings coincide in some aspects with geographical patterns in $\delta^{13}C$ based on the ecophysiological model by Suits et al. (2005). The southern Sonoran desert and Chihuahuan desert show enriched $\delta^{13}C$ values, although the rest of the burrowing owl distribution remains depleted (with low variation). And our inferred continental pattern in $\delta^{15}N$ coincides with the latitudinal pattern predicted by Amundson

et al. (2003) based on climate and soil properties, but does not show the enriched $\delta^{15}N$ disruption in the Sierra Madre Occidental due to our lack of samples there (burrowing owls do not breed in mountainous areas). Moreover, our geographic assignments of adult feathers based on comparisons to our nestling-derived base map showed some consistency with previous findings on burrowing owl breeding dispersal. Burrowing owls appear to be unconstrained in their breeding dispersal capabilities, with individual owls frequently crossing major physiographic barriers such as the continental divide (Duxbury 2004; G. Holroyd, pers. comm.). In addition, populations further north tend to receive more immigrants from more widespread locations compared to southern populations (also see Duxbury 2004). If the immigrants that our isotope analysis suggests are dispersing from southern populations into populations at the northern extent of the breeding range are primarily second-year birds, this finding supports a component of Rappole's (1995) hypothesis for the evolution of migratory behavior. Rappole (1995) suggested that migration evolved in tropical resident populations, where high site fidelity and competition forced juveniles towards northern breeding grounds (Rappole 1995). Future studies should test whether immigrants in northern populations are more likely to be second-year birds compared to immigrants in central and southern populations. We found some evidence that suggests that burrowing owls populations in Canada and the northern United States are declining due to northern owls dispersing to the agricultural areas in northwestern Mexico and the southwestern United States. However, the prediction we used to test our hypothesis did not provide the resolution we initially envisioned due to an unforeseen disruption in isotopic gradients in North America.

Nestling feathers in the Salton Sea in southern California and Mexicali Valley in northern Baja California showed similar isotopic signatures to those of northern populations. This similarity hinders our ability to correctly infer dispersal events between these 2 regions and their neighboring locations. Similarities in $\delta^{2}H$ can be explained by the influence of the Colorado River water. Contribution of local precipitation to the $\delta^{2}H$ signature of the agricultural ecosystem is extremely limited (89 mm of mean annual precipitation in Mexicali, Ruiz Corral et al. 2006). The water used to irrigate crops in the lower Colorado River region comes almost entirely from the Colorado River (rather than from local groundwater), which carries ^{2}H-depleted water from precipitation that originated throughout the Colorado River basin (which extends up into Colorado, Utah, and Wyoming). The reasons for the similarities in $\delta^{13}C$ and $\delta^{15}N$ in nestling feathers between Mexicali and Canadian populations are not as clear.

Our data suggests 100% philopatry for burrowing owls breeding in an isolated population in central Mexico (Texcoco) based on ^{13}C and ^{15}N only (Figs. 12 and 13), but with some immigration from local neighboring populations when using ^{2}H, ^{13}C, and ^{15}N (Fig. 17). These results suggest that the population at Texcoco (TEX) is non-migratory with little connectivity with other populations. Our isotopic results are corroborated by a genetic study that found genetic differentiation of this population from any other burrowing owl population (Chapter V).

In summary, our results suggest that burrowing owls have high levels of philopatry or localized breeding dispersal within most populations in the central and southern portions of their breeding distribution, but have a sufficient number of long-

range dispersal events to promote high connectivity among populations throughout most of their breeding range. This unconstrained movement has apparently lead to some dispersal of burrowing owls from Canada to agricultural areas in northwestern Mexico, in support of the migration-mediated range-shift hypothesis. These dispersal events likely allow owls to colonize suboptimal habitat on the periphery of their breeding range. Therefore, the patterns of breeding dispersal suggest that burrowing owls may have the capability to re-colonize former breeding habitat. Thus, documented population declines may actually represent extirpation processes being attenuated by breeding dispersal, which has even deeper implications for the viability of local burrowing owl populations at the northern edge of their range.

CHAPTER V. GENETIC VARIATION AND DIVERGENCE TIMES AMONG ISLAND AND CONTINENTAL POPULATIONS OF BURROWING OWL SUBSPECIES (*ATHENE CUNICULARIA*) IN NORTH AMERICA

1. Abstract

Subspecies diagnosis is highly relevant to adequately preserve genetic variation, especially for species of conservation concern with endangered insular populations. Burrowing owls (*Athene cunicularia*) are widespread in both North and South America and resident populations occur on many islands in the eastern Pacific Ocean and the Caribbean Sea. Eighteen subspecies have been identified, but these taxonomic relationships are based primarily on morphological traits. This study characterizes microsatellite and mitochondrial DNA variation among the western (*A. c. hypugaea*), Florida (*A. c. floridana*), and Clarion (*A. c. rostrata*) subspecies of the burrowing owl. We also characterized genetic variation in 2 geographically isolated populations of the western subspecies, one on Guadalupe Island (250 km off the coast of Baja California) and the other near Mexico City. We estimated mutation rates of 11 microsatellite loci for burrowing owls from Clarion Island and used those mutation rates to estimate divergence time between the western and Clarion subspecies. Clarion burrowing owls had no intrapopulation variation (i.e., fixation) at 5 out of 11 microsatellite loci. The Florida subspecies had only polymorphic loci but had reduced levels of genetic variation compared with the western subspecies (a more-widespread subspecies that occurs throughout western North America). We estimated a divergence time of 110,000 – 370,000 years between the Clarion and the western burrowing owl subspecies. We found

genetic differentiation between western burrowing owl populations and a geographically isolated population in central Mexico (near Texcoco Lake). We identified 3 haplotypes for the western burrowing owl based on our extended mitochondrial DNA survey; one of them was present in all individuals sampled on Clarion Island and another was present exclusively on Guadalupe Island. These data confirm the high connectivity among western burrowing owl despite several large geographic barriers. Our results suggest the need for further research to explore the possibility of re-classifying the burrowing owls on Guadalupe Island and highlight the need to protect the unique population of burrowing owls in the Texcoco Lake area of central Mexico.

2. Introduction

Subspecies are of particular interest to evolutionary biologists because they are often thought to represent evidence of speciation in progress. Most avian subspecies in North America have been described as such based on morphological, vocal, or plumage characteristics (Cicero 2010). With the advent of genetic markers, many of these subspecies designations have recently been revised. Solving these discrepancies is highly relevant to adequately preserve genetic variation for taxa of conservation concern, especially those with endangered insular subspecies (Pratt 2010). Burrowing owls (*Athene cunicularia*) are widespread in North and South America, inhabiting open arid and semiarid plains from southern Canada to Tierra del Fuego, including islands in the Caribbean Sea and Pacific Ocean (König et al. 1999). Eighteen subspecies are recognized, but these designations are based on variation in size, weight, and plumage coloration (König et al. 1999). Adaptive radiation in burrowing owls has apparently been

less intense in North America with only 3 recognized subspecies: Clarion (*A. c. rostrata*), Florida (*A. c. floridana*), and western (*A. c. hypugaea*) burrowing owls. The western burrowing owl is federally endangered in Canada and is a species of conservation concern in the U.S., and the Clarion burrowing owl is federally endangered in Mexico. Elucidating phylogeographic relationships among burrowing owl subspecies in North America can help inform appropriate scales for management and recovery efforts and potentially reveal major historical events that determined continental speciation patterns in other taxa.

The Clarion burrowing owl (*A. c. rostrata* Townsend) is the most isolated of all of the 3 recognized subspecies in North America, occupying a small island in the Pacific Ocean about 700 km southeast of the Baja California peninsula. The Clarion burrowing owl is listed as an endemic endangered species by the federal government in Mexico (Secretaria de Medio Ambiente y Recursos Naturales 2002), and was negatively affected by feral goats and pigs that were once common on Clarion Island before their removal in recent years (Everett 1988, Brattstrom 1990). Therefore, an assessment of the genetic variation and phylogeography of the Clarion burrowing owl is necessary to assess its taxonomic status as a required component of any population viability analysis. The Florida subspecies (*A. c. floridana* Ridgway) inhabits the Florida peninsula and is genetically differentiated from the western subspecies (Korfanta et al. 2005). The western burrowing owl (*A. c. hypugaea* Bonaparte) is the most widespread subspecies occupying a continuous breeding distribution over the western half of North America (Wellicome and Holroyd 2001). However, isolated western burrowing 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 (Enriquez-Rocha 1997), where burrowing owls have been observed during the breeding season.

In this study, we used mitochondrial DNA sequences and DNA microsatellite markers to characterize genetic variation among the 3 currently recognized North American subspecies of burrowing owls. We estimated divergence time between the western and Clarion subspecies based on microsatellite variation. We also examined samples from 2 geographically isolated populations that were both assumed to be western burrowing owls: Texcoco and Guadalupe Island. The Texcoco population of western burrowing owl inhabits the area around the ancient Texcoco Lake, near Mexico City. This resident population appears to be isolated from the rest of the subspecies' breeding distribution (the nearest known breeding population is ~400 km to the north) and genetic divergence from populations in the core breeding range might be expected. Guadalupe Island is an oceanic island 250 km west of Baja California, Mexico (Fig. 19). Our study intended to expand upon past genetic surveys of burrowing owl subspecies based on mitochondrial DNA (cytochrome b) by Desmond (2001) and DNA microsatellite markers by Korfanta et al. (2005).

3. Methods

A. Sample collection

We trapped burrowing owls during the spring and summer of 2004-2009. Within the range of the western burrowing owl, we obtained samples from 1,671 burrowing owls

from 37 locations representing 2 provinces in Canada, 11 states in the United States, and 8 states in Mexico, including 19 burrowing owls from Texcoco and 6 owls from Guadalupe Island. Study locations and number of owls sampled per location for the western burrowing owl are provided in Chapter III. Birds on Clarion Island are year-round residents and 19 samples were collected in November 2008 by G. Holroyd, E. Valdez, and H. Trefry. We did not include any birds that were known to be closely related (i.e., parents and their offspring, or juveniles from the same nest burrow). Our primary source of genomic DNA was blood. We obtained ~50 μL 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 performed bird handling, and blood and feather collection, as well as the import-export through international boundaries, under the compliance of Canadian, Mexican, and U. S. regulations. We also complied with the University of Arizona Institutional Animal Care and Use Committee regulations under protocols #01-089 and 04-196. We obtained DNA from museum specimens (American Museum of Natural History) for a subspecies of burrowing owl in South America (*A. c. cunicularia*) to determine the adequacy of our microsatellites for phylogenetic inference, and the little owl (*A. noctua*), a congener that is resident in Europe, Asia, and northern Africa.

B. Genotyping

We followed laboratory procedures for DNA extraction, polymerase chain reaction, and allele scoring for microsatellite loci as described in Appendix A and Chapter III. In

addition, we sequenced mitochondrial DNA (mtDNA) in the cytochrome b gene as outlined in Desmond et al. (2001).

C. Data analysis

We used MS Excel© macro *GENALEX* 3.6 (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* 4.0.10 (Rousset 2008) to calculate F_{ST} (Weir and Cockerham 1984) between the Texcoco population and each of the other 36 populations of western burrowing owl to reveal subtle genetic differentiation.

We used microsatellite repeat data to calculate divergence time (T) between the western and Clarion subspecies of burrowing owls. Under the stepwise mutation model (Ohta and Kimura 1973), divergence time is given by the expression (Goldstein et al. 1995):

$$T = \frac{(\delta\mu)^2}{2w}$$

where w is the mutation rate (mutation probability per meiosis) and $(\delta\mu)^2$ is the squared difference in the mean number of repeats between the 2 subspecies. Zhivotovsky (2001) suggested an estimator of divergence time T_D that does not assume mutation-drift equilibrium and is independent of population growth in the absence of gene flow. However, T_D requires one more unknown component, the variance in the number of repeats in the ancestral population, so we could not use this statistic. We used genotype data from Clarion Island only to estimate w because we can fairly assume that migration

is not affecting genetic variation within this subspecies and that all genetic variation is due to mutation-drift equilibrium. We estimated microsatellite mutation rates *w* at each locus by first estimating the population-scaled mutation parameter θ under mutation-drift equilibrium (where $\theta=4N_e w$, and N_e is the effective diploid population size) and used the θ_H estimator derived by Ohta and Kimura (1973) for the stepwise mutation model. The θ_H estimator is given by the equation:

$$\theta_H = \frac{1}{\left(1-\hat{H}\right)^2} - 1$$

where $\hat{H}$ is the expected heterozygosity. The simplicity of θ_H does not hinder its efficiency as an estimator of θ because θ_H performs as well as more advanced likelihood-based estimators (RoyChoudhury and Stephens 2007). We computed the mutation rate *w* with the expression $w = \theta_H (4N_e)^{-1}$. However, we observed allele fixation in 5 out of the 11 microsatellite loci studied (Table 6). In such situations, estimates of *H*, θ_H, and *w* become zero. We therefore assumed a population-scaled mutation rate $\theta_H = 0.1051$ for those 5 loci. This θ_H value amounts to capturing a heterozygous individual for a second allele in one additional sampling attempt at Clarion Island (capturing a 20th owl), which is a conservative estimate of θ_H. We used package *bootstrap* in program *R* (R Development Core Team 2009) to generate 1,000 bootstrap replicates of population-scaled divergence times ($T \cdot (2N_e)^{-1}$) over the 11 loci. We used these replicates to estimate the 95%-confidence interval of the average population-scaled divergence time between Clarion and western burrowing owls.

4. Results

The amount of genetic variation within subspecies varied considerably among the 3 currently recognized subspecies of burrowing owls in North America (Table 6). The western burrowing owl had high levels of genetic variation at all loci and all populations. Western burrowing owl populations had high observed heterozygosities ($\overline{H}_O$ = 0.823 ± 0.022), similar to that of the isolated population in central Mexico ($\overline{H}_O$ = 0.807 ± 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 rest of the populations of the western subspecies was evident in the mean number of alleles per loci (7.7 *vs.* 22.5 alleles, respectively). The Florida burrowing owl had lower levels of genetic variation ($\overline{H}_O$ = 0.548 ± 0.059 and N_a = 5.1±0.1) compared to the western burrowing owl, and 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 ($\overline{H}_O$ = 0.177 ± 0.090), had 5 fixed loci (45%; Table 6), and did not have any unique alleles. The virtual absence of unique alleles in our markers extended to other subspecies and congeneric species. We found only 1 allele at locus ATCU04 in 2 of 3 burrowing owls from Argentina (*A. c. cunicularia*), 1 homozygous and 1 heterozygous. We found no unique alleles in 2 European little owls (*A. noctua*) genotyped for these 11 loci.

F_{ST} statistics 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 ± 0.0011) is

significantly higher (t = 26.77, d.f. = 701, $P < 0.0001$) than the mean pairwise F_{ST} among all other burrowing owl populations (F_{ST} = 0.0085 ± 0.0003).

We estimated 108.6 years individual^{-1} (95% C. I. from 1,000 bootstrap replicates: 15.0 - 232.9) as the average population-scaled divergence time ($T \cdot (2N_e)^{-1}$) across loci between Clarion and western burrowing owls. Estimates of divergence time depend on the effective population size (N_e) and therefore a careful choice of N_e must be made to adequately estimate divergence time. Potential estimates of effective population size for Clarion burrowing owls vary by orders of magnitude, including 10 owls (Everett 1988), and 1,700 owls (850 pairs, Wanless et al. 2009). The latter estimate of N_e originated from a recent call-broadcast survey but may have overestimated the population size, which is probably closer to 500 owls (200-300 pairs; G. Holroyd, H. Trefry, and E. Valdez, pers. comm.). Divergence time varies considerably within this range of N_e estimates (Fig. 20). Estimated divergence time between the Clarion and the western burrowing owls occurred 370,000 years ago (95% C.I. = 51,000-790,000 years) if N_e = 1700, and 110,000 years ago (95% C.I. = 15,000-240,000 years) if N_e = 500, more than a million years after Clarion Island emerged as a volcanic island in the late Pliocene (Brattstrom 1990).

Mitochondrial DNA suggests a more recent evolutionary split of the Clarion subspecies from the western burrowing owl compared to that of the Florida burrowing owl. We only found 3 informative sites in the mtDNA cytochrome b sequence of 307 base pairs with substitutions in base pairs 85, 178, and 274. We did not attempt any statistical inference with phylogenetic trees in this chapter considering such a limited amount of informative sites. These 3 informative sites yielded 4 haplotypes. We found

haplotypes TGA and TGG in western burrowing owl populations, and TAG in Florida burrowing owls. In addition, we found haplotype CGG in 3 out of 5 owls on Guadalupe Island (Fig. 21). Surprisingly, we did not find exclusive haplotypes for Clarion Island, where all burrowing owls had the TGG haplotype (1 of the 2 found in western burrowing owl populations throughout their range). Haplotypes TGA and TGG were found in equal frequency among all western burrowing owl populations (Fisher's exact test, $P = 0.065$; Fig. 21), including the Guadalupe Island population. However, low sample size may have reduced our power to detect differences in haplotype frequencies among populations. This result suggests high connectivity among western burrowing populations on the mainland (except Texcoco) despite the presence of large geographic barriers such as the Rocky Mountains, the Sierra Madre Occidental, and the Gulf of California (Fig. 21).

5. Discussion

Avian endemism is high in the Revillagigedo Archipelago, with greatest affinity to the Sonora-Sinaloa area of mainland Mexico (Brattstrom 1990). Mitochondrial DNA supports this origin for the Clarion burrowing owl, which possesses a western haplotype, and not a Florida or South American haplotype (Desmond et al. 2001). Divergence time estimated by our microsatellite data is bounded above by the age of Clarion Island, and provides a time framework for the colonization of the Revillagigedo Archipelago. However, our estimates of divergence times between Clarion and the western burrowing owl, although realistic, may be biased low. The use of $(\delta\mu)^2 \cdot (2w)^{-1}$ to estimate divergence time relies on a mutation-drift equilibrium and a constant N_e (Ramakrishnan and Mountain 2004), assumptions which are likely not met on Clarion Island. Clarion

burrowing owls may have been near extinction recently (Everett 1988) and probably underwent a recent population expansion after the eradication of feral pigs in 2002 (Wanless et al. 2009). If populations are growing, $(\delta\mu)^2 \cdot (2w)^{-1}$ underestimates the actual divergence time (Zhivotovsky 2001). Furthermore, the surrogates of expected heterozygosity for fixed loci in Clarion burrowing owls may have also affected our divergence time estimates. However, the lack of unique mtDNA haplotypes on Clarion Island suggests that these 2 subspecies diverged less than 200,000 years ago based on the so-called 2% rule (Lovette 2004), which supports our estimate of divergence time based on microsatellite data. An extended genetic survey including more mtDNA and nDNA sequences will help refine the estimate of when Clarion burrowing owls diverged from the western burrowing owl. The Clarion burrowing owl was proposed as a different subspecies based entirely on morphology (Townsend 1890). Intriguingly, the lack of exclusive microsatellite alleles or exclusive haplotypes does not support subspecies status for the Clarion burrowing owl.

Desmond (2001) found that Florida haplotype (TAG) differs in 2 nucleotides from the one found in the western burrowing owl (TGA) and applied the 2% rule (Lovette 2004) to suggest a divergence time of 350,000 years. However, the presence of a second western haplotype (TGG) that differs in only 1 nucleotide from the Florida haplotype would suggest a more recent split of 160,000 years. This study shows that even with slow-evolving markers such as mtDNA, sample size within populations and subspecies, not only among species, is critical to obtain better inferences in phylogenetic relationships.

Our microsatellite marker data set confirmed the results from Korfanta et al. (2005) that the Florida burrowing owl had lower levels of genetic variation than the western burrowing owl. These authors found that the Florida subspecies had a 37% lower expected heterozygosity than that of western burrowing owl, quite similar to the difference found in our marker set (33%). However, the number of alleles in the western burrowing owl in our marker set was 4.1 times higher than that in the Florida subspecies, whereas this factor was only 1.7 times in Korfanta et al. (2005). This difference highlights again the importance of sample size to adequately assess levels of genetic differentiation at any level.

Microsatellite markers suggest that the resident burrowing owl population near Texcoco in central Mexico is genetically different from the rest of the western burrowing owl populations. This population inhabits the open areas in the former Texcoco Lake at an elevation above 2,000 m. Perhaps the genetic divergence from the other western burrowing owl populations reflects a recent colonization event of Texcoco Lake followed by drift due to small population size. The burrowing owl population at Texcoco Lake inhabits an area with a large human population in a highly urbanized landscape, and, if differentiated, this 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 (Enriquez-Rocha 1997) and more breeding populations probably exist south of the Neovolcanic Axis, which may also have diverged from the northern populations.

The existence of a unique mtDNA haplotype on Guadalupe Island is intriguing and suggests some genetic differentiation of this population at the subspecies level. Guadalupe Island haplotype (CGG) differs from Western (TGA and TGG) and Florida (TAG) haplotypes in a transition at the 85th base pair (T→C), as well as from 3 South American subspecies (*A. c. cunicularia*, *A. c. nanodes*, and *A. c. punensis*; Desmond 2001). This fact suggests that this transition is a derived character. The possibility of a Guadalupe Island subspecies should not be surprising given the high levels of avian endemism on Guadalupe Island (Quintana-Barrios et al. 2006). In addition, the presence of the 2 western haplotypes, not one, also suggests a recent colonization or recent gene flow from continental western burrowing owl populations. Future studies should focus their attention on the burrowing owl population on Guadalupe Island and additional samples (and more DNA markers) may help further resolve the phylogeography of this isolated island population.

In summary, our data reveal novel information about genetic variation of burrowing owl subspecies in North America. Clarion burrowing owls appear to have diverged from the western burrowing owl approximately 200,000 years ago. In addition, the Texcoco and Guadalupe Island populations appear to be genetically differentiated from the remainder of the continental populations and our data suggest complex historical mechanisms for genetic isolation unexplained by current separation by distance or geographic barriers.

APPENDIX A. NOVEL MICROSATELLITE LOCI FOR THE BURROWING OWL *ATHENE CUNICULARIA*

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 2 burrowing owl breeding populations, one from central Sinaloa, Mexico, and one from the Central Valley of California, U.S.A. All loci were at Hardy-Weinberg equilibrium, except 2 loci for the California population. Expected heterozygosity was relatively high ($\overline{H}_E$ = 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.

The western burrowing owl (*Athene cunicularia hypugaea*) has undergone an intriguing distributional change since the mid 20th 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 steadily increased during the second half of the 20th 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 desert thornscrub has created suitable breeding habitat in these otherwise wintering grounds (Enriquez-Rocha 1997). Breeding densities in the agricultural areas of coastal Sonora and Sinaloa may be as high as those in the Imperial Valley of California. We developed and characterized new 11 microsatellite loci to estimate migration rates among burrowing owl populations in North America and determine the breeding dispersal patterns underlying this odd distributional change. These loci more than doubles the existing library of 7 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 Pueblo Chemical Depot, Fort Carson, 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 fragments ranging from 300 to 1400bp 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, 10X 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 2 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 under an α=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 Culiacan, in the Mexican State of Sinaloa, and 40 non-related owls from Naval Air Station Lemoore, in the Central Valley of California, U.S.A. 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 7). 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.

APPENDIX B. ANIMAL SUBJECT APPROVAL

Institutional Animal Care
and Use Committee

THE UNIVERSITY OF
ARIZONA.
TUCSON ARIZONA

P.O. Box 210101
Tucson, AZ 85721-0101

Verification of Review
By The Institutional Animal Care and Use Committee (IACUC)
PHS Assurance No. A-3248-01 -- USDA No. 86-3

The University of Arizona IACUC reviews all sections of proposals relating to animal care and use. The following listed proposal has been granted ***Final Approval*** according to the review policies of the IACUC:

PROTOCOL CONTROL NUMBER/TITLE:

#04-196- "Determination of Wintering Grounds of Burrowing Owls in Northern Mexico"

PRINCIPAL INVESTIGATOR/DEPARTMENT:

Courtney Conway - School of Natural Resources

GRANTING AGENCY:

T & E inc.

SUBMISSION DATE: **November 9, 2004**

APPROVAL DATE: **December 16, 2004** APPROVAL VALID THROUGH*: **December 15, 2007**

*When projects or grant periods extend past the above noted expiration date, the Principal Investigator will submit a new protocol proposal for full review. Following IACUC review, a new Protocol Control Number and Expiration Date will be assigned.

REVIEW STATUS FOR THIS PROJECT WAS CONFIRMED ON: **December 17, 2004**

REVISIONS (if any):

MINORITY OPINIONS (if any):

Richard C. Powell

Richard C. Powell, PhD, MS
Vice President for Research

DATE: December 17, 2004

This approval authorizes only information as submitted on the Animal Protocol Review Form, Amendments, and any supplemental information contained in the file noted as reviewed and approved by the IACUC.

MEMORANDUM FROM THE IACUC OFFICE

Central Animal Facility - Building #101
P.O. Box #210101, Tucson, AZ 85721-0101
Phone: 621-9305, FAX: 621-3355
E-Mail: musgravl@u.arizona.edu

May 11, 2005

TO: Dr. Courtney J. Conway
School of Natural Resources
P.O. Box #210043
BioSciences East #104

FROM: Linda S. Musgrave, IACUC Coordinator

RE: AMENDMENT: #04-091 - "Comparative Demography of Burrowing Owls in North America"

The above noted amendment/revision dated April 20, 2005 requested the following:

*** Addition of survey sites and animal numbers**

The information you have provided has been reviewed by the IACUC and approval has been granted. It has also been added to your file for future reference.

Thank you for keeping our office and the IACUC informed as to modifications you wish to make in your animals work.

Your consideration in this matter is appreciated.

LSM

PLEASE NOTE:

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Table 1. Characteristics of irrigation districts in northwestern Mexico (Baja California, Sonora and Sinaloa, Direccion General de Distritos de Riego, 1973). These areas represent potential burrowing owl breeding habitat. Irrigation districts marked with (*) denote districts where our survey efforts represent the first documentation of breeding burrowing owl populations inhabiting irrigated agricultural areas in those districts. (–) denotes information not available in the reference above.

Irrigation district	Main water source	Start of operations	Irrigated surface (ha)	Area (ha)	Irrigation channels (km)	Drains (km)
Rio Colorado*	Colorado river	1939	171,561	203,055	1,900	1,815
Rio Altar, Pitiquito y Caborca*	Deep groundwater and Altar river	1951	48,753	59,585	71	–
Valle de Guaymas	Deep groundwater	1967	22,311	24,179	13	–
Costa de Hermosillo*	Deep groundwater	1963	119,056	149,382	–	–
Colonias Yaquis	Yaqui river	1937	19,926	20,000	249	191
Rio Yaqui*	Yaqui river	1951	203,603	215,880	2,424	2,060
Rio Mayo*	Mayo river	1951	84,000	93,287	1,348	785
Valle del Carrizo	Alamos river	1969	28,185	40,000	479	406
Rio Fuerte*	Fuerte river	1956	165,579	240,356	1,801	2,112
Rio Fuerte - Guasave	Sinaloa river	1944	21,423	48,040	338	–
Mocorito*	Mocorito and Humaya rivers	1958	10,556	12,168	114	–
Humaya	Humaya river	1965	75,977	93,159	1,287	726
Culiacan*	Tamazula, Humaya, and San Lorenzo	1923	87,128	98,894	1,256	1,336
San Lorenzo	San Lorenzo River	1923	12,282	15,766	364	190
Total			1,070,340	1,313,751	11,644	9,620

Table 2. Numbers of individuals sampled within each of 36 burrowing owl study locations in Canada, United States, and Mexico. Study location acronyms with (*) and (†) denote southern agricultural populations and northern declining migratory populations, respectively

Study location	Acronym	Individuals genotyped
Southern Alberta, Alberta, Canada	ALB†	37
Baja California Sur, Mexico	BCS	23
Buckley Air Force Base, Colorado, U.S.A.	BUC	33
Buffalo Gap National Grassland, South Dakota, U.S.A.	BUF	54
Caborca Valley, Sonora, Mexico	CAB*	25
Casa Grande, Arizona, U.S.A.	CAG*	59
Fort Carson Army Base, Colorado, U.S.A.	CAR	23
Coyame and Ahumada, Chihuahua, Mexico	CHI	34
Comanche National Grassland, Colorado, U.S.A	COM	40
Culiacan Valley, Sinaloa, Mexico	CUL*	63
Delicias, Chihuahua, Mexico	DEL	25
Dixon Naval Radio Transmitter Facility, California, U.S.A.	DIX	29
Dugway Air Force Base, Utah, U.S.A.	DUG	30
Edwards Air Force Base, California, U.S.A.	EDW	44
Rio Fuerte Valley, Sinaloa, Mexico	FUE*	67
Galeana, Nuevo Leon, Mexico	GAL	47
Grand River-Little Missouri Natl. Grasslands, North Dakota	GRL†	21
Hermosillo, Sonora, Mexico	HER*	60
Holloman Air Force Base, New Mexico, U.S.A.	HOL	22
Janos, Chihuahua, Mexico	JAN	62
Kiowa - Rita Blanca National Grasslands, NM, TX, U.S.A.	KIB	29
Kirtland Air Force Base, New Mexico, U.S.A.	KIR	73
La Laguna, Coahuila, Mexico	LAG	54
Naval Air Station Lemoore, California, U.S.A.	LEM	47
Mexicali Valley, Baja California, Mexico	MEX*	59
Mountain Home Air Force Base, Idaho, U.S.A.	MNH	62
Moses Lake, Washington, U.S.A.	MOS	55
Nellis Air Force Base, Nevada, U.S.A.	NEL	55
Nevada Test Site, Nevada, U.S.A.	NTS	25
Pawnee National Grassland, Colorado, U.S.A.	PAW	54
Grasslands National Park and Regina Plains, Saskatchewan	SAK†	61
Tri-Cities, Washington, U.S.A.	TCY	54
Tucson, Arizona, U.S.A.	TUC	25
Texas Panhandle, Texas, U.S.A.	TXP	15
White Sands Missile Range, New Mexico, U.S.A.	WSM	24
Yaqui-Mayo Valley, Sonora, Mexico	YAQ*	70

Table 3. Mean number of alleles (N_a), number of effective alleles (N_e), number of private alleles (N_p), observed heterozygosity (H_O), expected heterozygosity (H_E), and fixation index (F) averaged across all 11 loci for each of 36 study locations of burrowing owls in North America. Population acronyms are shown in Table 2.

Population	N_a	N_e	N_p	H_O	H_E	F
ALB	13.40±1.72	7.24±1.09	0.20±0.13	0.83±0.02	0.84±0.02	0.00±0.02
BCS	11.00±1.26	6.36±0.96	0.20±0.13	0.81±0.04	0.82±0.02	0.02±0.03
BUC	12.00±1.62	7.04±1.12	0.00±0.00	0.82±0.04	0.82±0.03	0.01±0.03
BUF	14.20±1.76	7.09±1.33	0.20±0.13	0.84±0.02	0.83±0.02	-0.01±0.01
CAB	10.80±1.27	6.70±0.78	0.00±0.00	0.84±0.03	0.83±0.02	-0.01±0.02
CAG	14.70±1.76	6.88±1.07	0.00±0.00	0.84±0.02	0.83±0.02	-0.01±0.01
CAR	10.40±0.79	5.77±0.65	0.00±0.00	0.86±0.03	0.81±0.02	-0.06±0.03
CHI	12.30±1.29	6.66±0.92	0.00±0.00	0.82±0.03	0.83±0.02	0.01±0.02
COM	13.40±1.76	7.42±1.15	0.00±0.00	0.87±0.03	0.84±0.02	-0.04±0.02
CUL	13.00±1.81	7.29±1.28	0.00±0.00	0.81±0.04	0.82±0.04	0.02±0.01
DEL	11.20±1.11	6.60±0.87	0.30±0.21	0.83±0.03	0.82±0.02	-0.01±0.03
DIX	10.00±1.03	6.17±0.72	0.00±0.00	0.86±0.03	0.82±0.02	-0.04±0.04
DUG	11.70±1.14	6.66±0.87	0.00±0.00	0.86±0.02	0.83±0.02	-0.04±0.02
EDW	12.30±1.10	6.58±0.95	0.10±0.10	0.83±0.02	0.83±0.02	0.00±0.02
FUE	13.50±1.68	6.85±0.98	0.10±0.10	0.83±0.03	0.82±0.03	0.00±0.02
GAL	13.30±1.56	7.25±1.17	0.00±0.00	0.84±0.02	0.84±0.02	-0.01±0.02
GRL	11.10±1.29	6.98±1.02	0.00±0.00	0.85±0.03	0.83±0.03	-0.03±0.03
HER	13.40±1.30	6.92±1.09	0.00±0.00	0.83±0.02	0.83±0.02	0.00±0.02
HOL	10.70±1.18	6.81±1.03	0.00±0.00	0.86±0.04	0.83±0.02	-0.04±0.03
JAN	14.10±1.63	7.22±1.21	0.20±0.13	0.86±0.02	0.83±0.02	-0.03±0.01
KIB	11.90±1.34	6.80±1.05	0.00±0.00	0.80±0.03	0.82±0.03	0.03±0.02
KIR	15.00±1.56	7.34±1.11	0.30±0.30	0.84±0.02	0.84±0.02	0.00±0.02
LAG	14.20±1.73	7.42±1.19	0.20±0.13	0.83±0.03	0.84±0.02	0.01±0.03
LEM	12.60±1.50	6.82±1.27	0.10±0.10	0.83±0.03	0.82±0.02	0.00±0.02
MEX	13.70±1.67	7.20±1.22	0.10±0.10	0.83±0.03	0.83±0.02	0.00±0.02
MNH	14.50±1.90	7.36±1.29	0.00±0.00	0.84±0.03	0.83±0.02	-0.01±0.01
MOS	14.20±1.36	7.37±1.15	0.50±0.40	0.84±0.03	0.84±0.02	0.00±0.01
NEL	12.50±1.34	6.15±0.57	0.00±0.00	0.83±0.02	0.82±0.02	-0.01±0.01
NTS	11.30±1.24	6.95±0.70	0.00±0.00	0.81±0.03	0.84±0.02	0.04±0.03
PAW	14.20±1.65	7.82±1.37	0.00±0.00	0.84±0.02	0.84±0.02	0.00±0.02
SAK	15.70±2.09	7.64±1.38	0.20±0.13	0.84±0.02	0.84±0.02	0.00±0.03
TCY	13.10±1.68	7.13±0.93	0.00±0.00	0.86±0.03	0.84±0.02	-0.02±0.03
TUC	9.40±1.13	5.70±0.76	0.00±0.00	0.78±0.04	0.79±0.03	0.01±0.03
TXP	9.50±1.26	5.95±0.93	0.00±0.00	0.78±0.05	0.78±0.04	0.00±0.04
WSM	11.70±1.09	7.11±0.98	0.10±0.10	0.85±0.03	0.84±0.02	-0.02±0.02
YAQ	13.50±1.55	6.79±0.96	0.20±0.20	0.82±0.02	0.83±0.02	0.01±0.01

Table 4. Statistical significance (*P*-values) of Analyses of Molecular Variance (*AMOVA*) based on the F_{ST} statistics for each of 8 two-group classifications of 36 burrowing owl study sites. Group 1 includes the southern agricultural study sites (CAB, CAG, CUL, FUE, HER, MEX, and YAQ) and the study sites listed in the table below. Group 2 includes the remainder of the study sites. Acronyms are listed in Table 2. Bold-face values denote significant comparisons for $\alpha = 0.05$

Study sites in Group 1	*P*-value	
	Standard	Weighted averaged over all loci
ALB, GRL, SAK	**0.028**	**0.012**
MNH, MOS, TCY	0.240	0.218
BUF, CAR, PAW	0.131	0.117
EDW, NEL, NTS	0.220	0.238
COM, KIB, KIR	0.184	0.174
DEL, GAL, LAG	0.060	**0.046**
DIX, LEM	0.329	0.328
CHI, JAN, TUC	**0.027**	**0.008**

Table 5. Burrowing owl study populations. Study population acronyms with (*) and (†) denote southern agricultural populations and northern declining migratory populations, respectively.

Study population (Location, State/Province, Country)	Acronym
Southern Alberta, Alberta, Canada	ALB†
Buckley Air Force Base – Rocky Mountain Arsenal, Colorado, U.S.A.	BRM
Caborca Valley, Sonora, Mexico	CAB*
Casa Grande, Arizona, U.S.A	CAG*
Fort Carson Army Base, Colorado, U.S.A	CAR
Ahumada and Coyame, Chihuahua, Mexico	CHI
Culiacan Valley, Sinaloa, Mexico	CUL*
Delicias, Chihuahua, Mexico	DEL
Dixon Navy Radio Station, California, U.S.A.	DIX
Dugway Air Force Base, Utah, U.S.A.	DUG
Edwards Air Force Base, California, U.S.A.	EDW
Fort Bliss, New Mexico, U.S.A.	FBL
Rio Fuerte Valley, Sinaloa, Mexico	FUE*
Galeana, Nuevo Leon, Mexico	GAL
Hermosillo, Sonora, Mexico	HER*
Holloman Air Force Base, New Mexico, U.S.A.	HOL
Janos, Chihuahua, Mexico	JAN
Kirkland Air Force Base, New Mexico, U.S.A.	KIR
La Laguna, Coahuila, Mexico	LAG
Naval Air Station Lemoore, California, U.S.A.	LEM
Mexicali Valley, Baja California, Mexico	MEX*
Mount Home Air Force Base, Idaho, U.S.A.	MNH
Moses Lake City, Washington, U.S.A.	MOS
Mulege, Baja California Sur, Mexico	MUL
Nellis Air Force Base, Nevada, U.S.A.	NEL
Nevada Test Site, Nevada, U.S.A.	NTS
Grasslands National Park and Regina Plains, Saskatchewan, Canada	SAK†
Santo Domingo Valley, Baja California Sur, Mexico	SDO
St. George, Utah, U.S.A.	SGE
Salton Sea National Wildlife Refuge, California, U.S.A.	SSW*
Tri Cities, Washington, U.S.A	TCY
Texcoco, Estado de Mexico, Mexico	TEX
Tucson, Arizona, U.S.A	TUC
Texas Panhandle, Texas, U.S.A.	TXP
White Sands Missile Range, New Mexico, U.S.A.	WSM
Thunder Basin, Wyoming, U.S.A.	WYO
Yaqui-Mayo Valley, Sonora, Mexico	YAQ*

Table 6. 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. Boldface numbers show fixed loci. Locus names provided in Appendix A. We do not provide estimates for the Guadalupe Island populations given our small sample of owls at that location (n = 6).

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
S.E.	0.9	0.090	0.088	0.9	0.059	0.044	0.9	0.037	0.022	2.3	0.022	0.018

Table 7. Eleven microsatellite loci developed for the burrowing owl. Number of alleles (N_A), and the observed (H_O) and expected (H_E) heterozygosities for populations in central Sinaloa, Mexico and in Naval Air Station Lemoore, California. All individuals successfully amplified for all loci. The 2 boldface H_E values denote loci that deviated significantly from Hardy-Weinberg Equilibrium.

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 R: AGCCATTCCCTTCAGTCTTC	349-367	335	5	0.500	0.515	10	0.800	0.764
ATCU06 (GU167942)	$(CT)_8CA(CT)_{13}$	F: GAAATGGAAGGAGGAGTGC R: GCCATCCCTAATGCTTGTG	201-255	199	15	0.925	0.888	13	0.875	0.863
ATCU08 (GU167943)	$(CA)_{20}$	F: GCCCTCATATCATTAAGATCCTTC R: GGATTGTCATTTCCCCTCAG	223-293	211	25	0.925	0.942	25	0.925	0.942
ATCU13 (GU167944)	$(GT)_{17}$	F: ACCCCGAGTGCTCTAGTCAG R: GTTGTGAAGCGAGGGATG	222-258	221	10	0.725	0.821	10	0.775	0.733
ATCU20 (GU167945)	$(CA)_{15}$	F: GTTGCCATCATAGCAGCAG R: GCCAGATAACTACCCCAAATG	171-197	154	11	0.900	0.881	11	0.925	0.875
ATCU28 (GU167946)	$(GT)_{10}AT(GT)_9$	F: CAGTGTCAGAGTCAAGACATGC R: TGGAGAGGTTTAGGGCTAGG	328-352	312	10	0.875	0.833	9	0.775	0.800
ATCU36 (GU167947)	$(GT)_{13}$	F: TTGCACAGAAAATCCTGAGTC R: AACAAGAGTTACCTGAAGAGATGC	397-413	374	8	0.725	0.812	7	0.675	0.682
ATCU39 (GU167948)	$(GT)_{18}$	F: GTGTGGGTTGCCTCACATC R: AACATCCAGGAAACAAGATGC	159-189	160	13	0.800	0.851	13	0.725	**0.848**
ATCU41 (GU167949)	$(CA)_{12}$	F: AGAGATAGTAGTTTAGGGTAGGCTC R: ACGACACTTCTAGCACGTTG	201-223	188	7	0.725	0.768	9	0.550	**0.728**
ATCU43 (GU167950)	$(CA)_{19}$	F: GATCAGCTTGCAGCAAAGG R: GGGAGATGTTGAGGAAATCG	174-212	174	14	0.825	0.843	12	0.900	0.821
ATCU45 (GU167951)	$(GATA)_8GGTA(GATA)_2$	F: CTACCGAGCAGTGACAGTTTG R: GGGTGGACAGTTCCTCATTC	242-282	215	9	0.775	0.824	10	0.800	0.847

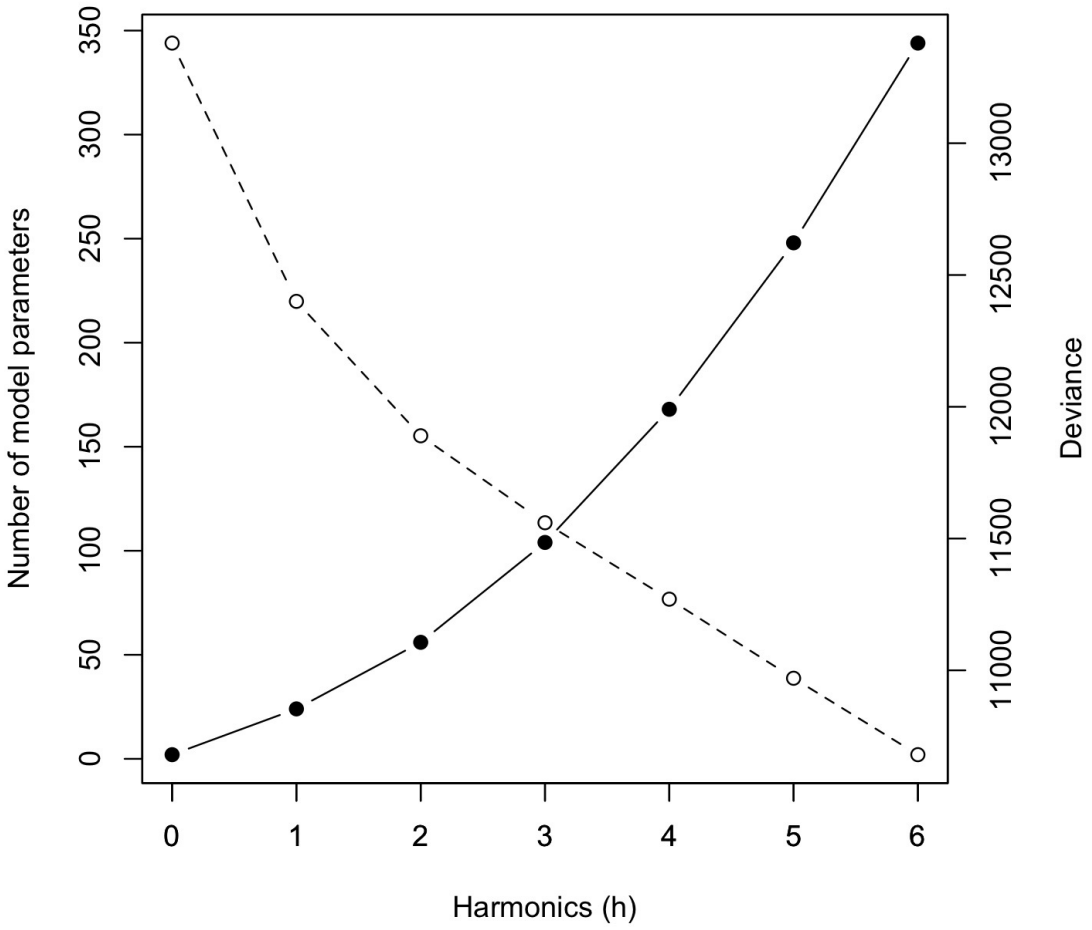

Figure 1. Relationship between the number of harmonics used in the double Fourier series (to model intercept and slope of the linear relationship between year and logit of presence; see text) and the number of model parameters (filled circles; solid line) and deviance (empty circles; dashed line) in logistic regression models for predicting presence of burrowing owls based on Breeding Bird Survey data in North America.

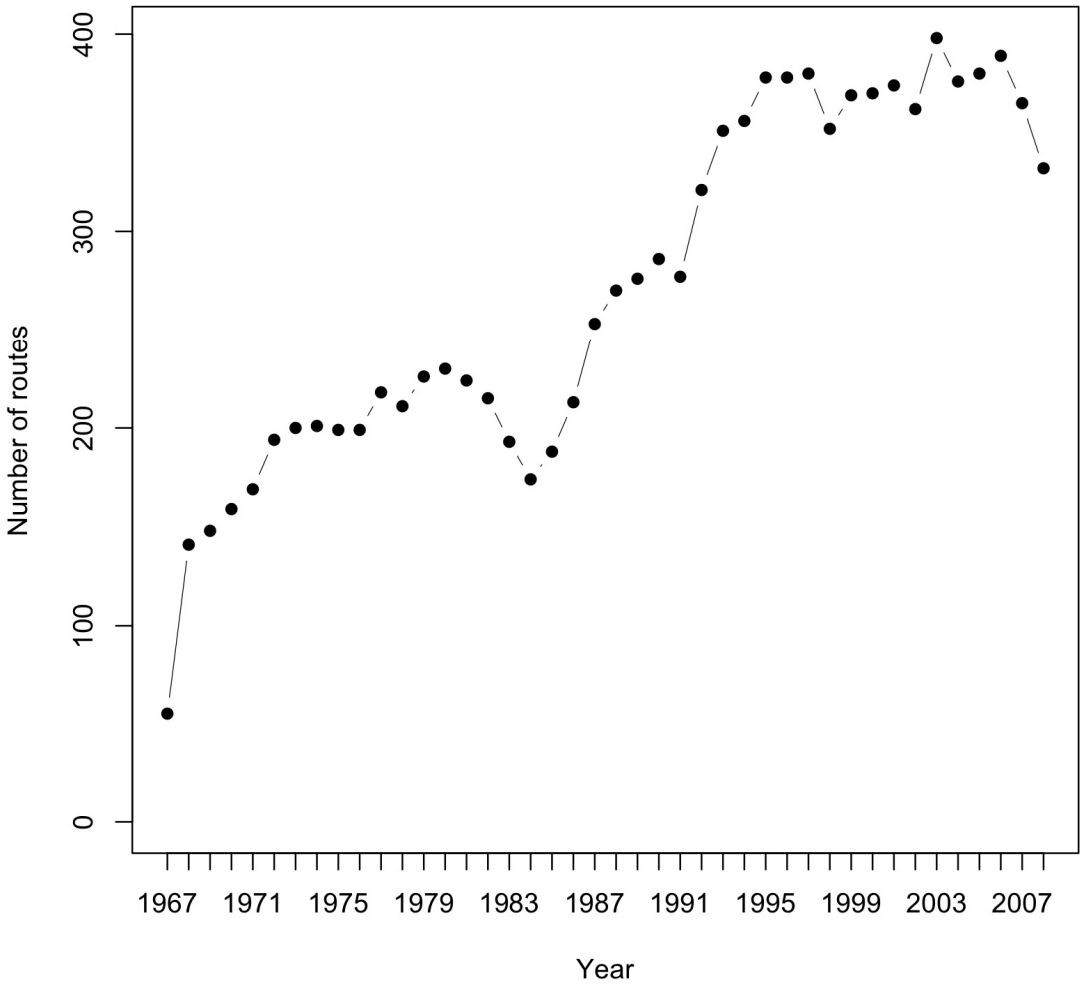

Figure 2. Number of Breeding Bird Survey routes per year at which ≥1 burrowing owl was detected.

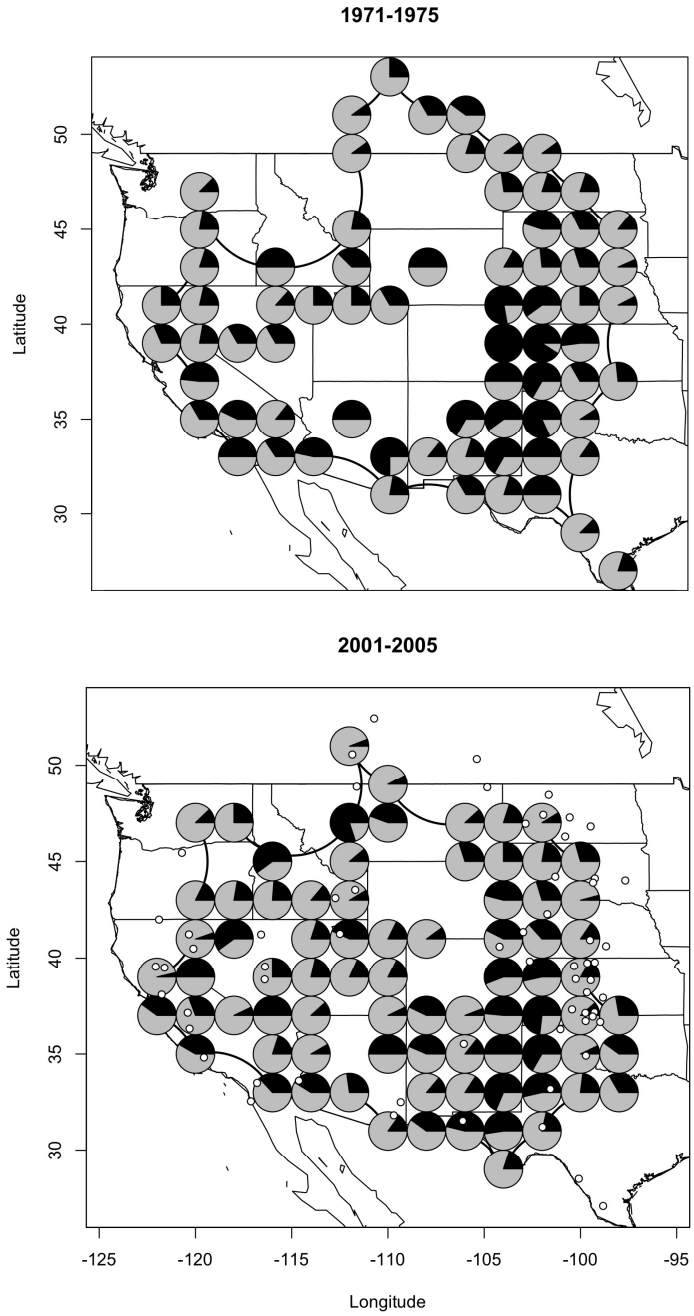

Figure 3. Spatial and temporal (1971-1975 vs. 2001-2005) variation in the proportion of Breeding Bird Survey (BBS) routes at which ≥1 burrowing owl was detected in 2-degree blocks. Black and gray sectors in each pie chart denote the proportion of BBS routes with and without owls, respectively. All circles include some black because the map includes only BBS routes at which ≥1 burrowing owl was detected and the map of 2001-2005 includes more circles because the number of BBS routes surveyed increased over time. White dots indicate routes where owls were detected in 1971-1975 but not in 2001-2005.

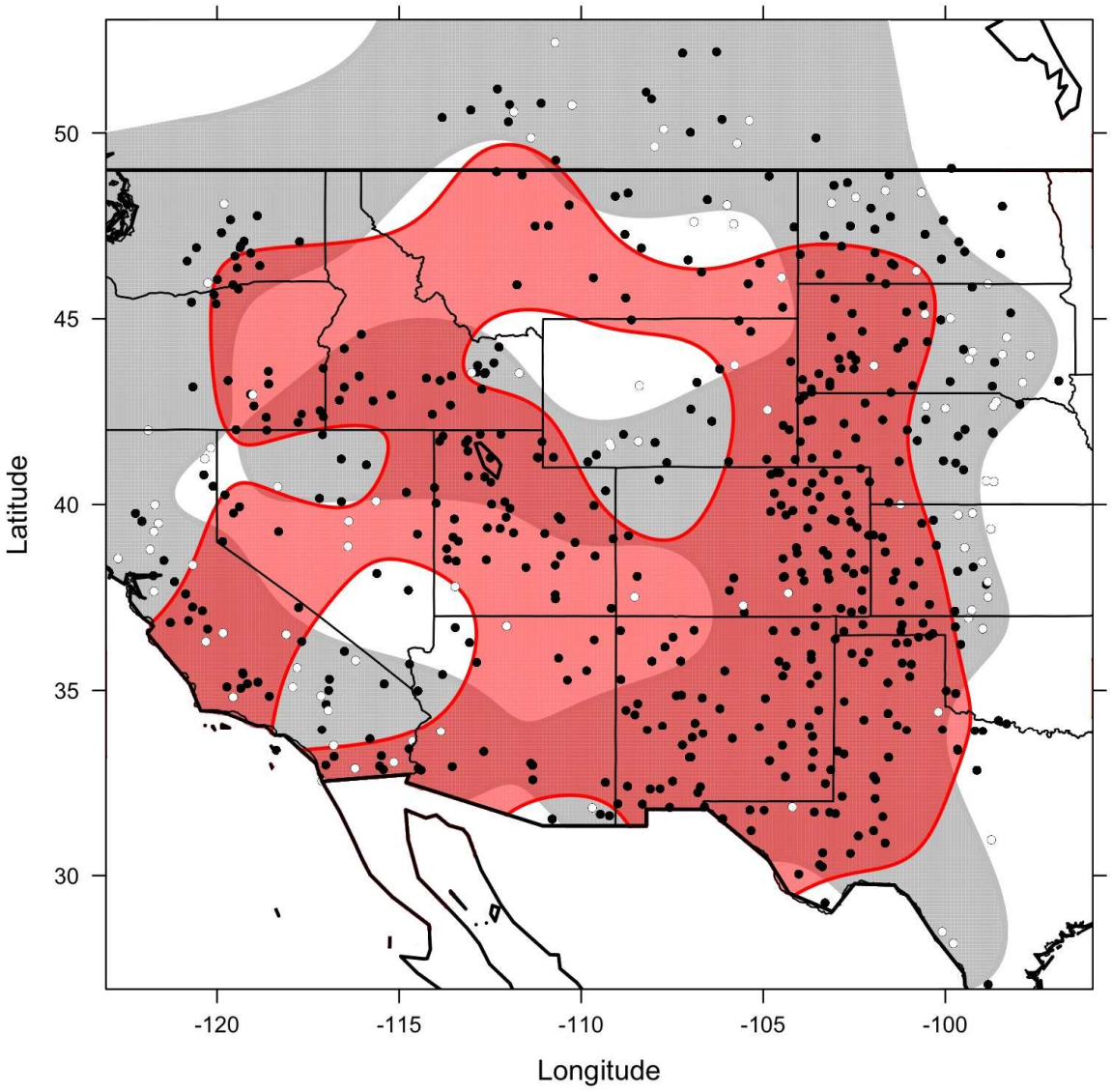

Figure 4. Estimated change in the breeding range of burrowing owls from 1967 to 2008 based on logistic regression of Breeding Bird Survey (BBS) data. The gray area denotes the owl's breeding range in 1967 as predicted by the model whereas the red area denotes the owl's breeding range in 2008 as predicted by the model. All dots show BBS routes at which ≥1 burrowing owl was detected. Empty dots indicate BBS routes where ≥1 burrowing owl was detected before 1987 but none after 1987. Black dots indicate BBS routes where ≥1 burrowing owl was detected after 1987 (regardless of how many were detected prior to 1987).

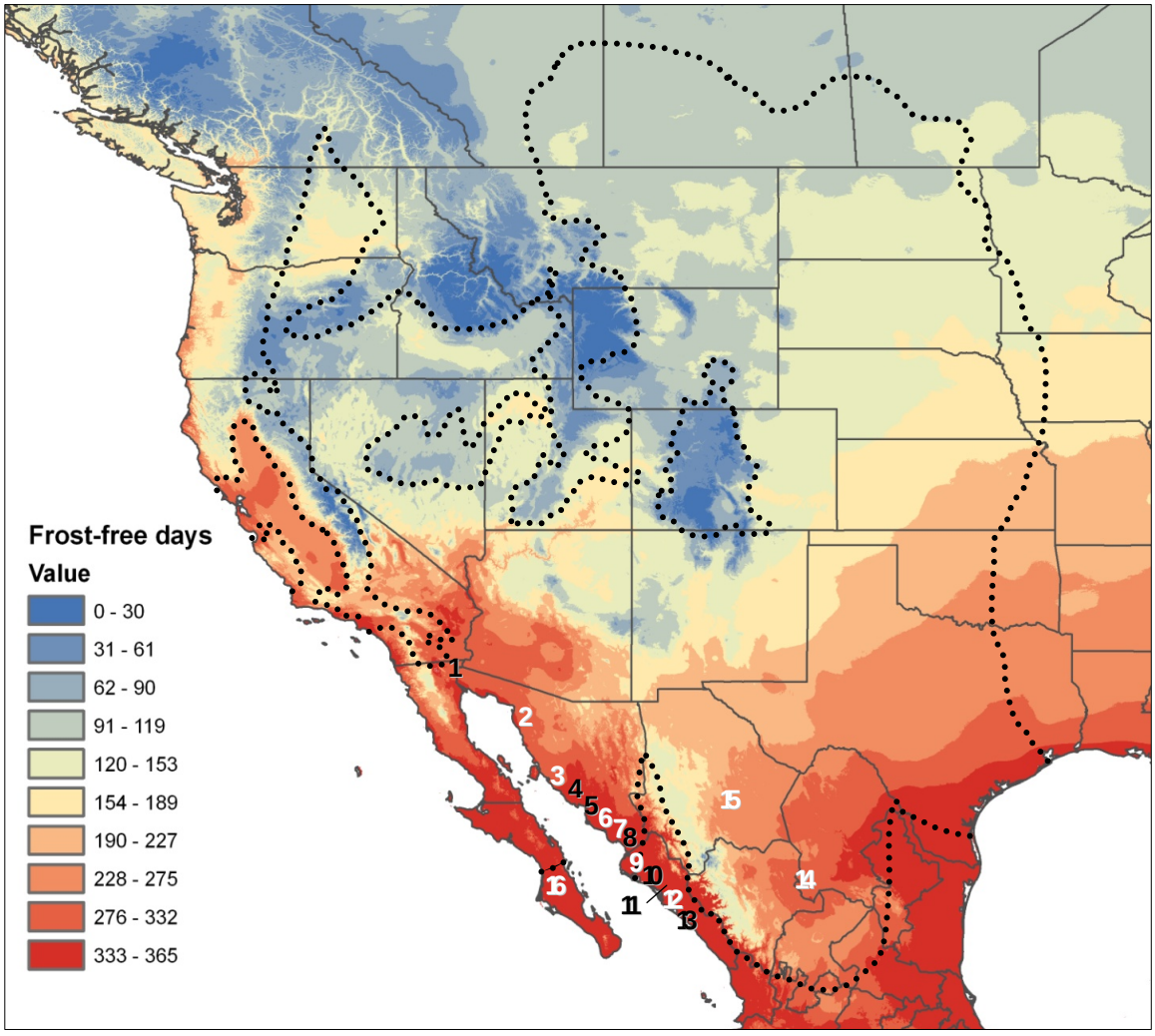

Figure 5. Frost-free days per year across the breeding range of the western burrowing owl in North America. Dotted line denotes the limit of the species' historic breeding distribution (from Wellicome and Holroyd 2001). Numbers on the map denote irrigation districts in northern Mexico: (1) Rio Colorado, (2) Rio Altar, Pitiquito y Caborca, (3) Costa de Hermosillo, (4) Valle de Guaymas (5) Colonias Yaquis, (6) Rio Yaqui, (7) Rio Mayo, (8) Valle del Carrizo, (9) Rio Fuerte, (10) Rio Fuerte – Guasave, (11) Mocorito, (12) Humaya and Culiacan, (13) San Lorenzo, (14) Region Lagunera, (15) Delicias, and (16) Santo Domingo. White numbers indicate locations where our survey efforts represent the first documentation of breeding burrowing owl populations inhabiting those irrigated agricultural areas.

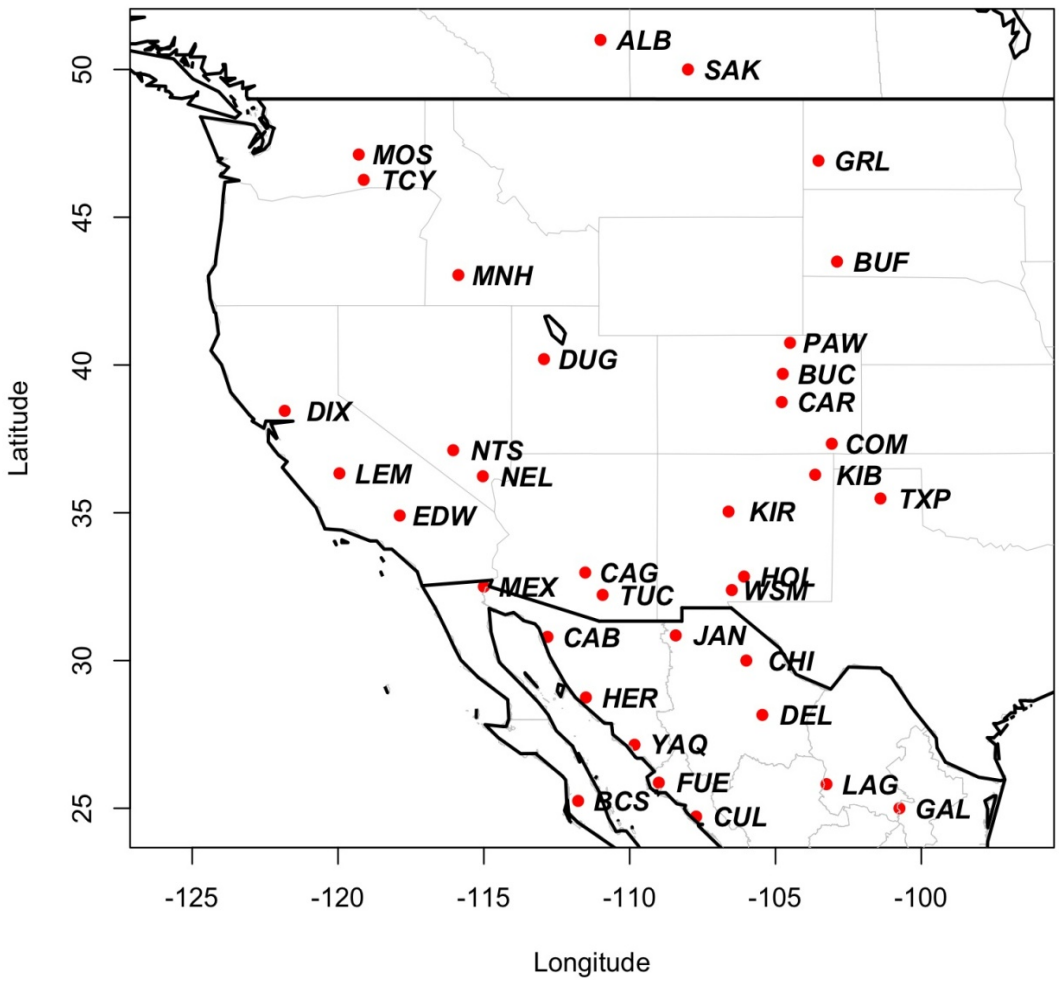

Figure 6. Burrowing owl study locations in Canada, Mexico, and the United States. Acronyms for study locations are listed in Table 2.

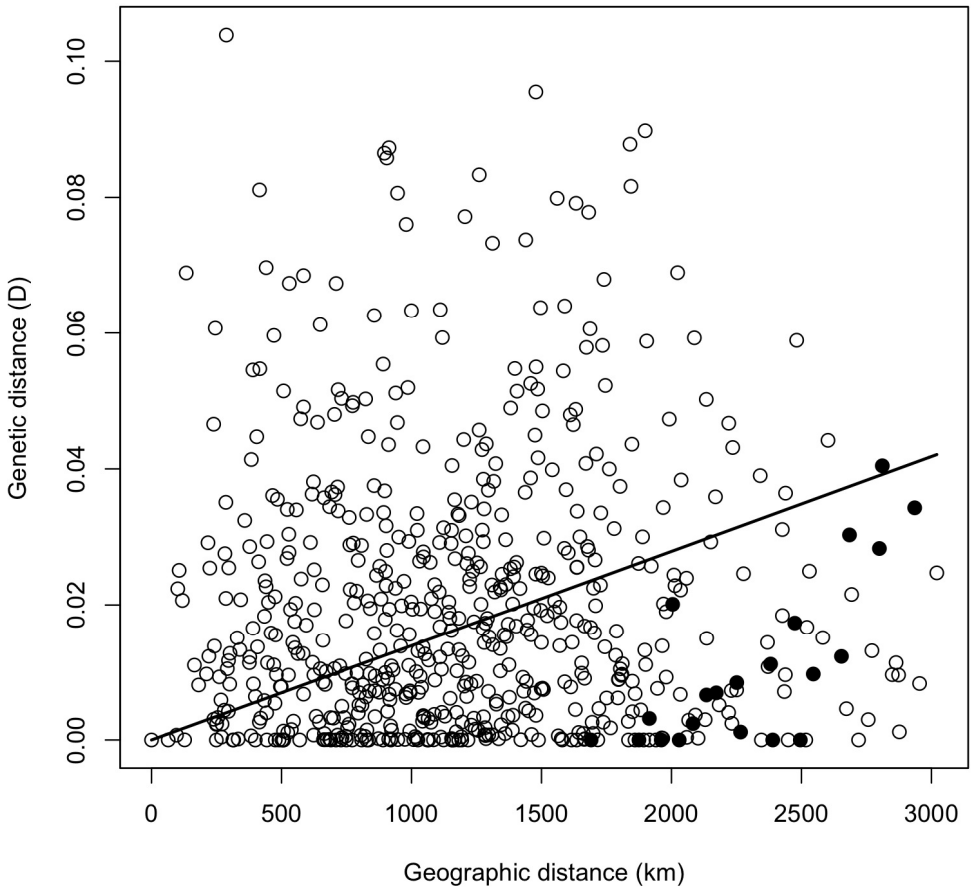

Figure 7. Scatterplot of actual differentiation *D vs.* geographic distances for all pairwise comparisons (*n*=630) among our 36 burrowing owl study locations across North America. Black dots indicate pairwise comparisons between northern study locations and southern agricultural locations, whereas empty dots indicate pairwise comparisons among the remainder of the study locations. Mantel correlation between geographic and genetic distance is not significantly different from zero (95% C. I. from -0.05 to 0.08).

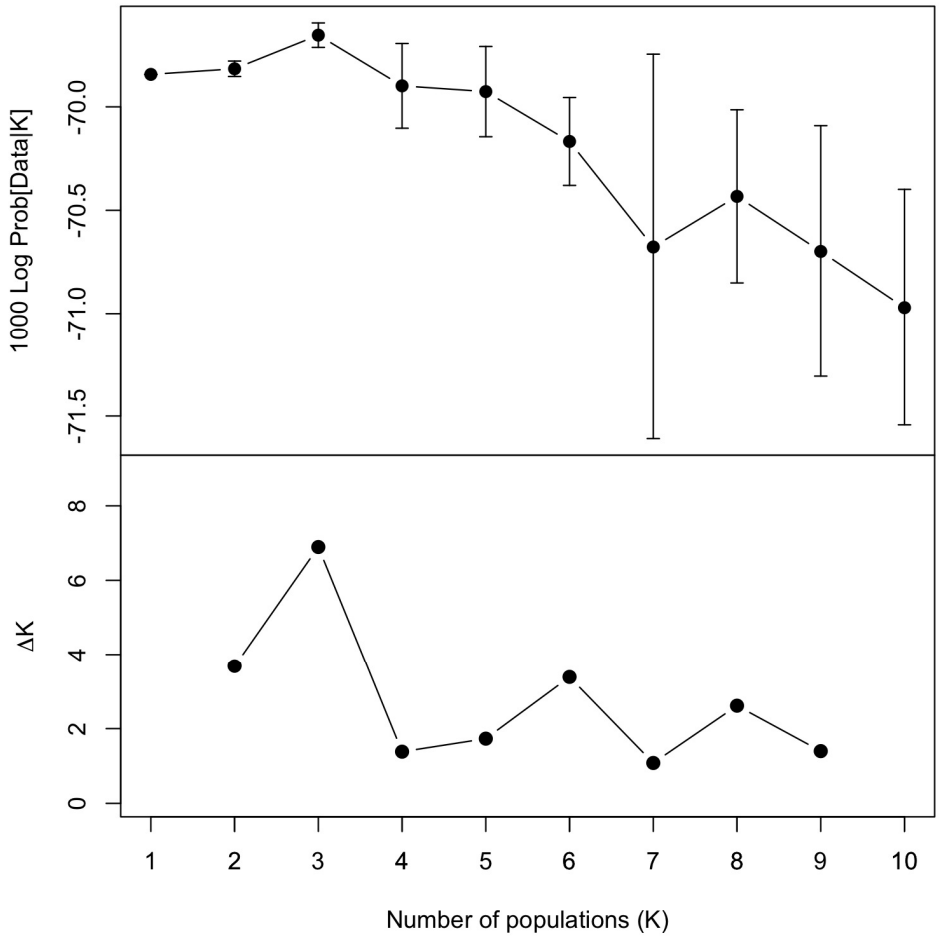

Figure 8. Estimated number of populations (*K*) based on the distribution of the log-likelihood of burrowing owl genotypic data as estimated by program *STRUCTURE* (upper plot), and the distribution of parameter Δ*K* as a function of the number of populations (*K*) in the same interval (lower plot).

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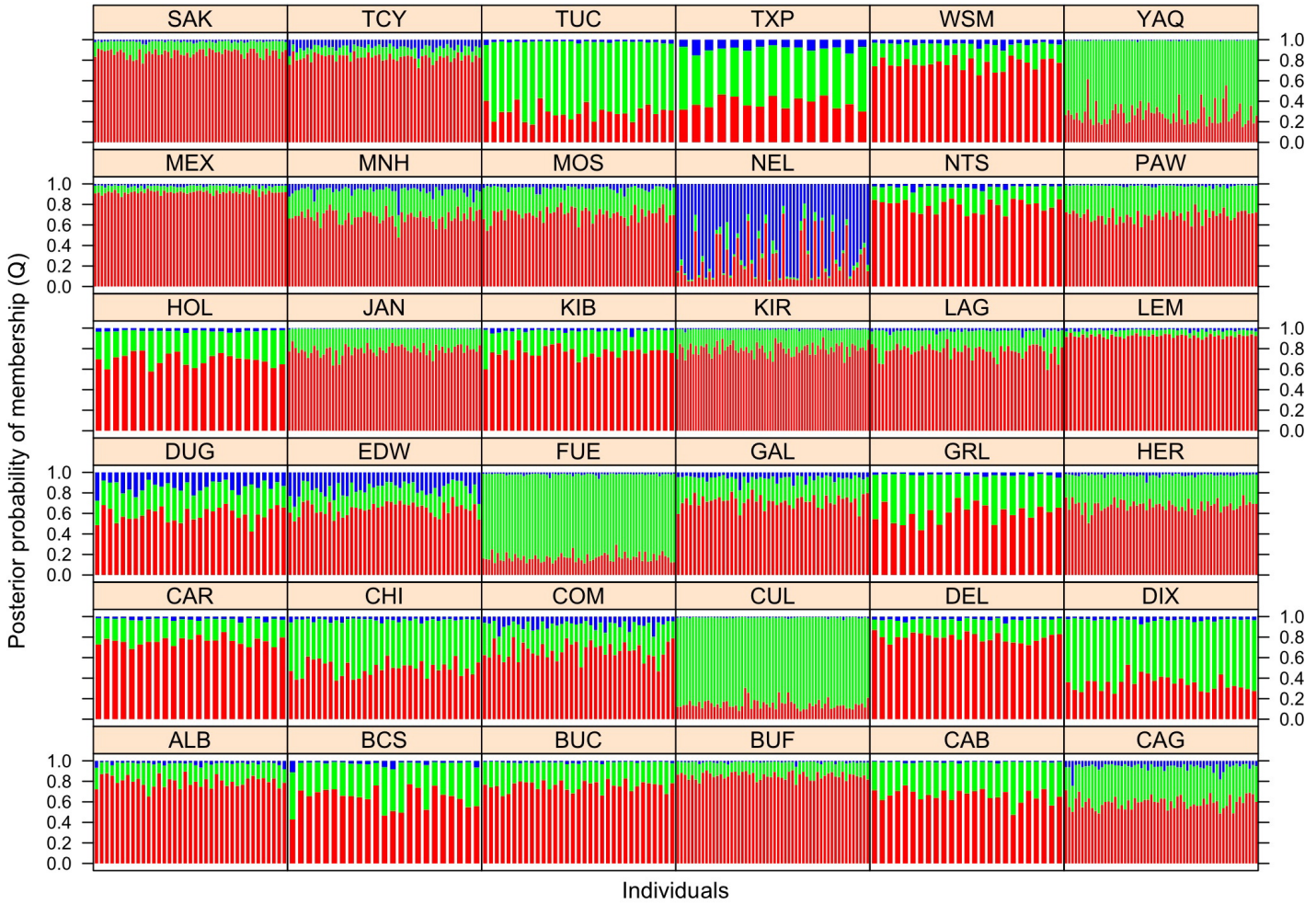

Figure 9. Posterior probability of membership of 1,560 burrowing owls to the 3 distinct populations identified by program *STRUCTURE*. Acronyms of the burrowing owl study locations are listed in Table 2.

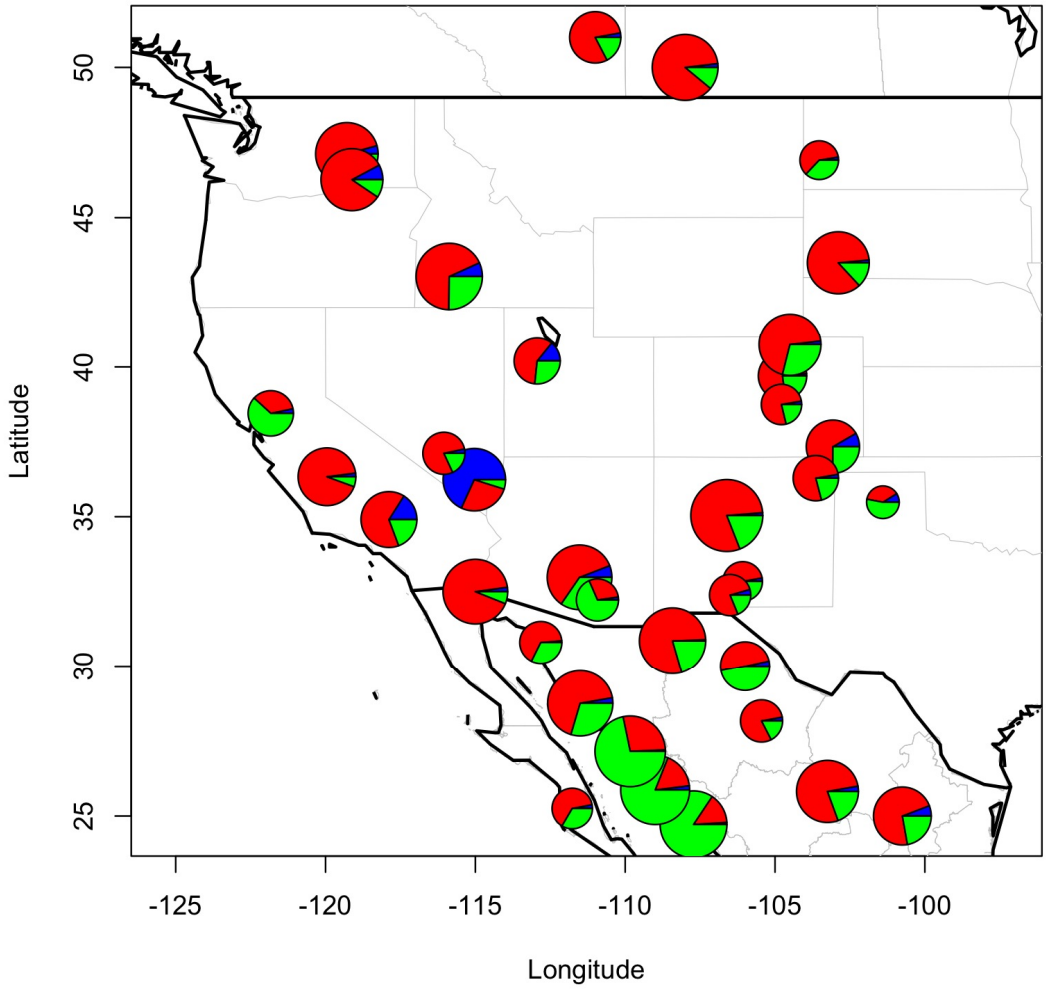

Figure 10. Geographic variation among study locations in the posterior probability of membership to each of the 3 populations inferred by program *STRUCTURE*. Pie chart sizes are proportional to the number of individuals genotyped at each study location.

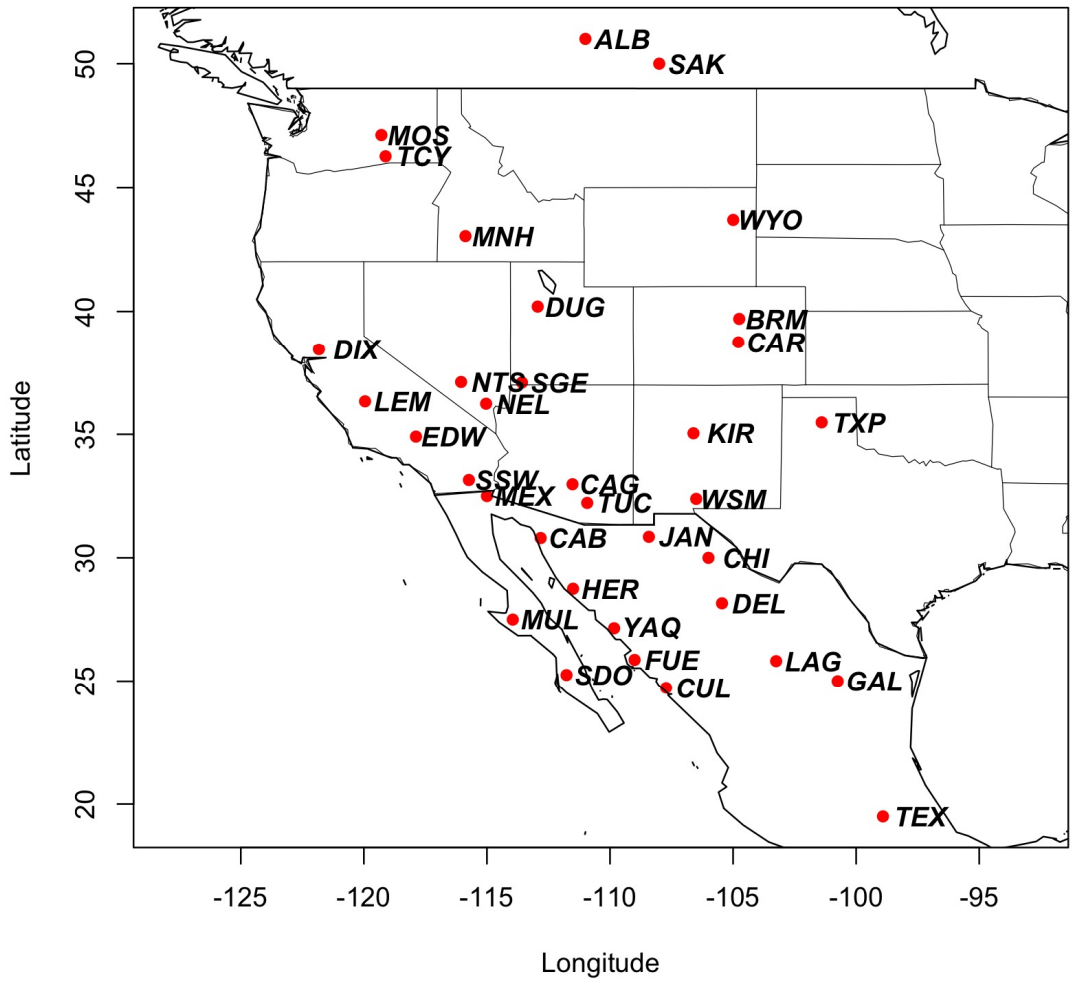

Figure 11. Burrowing owl study populations in Canada, Mexico, and the United States. Study population acronyms are provided in Table 5.

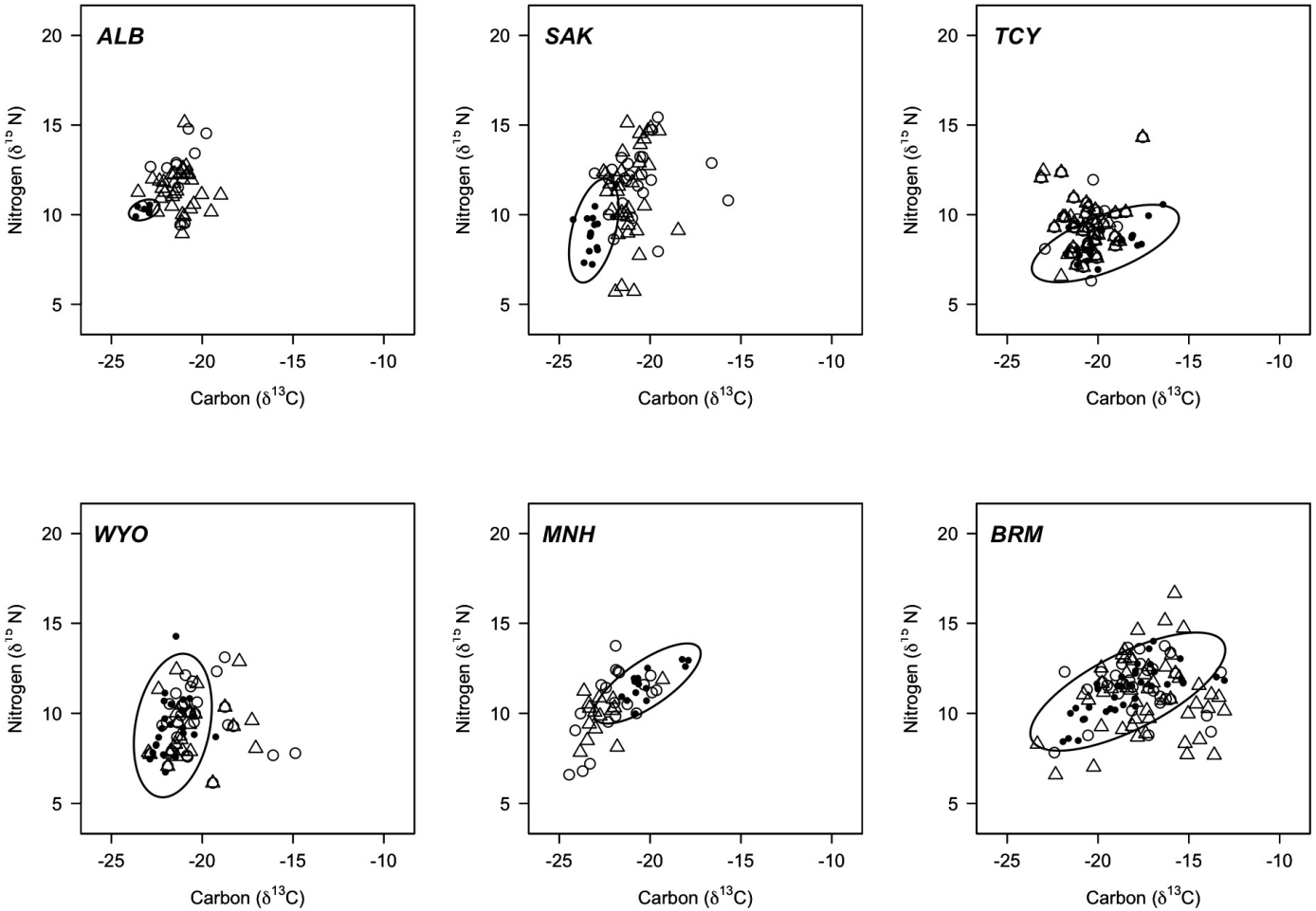

Figure 12. Stable isotope signatures of ^{13}C and ^{15}N in nesting and adult feathers collected at 27 burrowing owl study study locations. Filled and open circles show the isotope signature of nestling and adult feathers, respectively. Circles and triangles denote males and females, respectively. Ellipses show the 95th-percentile ellipses for the bivariate normal distribution based on isotope data on nestling feathers. Study population acronyms are shown in Table 5.

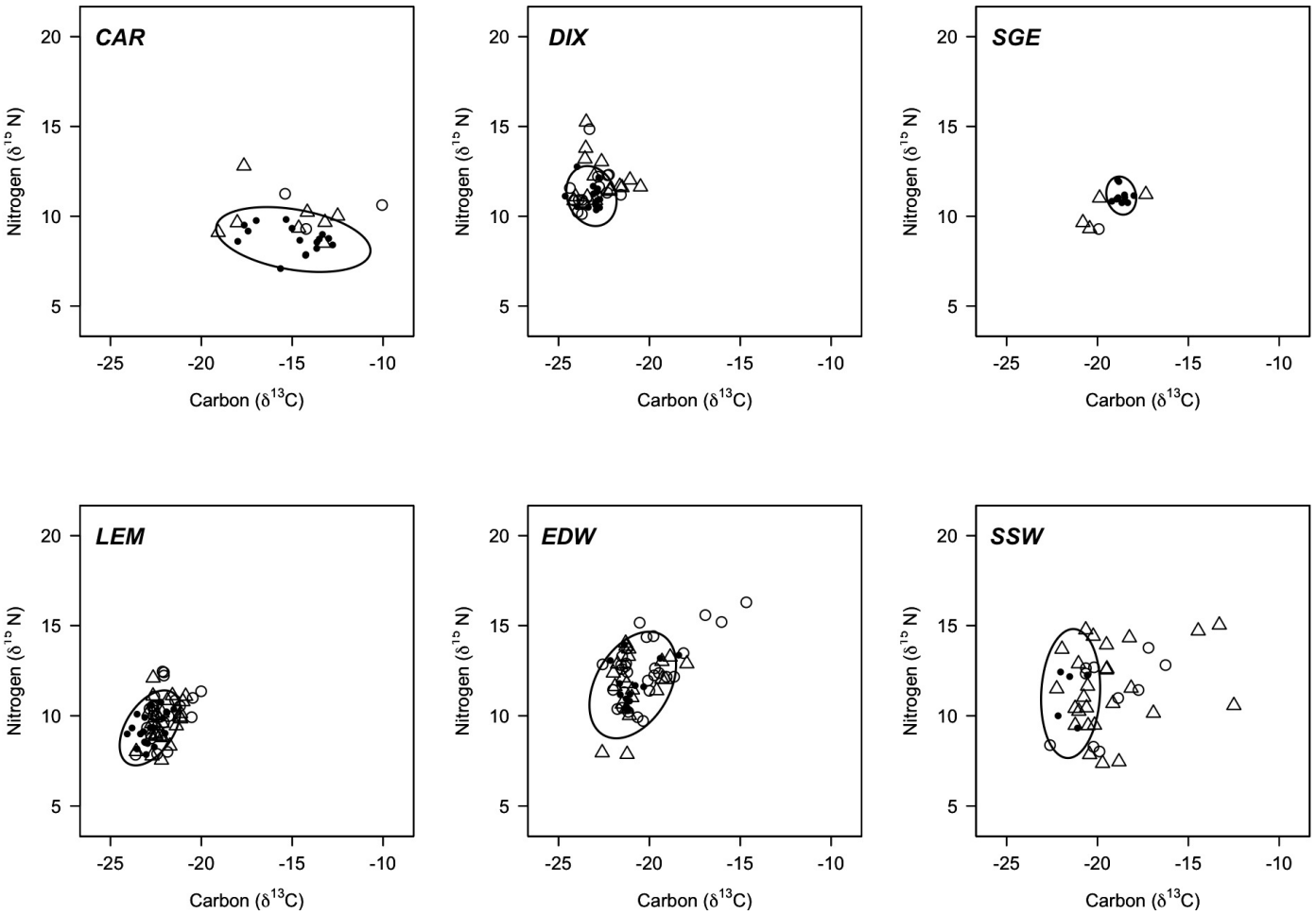

Figure 12 (continued). Stable isotope signatures of ^{13}C and ^{15}N in nesting and adult feathers collected at 27 burrowing owl study locations. Filled and open circles show the isotope signature of nestling and adult feathers, respectively. Circles and triangles denote males and females, respectively. Ellipses show the 95th-percentile ellipses for the bivariate normal distribution based on isotope data on nestling feathers. Study population acronyms are shown in Table 5.

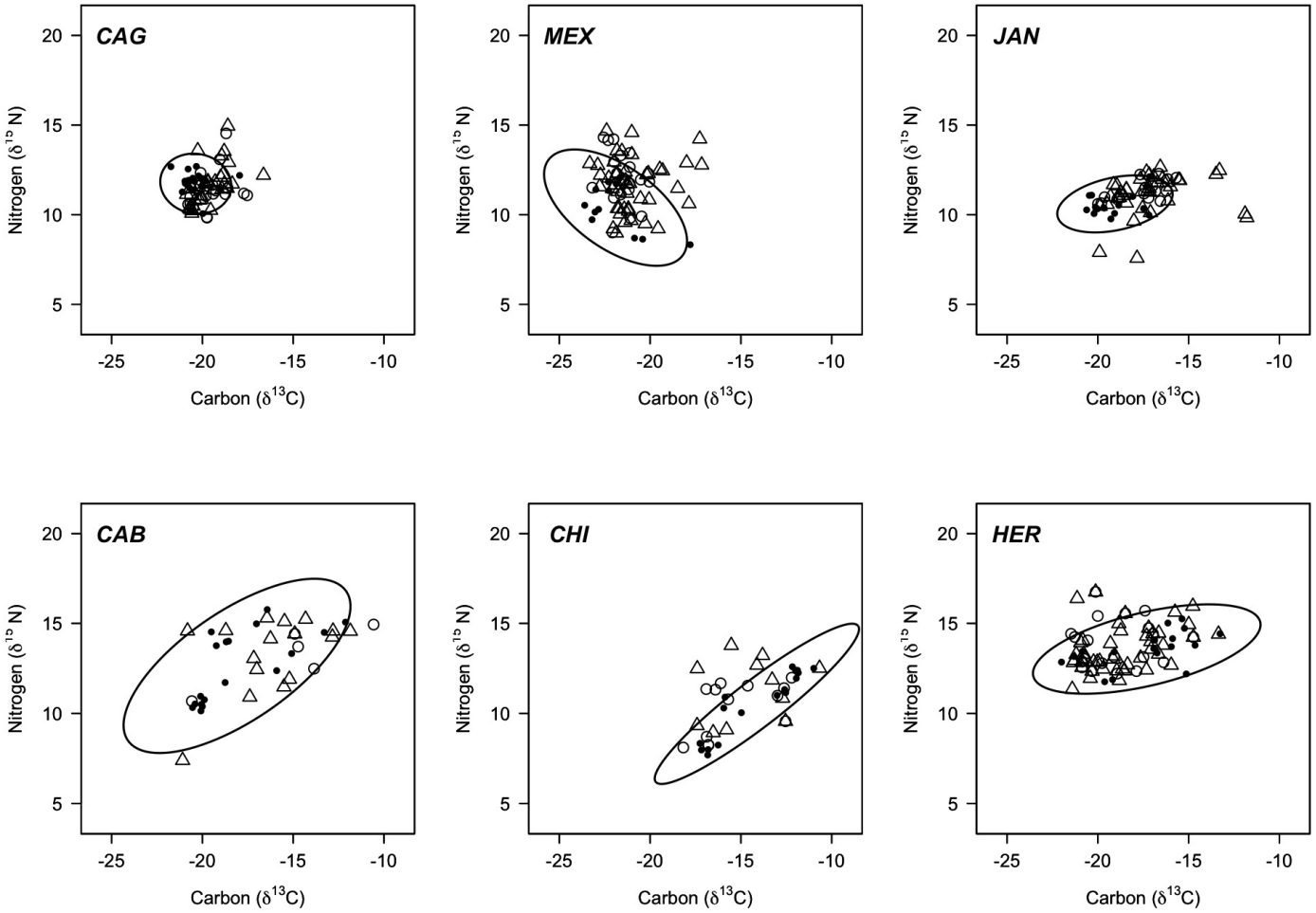

Figure 12 (continued). Stable isotope signatures of ^{13}C and ^{15}N in nesting and adult feathers collected at 27 burrowing owl study locations. Filled and open circles show the isotope signature of nestling and adult feathers, respectively. Circles and triangles denote males and females, respectively. Ellipses show the 95th-percentile ellipses for the bivariate normal distribution based on isotope data on nestling feathers. Study population acronyms are shown in Table 5.

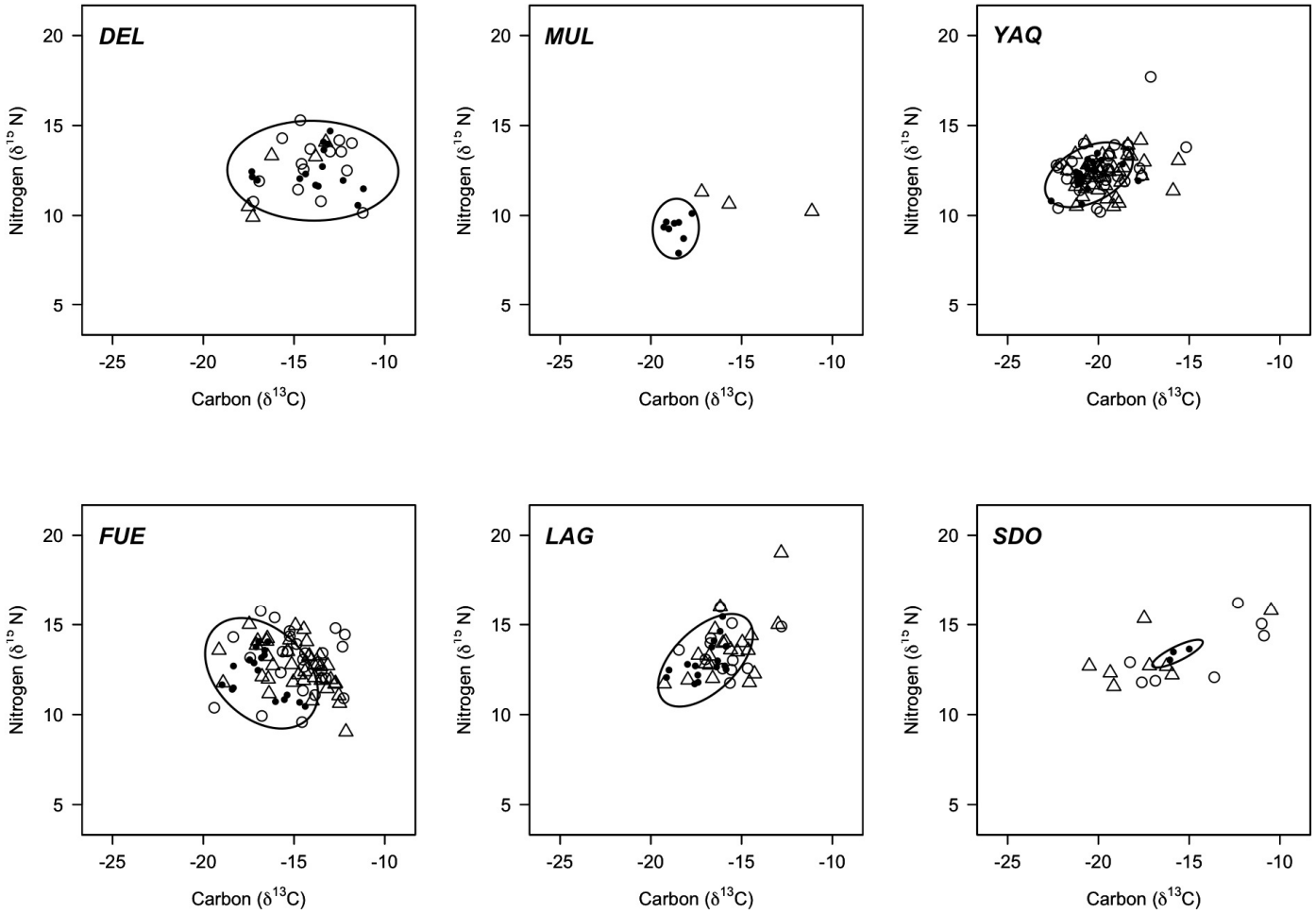

Figure 12 (continued). Stable isotope signatures of ^{13}C and ^{15}N in nesting and adult feathers collected in 27 burrowing owl study populations. Filled and open circles show the isotope signature of nestling and adult feathers, respectively. Circles and triangles denote males and females, respectively. Ellipses show the 95th-percentile ellipses for the bivariate normal distribution based on isotope data on nestling feathers. Study population acronyms are shown in Table 5.

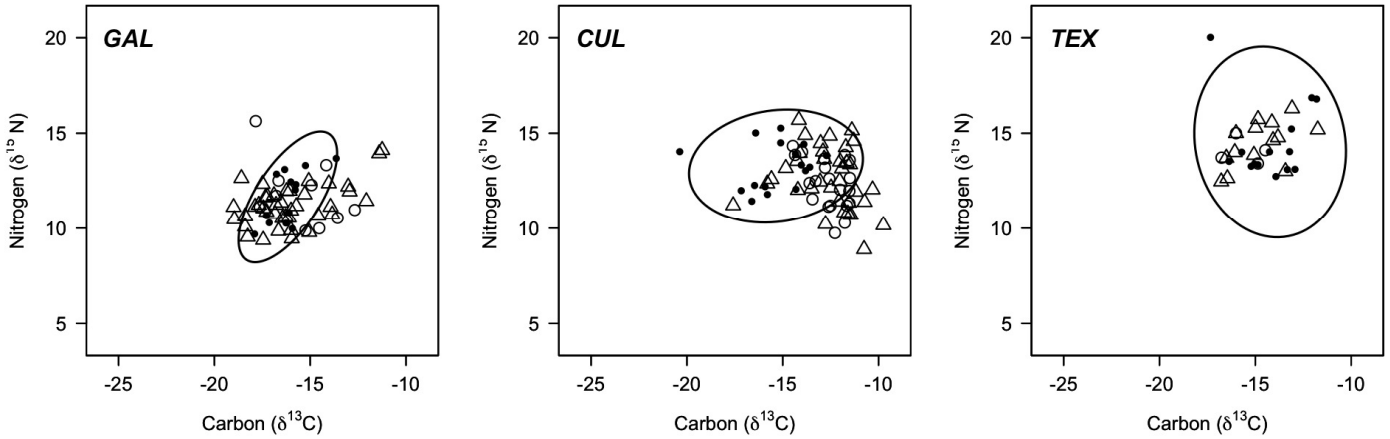

Figure 12 (continued). Stable isotope signatures of ^{13}C and ^{15}N in nesting and adult feathers collected at 27 burrowing owl study locations. Filled and open circles show the isotope signature of nestling and adult feathers, respectively. Circles and triangles denote males and females, respectively. Ellipses show the 95th-percentile ellipses for the bivariate normal distribution based on isotope data on nestling feathers. Study population acronyms are shown in Table 5.

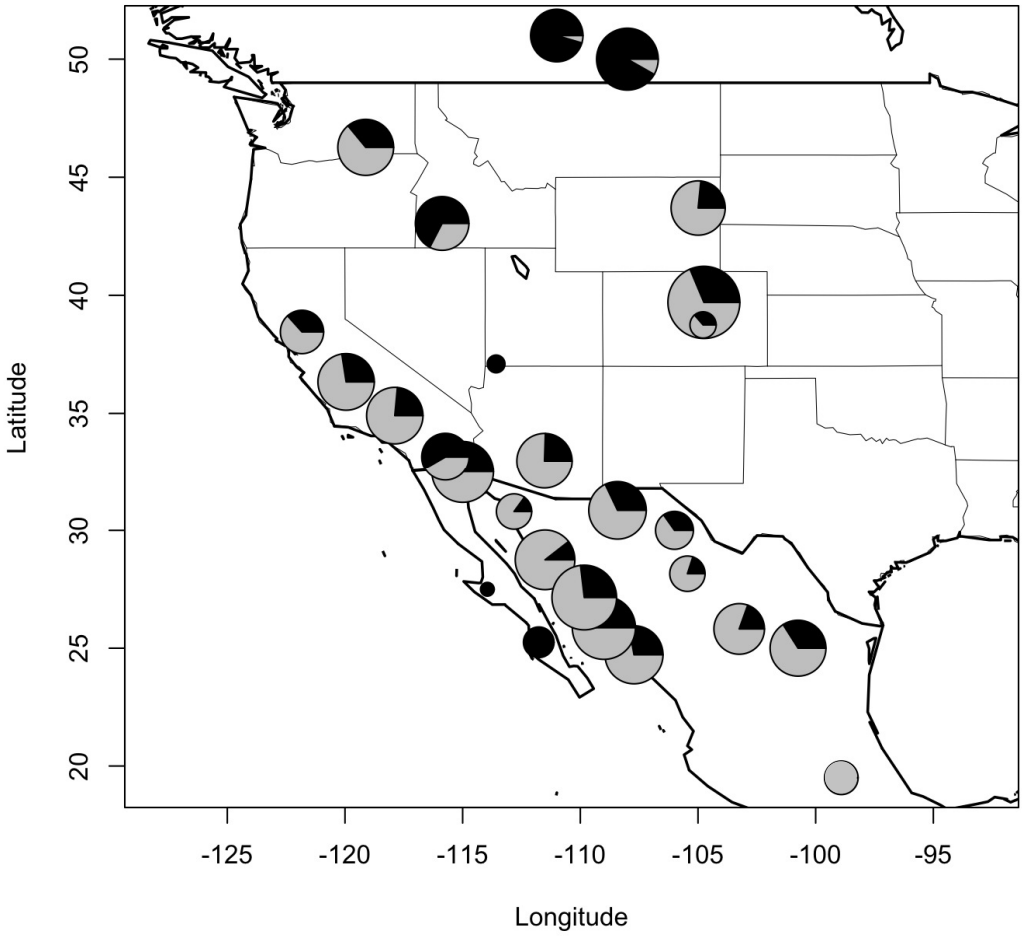

Figure 13. Geographic patterns of philopatry and immigration in burrowing owl populations across North America, as suggested by stable isotopes ^{13}C and ^{15}N. Each pie chart indicates the proportion of inferred phylopatric (gray) and immigrant (black) burrowing owls at each study population. The area of each pie chart is proportional to number of adult owls sampled at that location ($n_{max} = 83$).

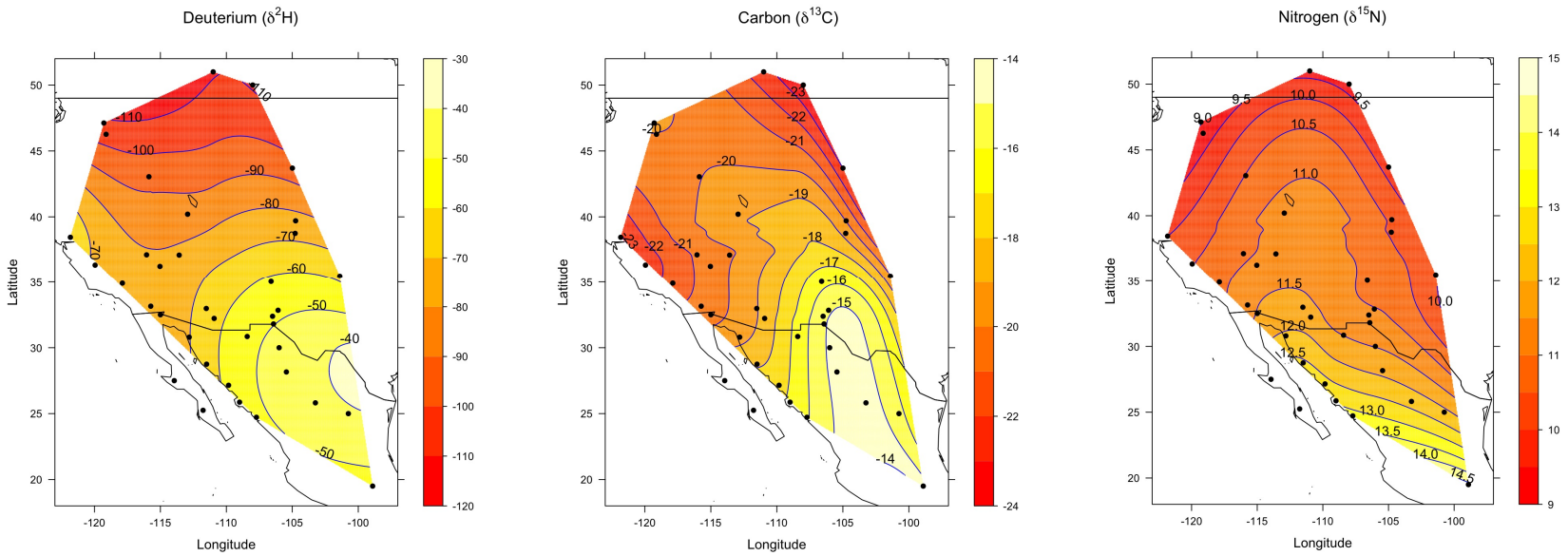

Figure 14. Maps representing the geographic variation in δ^2H, $\delta^{13}C$, and $\delta^{15}N$ (from left to right) of nestling feathers (i.e., local isotope ratios) as inferred by local regression analysis (LOESS) with latitude and longitude as explanatory variables. Black dots represent study locations (see Figure 11).

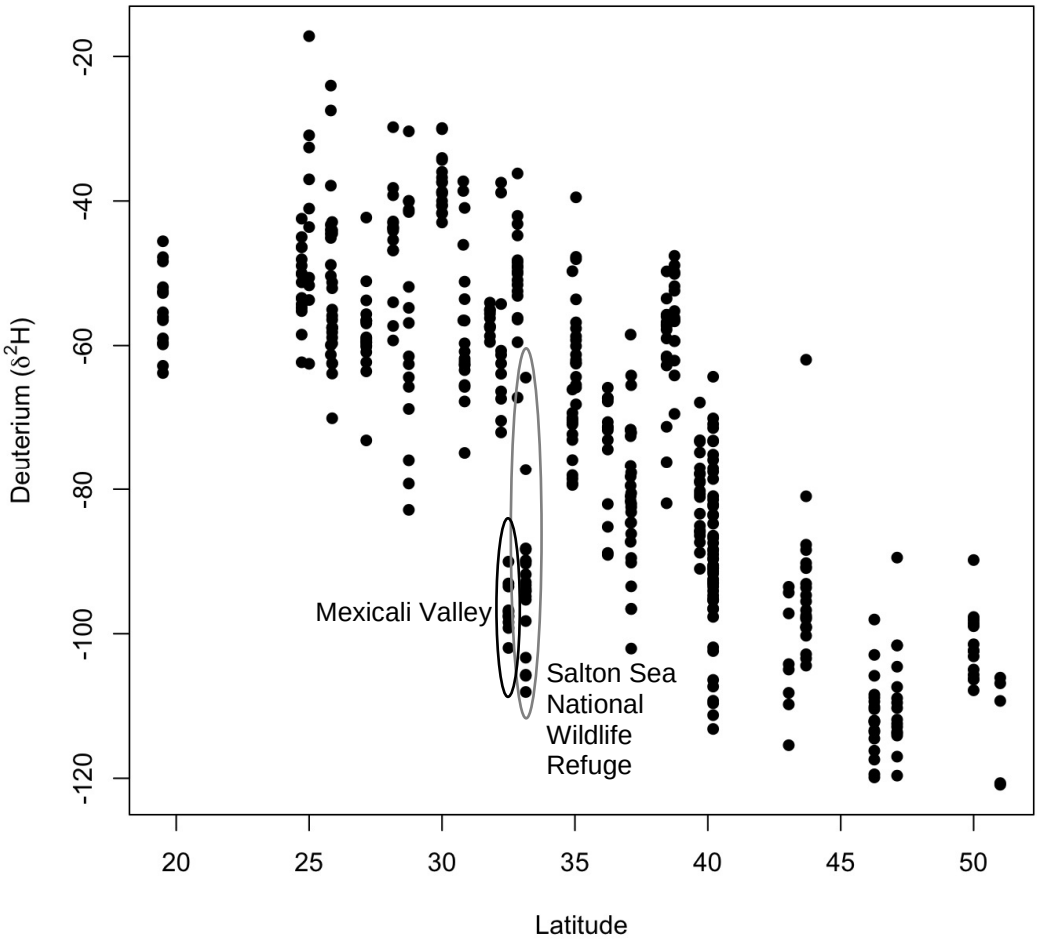

Figure 15. Latitudinal variation in δ^2H values in burrowing owl nestling feathers. The black dots represent individual feathers sampled from different latitudes (see Figure 1). Samples from the lower Colorado River Valley in Mexicali and Salton Sea deviate noticeably from the general latitudinal pattern. Longitude introduces variation in δ^2H not illustrated in this graph (Figure 14).

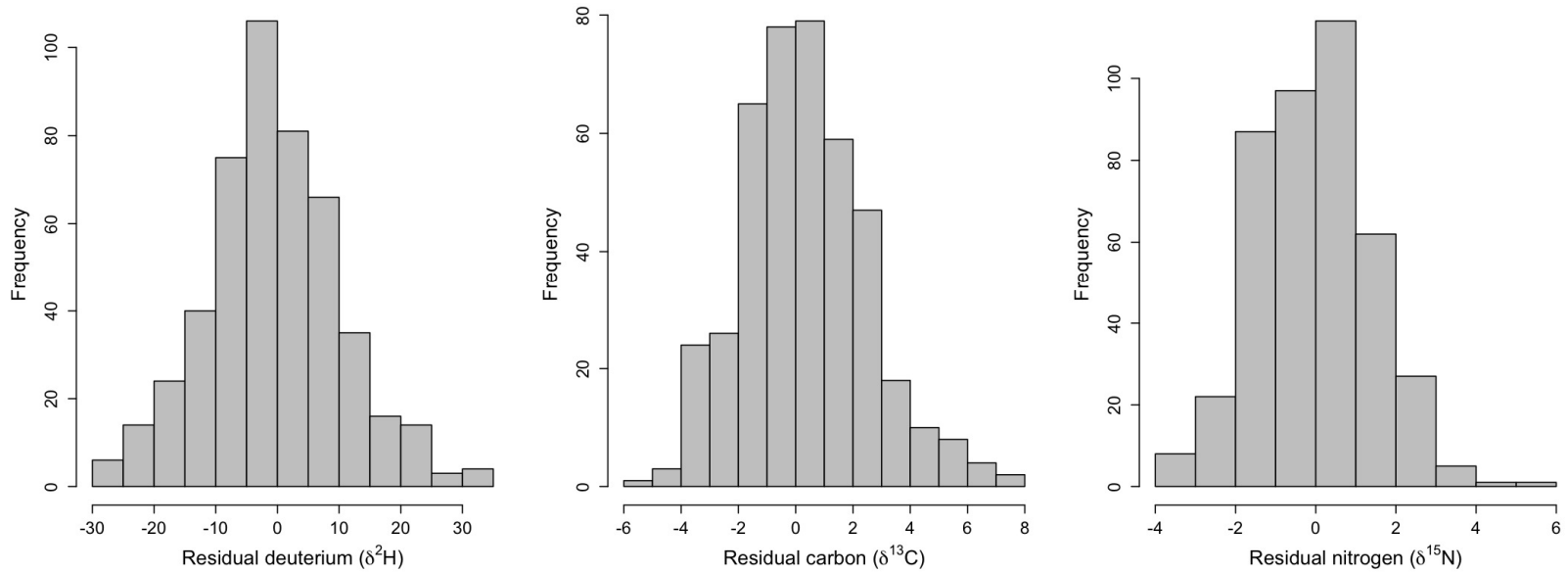

Figure 16. Local variation in δ^2H, $\delta^{13}C$, and $\delta^{15}N$ values shown as the distribution of residuals from a local regression analysis (LOESS) with latitude and longitude as explanatory variables.

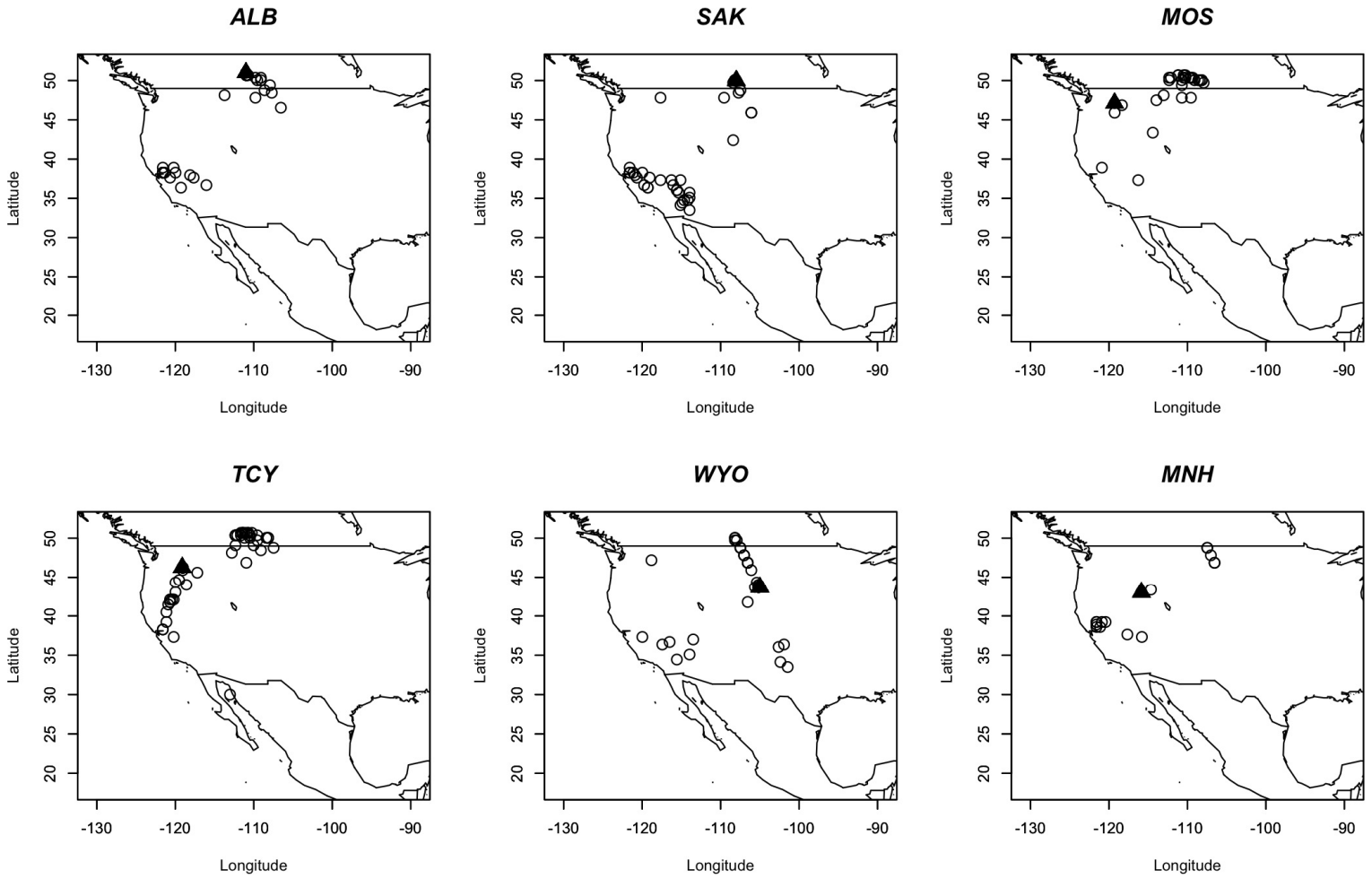

Figure 17. Geographic origin of breeding adult burrowing owls (open circles) at each of 21 study locations (triangles), as inferred from nestling base maps of $\delta^{2}H$, $\delta^{13}C$ and $\delta^{15}N$ data.

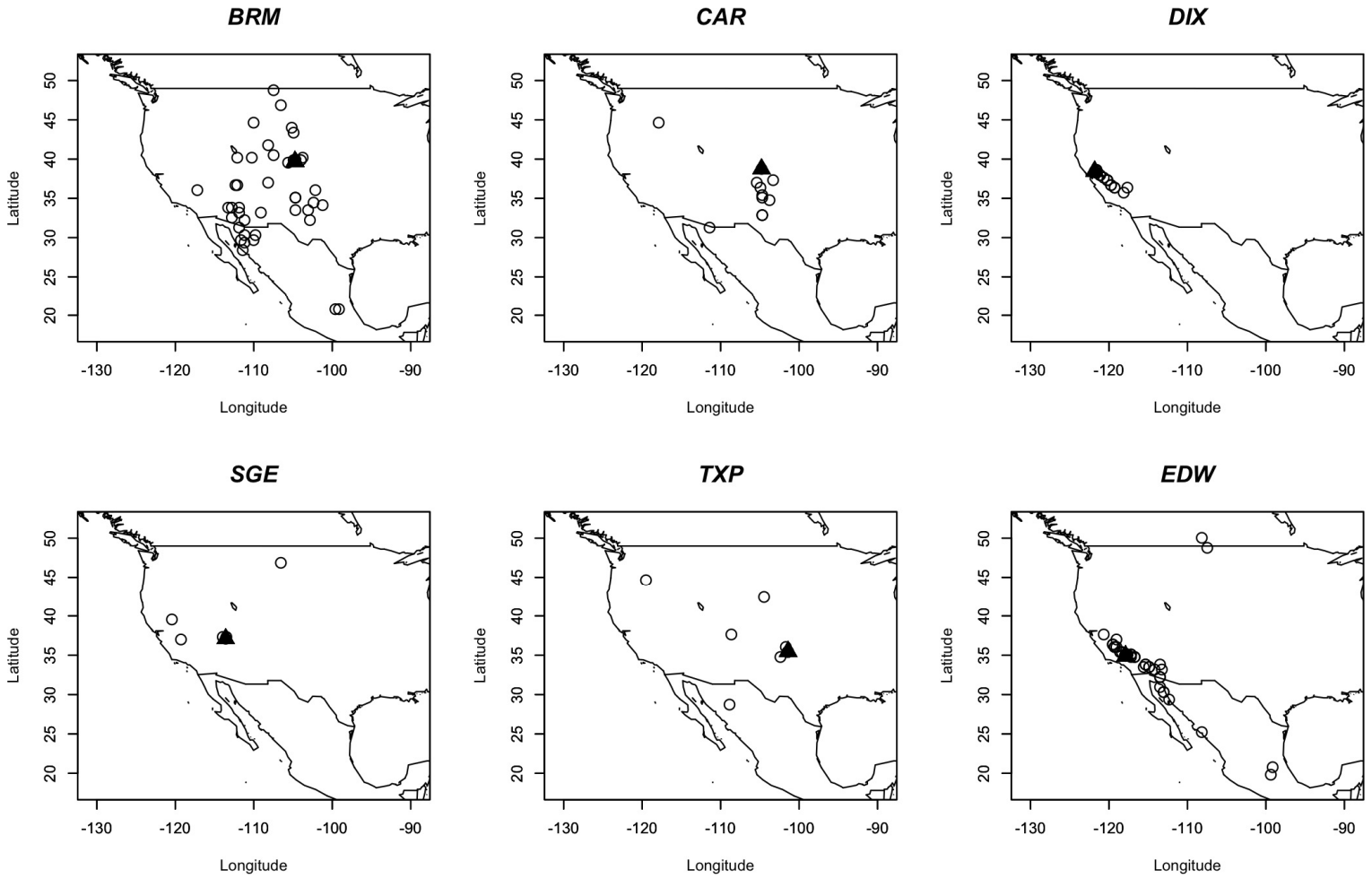

Figure 17 (Continued). Geographic origin of breeding adult burrowing owls (open circles) at each of 21 study locations (triangles), as inferred from nestling base maps of $\delta^{2}H$, $\delta^{13}C$ and $\delta^{15}N$ data.

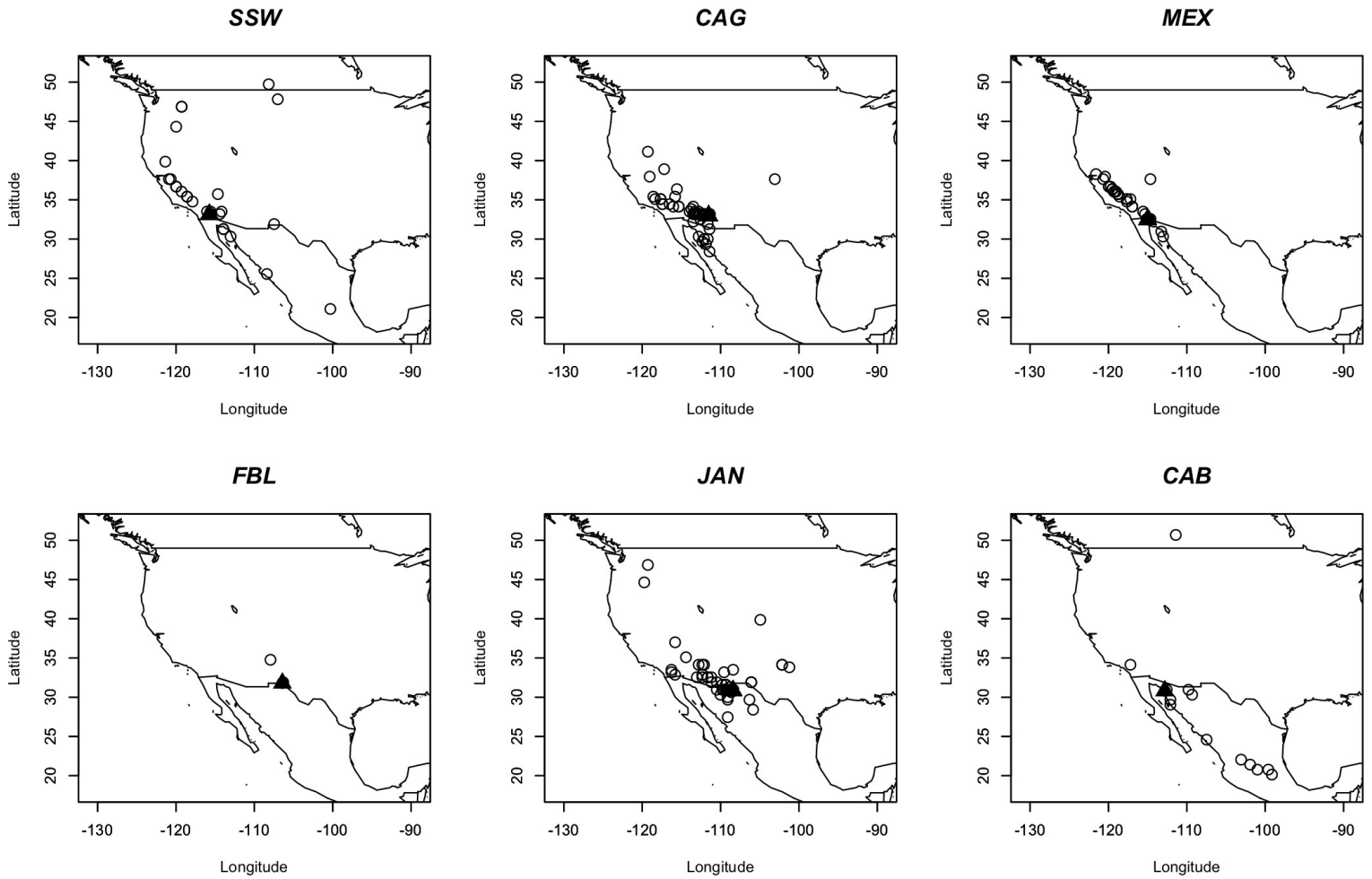

Figure 17 (Continued). Geographic origin of breeding adult burrowing owls (open circles) at each of 21 study locations (triangles), as inferred from nestling base maps of $\delta^{2}H$, $\delta^{13}C$ and $\delta^{15}N$ data.

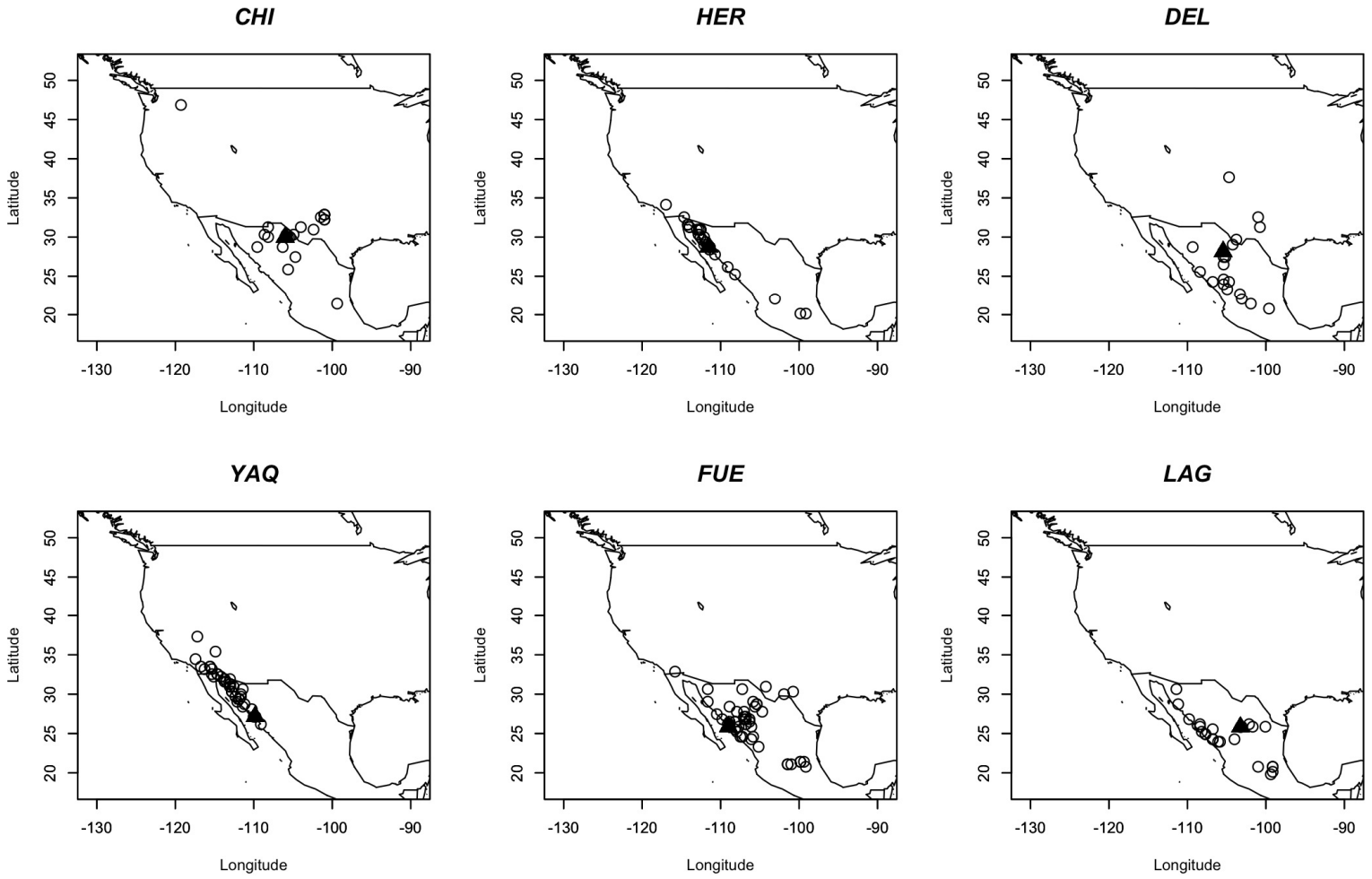

Figure 17 (Continued). Geographic origin of breeding adult burrowing owls (open circles) at each of 21 study locations (triangles), as inferred from nestling base maps of $\delta^{2}H$, $\delta^{13}C$ and $\delta^{15}N$ data.

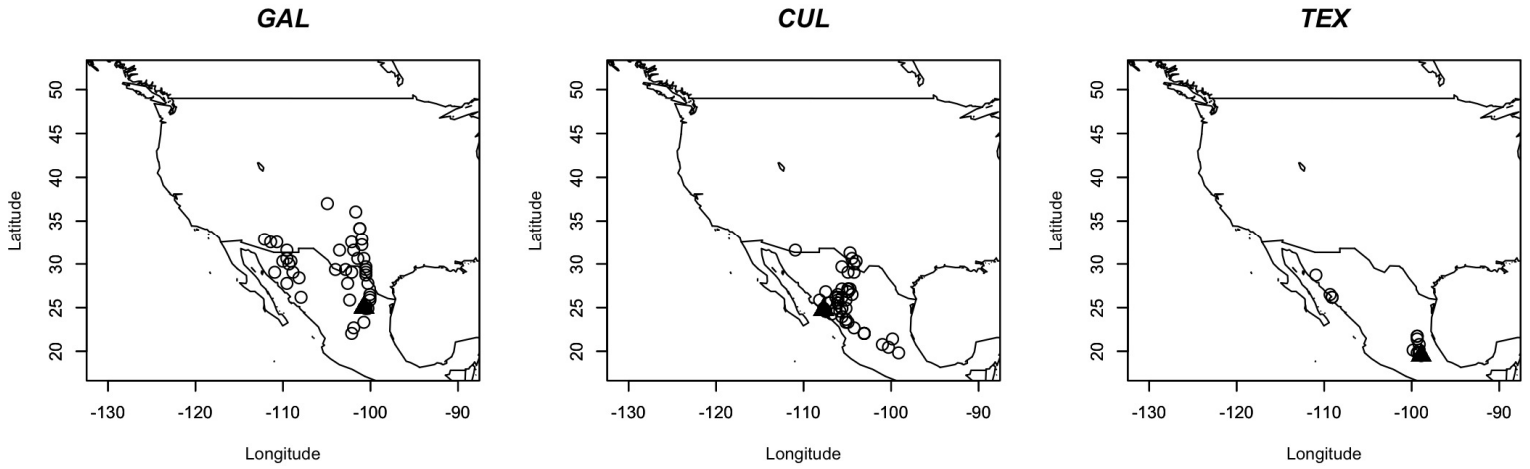

Figure 17 (Continued). Geographic origin of breeding adult burrowing owls (open circles) at each of 21 study locations (triangles), as inferred from nestling base maps of $\delta^{2}H$, $\delta^{13}C$ and $\delta^{15}N$ data.

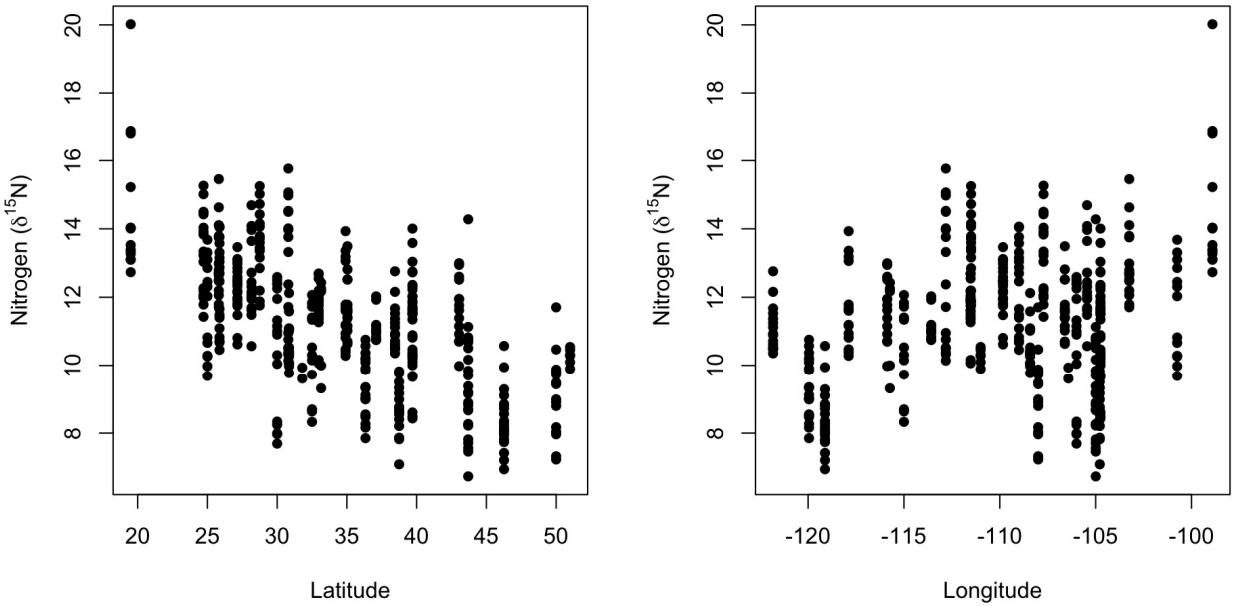

Figure 18. Latitudinal and longitudinal variation in δ^{15}N in burrowing owl nestling feathers in North America.

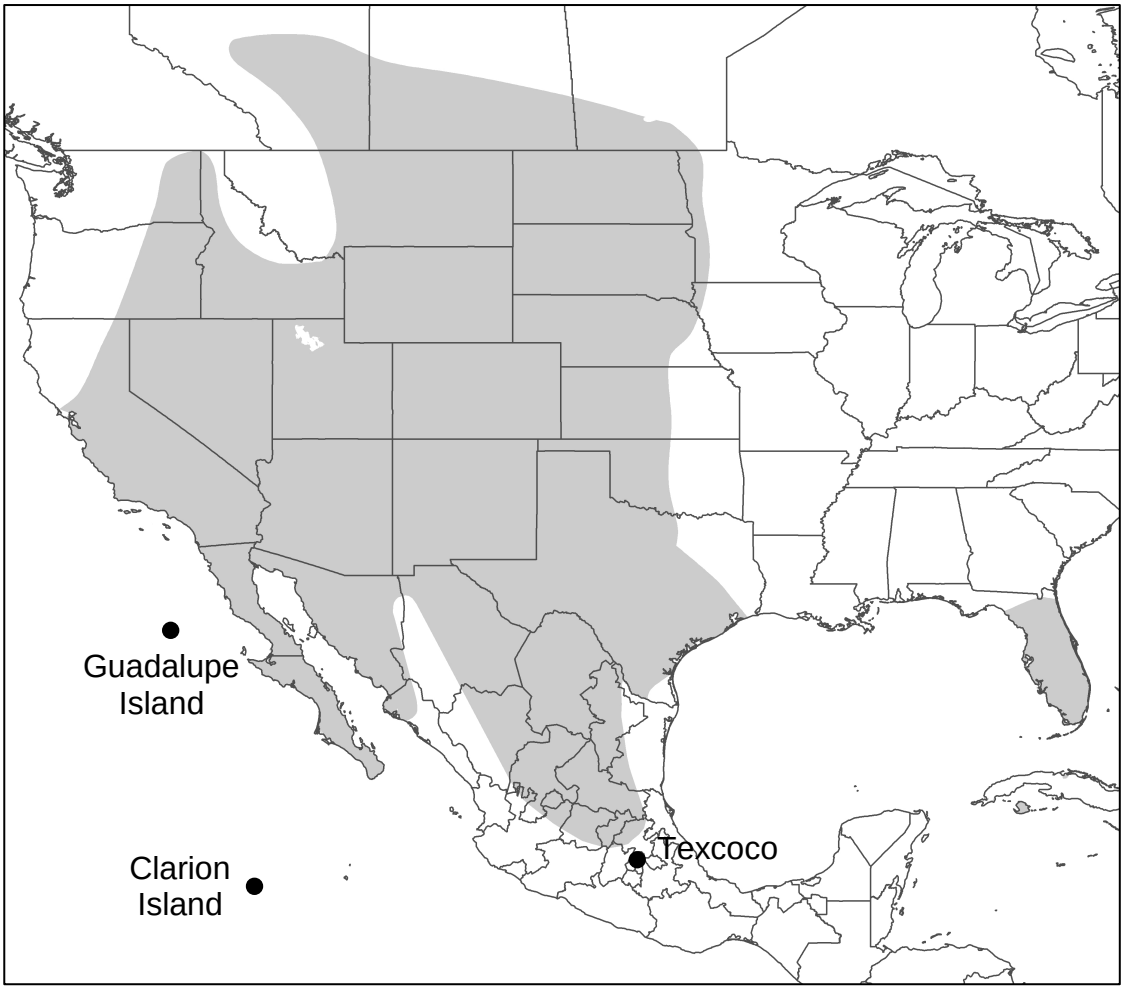

Figure 19. Insular (Clarion and Guadalupe) and isolated mainland (Texcoco) study populations of burrowing owls in Mexico. The gray area denotes the western burrowing owl's and the Florida burrowing owl's breeding distribution (as in Haug et al. 1993).

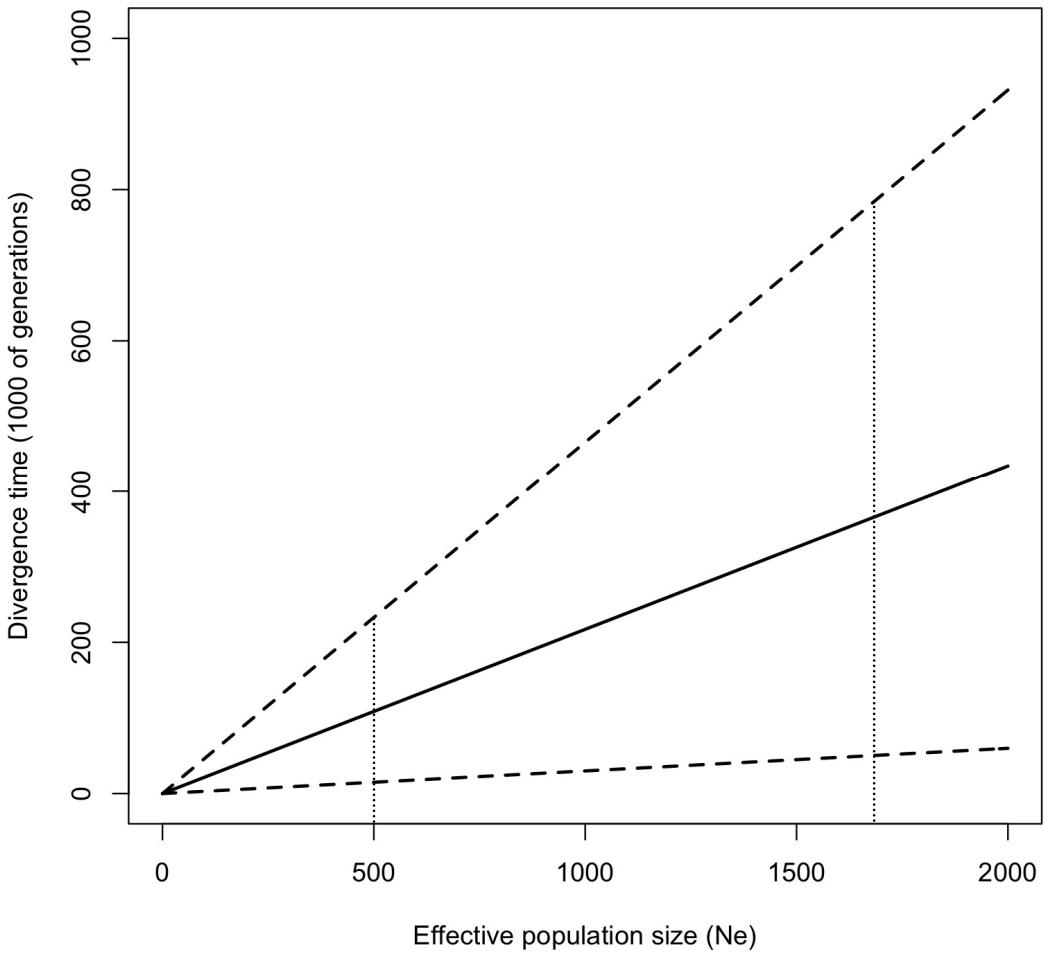

Figure 20. Mean divergence time (dashed lines are the 95% C. I. limits) between the Clarion and the western burrowing owl as a function of effective population size (N_e). The vertical dotted line indicated the hypothesized effective population sizes on Clarion Island (500 owls, G. Holroyd, pers. comm; 1,700 owls, Wanless et al. 2009).

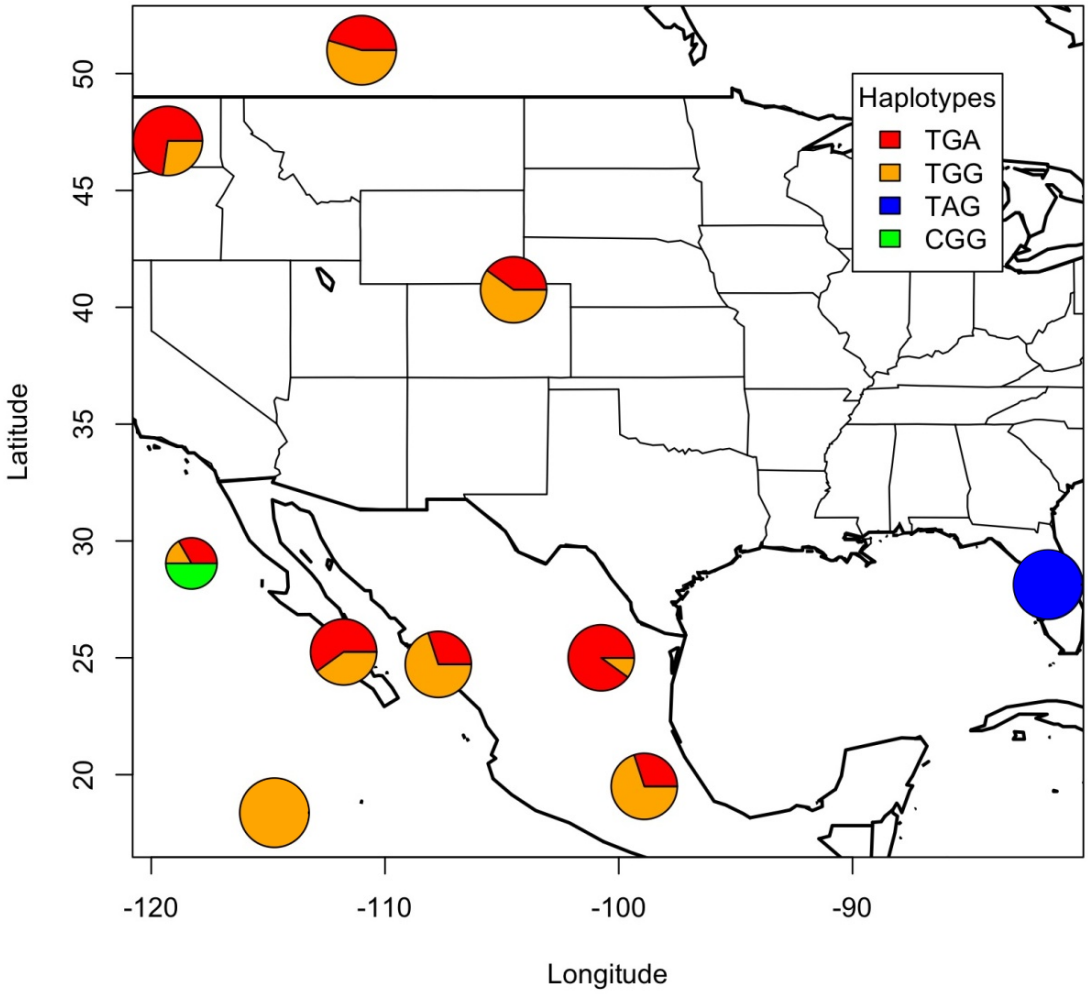

Figure 21. Geographic variation in the frequency of 4 cytochrome-b haplotypes (307 bp) found in burrowing owl populations in continental North America and Pacific Ocean Islands. The area of the pie charts are proportional to sample size (n_{max} = 11)

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