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Burrowing owl (*Athene cunicularia*) Population Genetics: A Comparison of North American Forms and Migratory Habits (Genética Poblacional de *Athene cunicularia*: Una Comparación entre las Formas de Norteamérica y Entre Hábitos Migratorios)

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## BURROWING OWL (*ATHENE CUNICULARIA*) POPULATION GENETICS: A COMPARISON OF NORTH AMERICAN FORMS AND MIGRATORY HABITS

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**ABSTRACT.**—We assessed the effects of range disjunction, migratory habit, coloniality, and habitat structure on the genetic differentiation of North American Burrowing Owl (*Athene cunicularia*) populations. Burrowing Owls in North America comprise two forms or subspecies: *A. c. floridana* in Florida, separated by ~1,500 km from the western form, *A. c. hypugaea*, which ranges from Texas to California and north to southern Canada. Burrowing Owls tend to be loosely colonial, and both the Florida populations and southerly populations of *A. c. hypugaea* from California to Texas are nonmigratory. To assess genetic structure, we examined 201 individuals from nine western and six Florida populations at seven highly variable microsatellite DNA loci. Mean gene diversity ( $H_{exp}$ ) was higher in the west than in Florida (0.539 and 0.341, respectively;  $P < 0.05$ ). Populations within subspecies were essentially panmictic (*A. c. floridana*:  $\theta = 0.038$ ,  $\rho = 0.014$ ; *A. c. hypugaea*:  $\theta = 0.014$ ,  $\rho = 0.009$ ) and even genetic differentiation across subspecies was modest ( $\theta = 0.051$ ,  $\rho = 0.014$ ). Nevertheless, the western and Florida forms were easily distinguished by any of several criteria, such as allelic absences in Florida, assignment tests, and well-supported branches on the inferred phylogenetic tree. Genetic differentiation was at least twice as great in resident Florida ( $\theta = 0.038$ ) and California ( $\theta = 0.021$ ) populations as in migratory western populations ( $\theta = 0.012$ ), though 95% confidence intervals of theta estimates overlapped. We found no evidence of a genetic bottleneck that would result in evolutionary disequilibrium within subspecies. In the west, high observed heterozygosity values and evidence of gene flow suggest that population declines and patchy habitat, which currently imperil this species throughout much of its range, have not led to inbreeding or biologically meaningful genetic differentiation among the sampled populations. Received 21 December 2003, accepted 12 September 2004.

**Key words:** *Athene cunicularia*, Burrowing Owl, genetic differentiation, microsatellite DNA markers, migration, philopatry.

### Genética Poblacional de *Athene cunicularia*: Una Comparación entre las Formas de Norteamérica y Entre Hábitos Migratorios

**RESUMEN.**—Determinamos los efectos de las disyunciones del rango, de los hábitos migratorios, de la colonialidad y de la estructura del hábitat sobre la diferenciación genética de poblaciones de *Athene cunicularia*. En Norteamérica esta

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especie comprende dos formas o subespecies: *A. c. floridana* en Florida, separada por ~1500 km de la forma del oeste, *A. c. hypugaea*, que se distribuye desde Texas hasta California y hacia el norte hasta el sur de Canadá. *Athene cunicularia* tiende a ser semi-colonial y tanto las poblaciones de Florida como las poblaciones sureñas de *A. c. hypugaea* de California hasta Texas no son migratorias. Para determinar la estructura genética, examinamos siete loci variables de ADN microsatelital en 201 individuos de nueve poblaciones del oeste y de seis poblaciones de Florida. La diversidad genética promedio ( $H_{\text{esp}}$ ) fue mayor en la población del oeste que en la de Florida (0.539 y 0.341, respectivamente;  $P < 0.05$ ). Las poblaciones dentro de las subespecies fueron esencialmente panmícticas (*A. c. floridana*:  $\theta = 0.038$ ,  $\rho = 0.014$ ; *A. c. hypugaea*:  $\theta = 0.014$ ,  $\rho = 0.009$ ), e incluso la diferenciación entre las subespecies fue modesta ( $\theta = 0.051$ ,  $\rho = 0.014$ ). Sin embargo, las formas del oeste y las de Florida se distinguieron fácilmente por cualquiera de varios criterios, tales como la ausencia de alelos en Florida, pruebas de asignación, y ramas fuertemente apoyadas en el árbol filogenético inferido. La diferenciación genética fue por lo menos dos veces mayor en las poblaciones residentes de Florida ( $\theta = 0.038$ ) y California ( $\theta = 0.021$ ) que en las poblaciones migratorias del oeste ( $\theta = 0.012$ ), aunque los intervalos de confianza del 95% de las estimaciones de zeta se sobrepusieron. No encontramos evidencia de un cuello de botella que podría resultar en un desequilibrio evolutivo dentro de las subespecies. En el oeste, los valores altos de heterocigocidad y la evidencia de flujo génico sugieren que las disminuciones poblacionales y la fragmentación del hábitat, que actualmente amenaza a esta especie a través de gran parte de su rango, no han causado endogamia o alguna diferenciación genética biológicamente significativa entre las poblaciones muestreadas.

SENSITIVE POPULATION GENETICS tools, such as microsatellite DNA markers, can reveal fine-scale differentiation and provide insights into population connectivity and demographics, characteristics that are often difficult to assess through field studies (Koenig et al. 1996). Range disjunctions and degree of philopatry are among the host of factors that can drive differentiation among populations. Those forces may be important in determining the genetic structure of the Burrowing Owl (*Athene cunicularia*), a grassland species that tends to occur in a patchy distribution on the landscape and was traditionally considered fairly philopatric. Here, we assess the influence of those forces and, to a lesser extent, the influence of coloniality and habitat structure on the genetic structure of populations of Burrowing Owls in the United States. The North American forms have at times been classified as distinct subspecies (e.g. American Ornithologists' Union 1983)—*A. c. hypugaea* in western North America (hereafter referred to as "the western form") and *A. c. floridana* in Florida ("the Florida form"). The range of the western form stretches from northern Mexico through Texas and California in the south and from Washington and North Dakota in the north,

with populations extending into three of the western Canadian provinces (Fig. 1).

The western form comprises three main population types, including migratory, resident, and nonbreeding (Fig. 1). Most individuals that breed in the United States and Canada migrate south during September and October and return north in March and April, though timing can vary and little is known about migration routes and destinations (Haug et al. 1993). A small percentage of Burrowing Owls from migratory populations may remain on their breeding grounds year-round (e.g. Martin 1973, Butts 1976). Individuals of resident populations in southern California, northern Mexico, and Florida breed and overwinter in an area without a significant migration (Haug et al. 1993); whereas nonbreeding populations in Mexico consist of individuals from migratory populations during the winter months.

Breeding populations of the western form are separated from the Florida form by ~1,500 km. Desmond et al. (2001) found a mitochondrial cytochrome-*b* sequence divergence of 0.7% across that range disjunction, which leads to an estimate of 350,000 years since divergence. The range disjunction is similar to that found

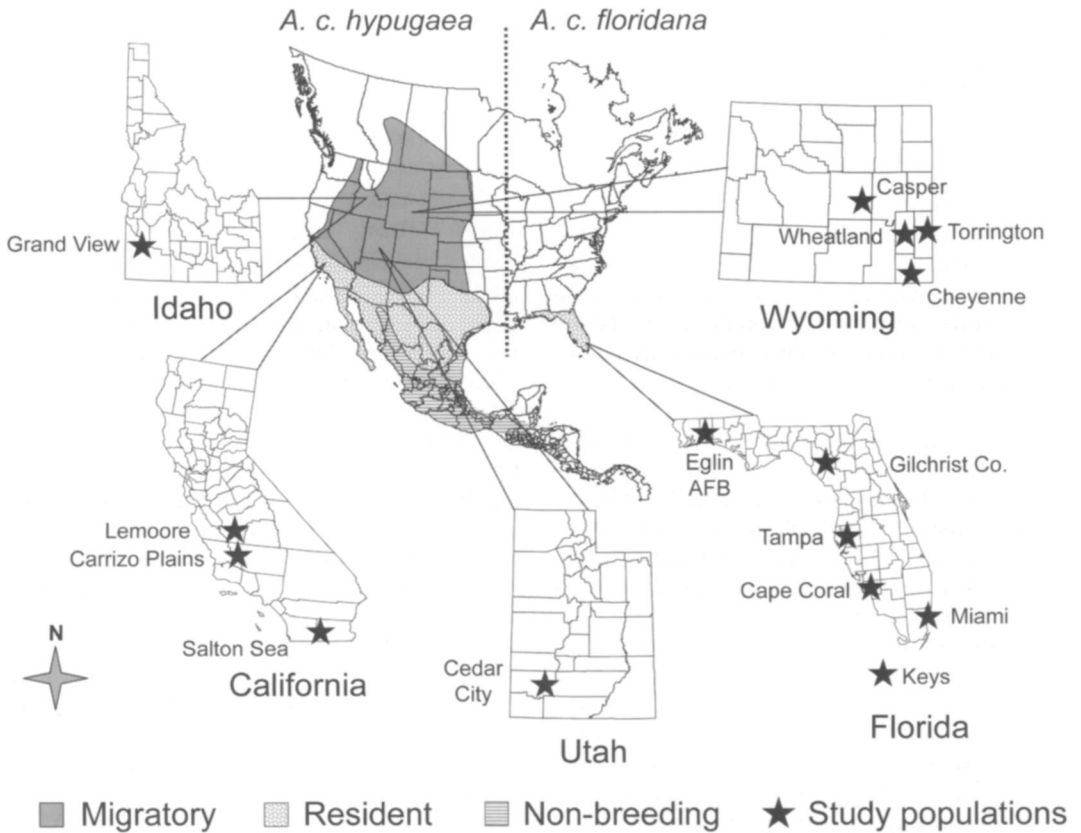


FIG. 1. Burrowing Owl distribution in North America and Mexico, including *A. c. hypugaea* in the west and *A. c. floridana* in Florida. Six migratory and nine resident populations were sampled for genetic analyses.

between the Western Scrub-Jay (*Aphelocoma californica*) and the Florida Scrub-Jay (*A. coerulescens*), which are currently considered distinct species (American Ornithologists' Union 1995). In contrast to the sequence divergence between the western and Florida forms of Burrowing Owl, Desmond et al. (2001) found no sequence differences among three individuals from Nebraska, California, and Baja California.

High philopatry will tend to lead to low gene flow and increased differentiation among populations. Migratory habit is related to philopatry, because resident species of birds tend to have lower dispersal rates than migratory species (Paradis et al. 1998). Millsap and Bear (1997) found a much higher re-encounter rate for banded Burrowing Owls in a resident Florida population (41%,  $n = 601$ ) than did Lutz and Plumpton (1999) for a migratory Colorado

population (8%,  $n = 555$ ). Mean natal dispersal distance was much lower in the Florida population than in a migratory Manitoba population (Haug et al. 1993), and breeding-site fidelity was much higher in the Florida population as compared with migratory populations. Finally, mate fidelity was very high in Florida (92%,  $n = 59$  pairs), whereas migration tends to separate breeding pairs in migratory populations (Lutz and Plumpton 1999), a trend that may be applicable to the resident and migratory populations of partially migratory species (i.e. those with resident and migratory components). On the basis of the evidence just presented, we predicted that genetic differentiation would be more pronounced in resident California and Florida populations than in migratory populations.

Two additional factors that may promote genetic differentiation among populations

are colonial breeding and the landscape-level structure of breeding habitat. Burrowing Owls tend to nest in loose aggregations or colonies, though they also nest as single pairs. Friesen (1997) suggested that coloniality may contribute to differentiation among colonial waterbirds by producing geographic clumping, with individuals more likely to return and breed with individuals in their natal or neighboring colonies. Habitat structure may also play a role in patterns of differentiation, even among taxonomically similar groups. McDonald et al. (1999) showed that gene flow was considerably higher in Western Scrub-Jays than in Florida Scrub-Jays, which was attributable, at least in part, to the patchy, discontinuous nature of the sand-pine scrub habitat to which the Florida species is restricted. Burrowing Owl habitat in Florida has been historically restricted to the prairies of central Florida, whereas suitable habitat for the Western Burrowing Owl has historically been more extensive and well connected.

Burrowing Owls have been declining in many parts of their range, particularly at the periphery (Wellicome and Holroyd 2001). Gene flow among declining populations may decrease as a result of limited recruitment and the extirpation of stepping-stone populations. Those declines raise the prospect of an increase in inbreeding levels, with exacerbating effects on population decline. Tentative support for that scenario comes from the work of Johnson (1997a), who found high homozygosity in mini-satellite DNA profiles for a declining population in Davis, California, that went extinct a few years after completion of the study. Any factors that might limit gene flow among populations may therefore have important conservation implications.

Here, we assess the genetic structure of populations of Burrowing Owls from California, Wyoming, Utah, Idaho, and Florida using microsatellite DNA. We examine how range disjunction, migratory habit, coloniality, and habitat structure may influence the genetic structure. We ask three major questions: (1) Do Florida and western forms show genetic differentiation among populations within forms, and does the degree or pattern of genetic differentiation differ between forms? (2) Do behavioral differences associated with migratory habit lead to discernible differences in the degree of differentiation among populations? (3) Do populations

show evidence of inbreeding, as measured by Wright's (1978) fixation index,  $F_{is}$ ?

## METHODS

### SAMPLE COLLECTION

Between 29 May 1998 and 17 July 1999, we captured Burrowing Owls from four eastern Wyoming populations (Fig. 1). Burrowing Owls were located primarily through surveys of historical nesting sites identified in the Wyoming Game and Fish Department's Wildlife Observation System, a database of wildlife sightings reported by biologists and the general public. Male Burrowing Owls were located during daylight hours through playbacks of male territorial *coo coo* calls (Haug and Didiuk 1993) projected from a megaphone. Call playbacks elicited male territorial behavior, including bobbing, flying, and the "white-and-tall" stance, all of which increase the visibility of the male (Haug and Didiuk 1993). Male and female adults were trapped using bal-chatri traps (Berger and Mueller 1959) modified for capturing Burrowing Owls (Bloom 1987), and most juveniles were captured on noose carpet traps (Barrentine and Ewing 1988). To minimize sampling of related individuals between years, we did not sample 1998 sites again in 1999. Individuals captured in 1999 nested  $\geq 10$  km from previously sampled areas.

Captured individuals were banded with a federal aluminum leg band and were sexed using coloration and behavioral criteria. Females tended to have more distinct breast-barring and darker coloration (Haug et al. 1993) and were more closely associated with the primary nest burrow. Approximately 100  $\mu$ L of blood were collected using ulnar venipuncture and stored in 1.0 mL of lysis buffer (Seutin et al. 1991). Blood samples were stored at 10°C until they could be processed. Collaborators generously donated samples from all other locations (Fig. 1; see Acknowledgments).

### LABORATORY METHODS

DNA was extracted from blood samples using QIAmp DNA Blood Mini Kits (Qiagen, Valencia, California). Twenty-two microsatellite primer pairs developed from a Burrowing Owl genomic library (Korfanta et al. 2002) were screened for



TABLE 1. Mean heterozygosity values per locus.

| Locus   | Number of alleles | $H_{obs}$ | $H_{exp}$          |
|---------|-------------------|-----------|--------------------|
| BUOW1   | 12                | 0.712     | 0.720              |
| BUOW4   | 19                | 0.698     | 0.793              |
| BUOW6   | 3                 | 0.126     | 0.125              |
| BUOW7   | 5                 | 0.296     | 0.382 <sup>a</sup> |
| BUOW11  | 12                | 0.674     | 0.717              |
| BUOW13  | 3                 | 0.043     | 0.067              |
| BUOW16  | 4                 | 0.311     | 0.346              |
| Overall |                   | 0.408     | 0.450              |

<sup>a</sup>  $H_{exp} > H_{obs}$  ( $P < 0.05$ ).

polymorphisms, and seven of those were used in the genetic analyses (GenBank Accession numbers AY094989–AY094995) (Table 1).

We performed the polymerase chain reaction (PCR) using a PTC-200 Peltier thermal cycler (MJ Research, Waltham, Massachusetts) to amplify 100 ng of template DNA. Further details of PCR reaction conditions are given in Korfanta et al. (2002). For each sample, 1.0  $\mu$ L of PCR-amplified product was resolved by electrophoresis on a 25-cm, 7% polyacrylamide gel and genotyped using a 4200-S Automated DNA Sequencer (Li-Cor, Lincoln, Nebraska). The 5' end of the shorter primer of each pair was labeled with a fluorescent third primer, which allowed the DNA amplicons to be detected by infrared laser fluorescence. Allele sizes were ascertained by comparison to known reference marker lanes and GENE IMAGIR, version 3.0 (Li-Cor) was used to determine genotypes of individuals. At least 20% of all individuals were run  $\geq 2\times$  for validation of scores; however, for some individuals, particularly in Florida populations, there was an insufficient quantity of template DNA to obtain genotypes at all loci. In those cases, statistical analyses were performed with the partial genotypes.

**Data analysis.**— Allele sizes were determined by subtracting the 19 base-pair fluorescent tails from the raw fragment lengths. We tested for deviation from expectations of Hardy-Weinberg equilibrium for each locus and population-locus pair using the probability subroutine in GENEPOP, which uses the exact test of Haldane (1954), Guo and Thompson (1992), and others. Nei's unbiased estimate of expected heterozygosity ( $H_{exp}$ ) was calculated using TFPGA (see Acknowledgments). We also assessed nonrandom mating through comparison of inbreeding coefficients (Weir and

Cockerham's [1984]  $f$  analog of  $F_{IS}$ ) using FSTAT (Goudet 1995) for each population-locus pair and for the overall population.

For microsatellite loci, the sometimes small sample sizes obtainable from natural populations may affect allelic presence or absence and potentially influence conclusions concerning population structure. We used a binomial probability approach (McDonald 2003) to calculate the likelihood that private alleles (i.e. alleles unique to a population; Neel 1973, Slatkin 1987) were truly missing in the population from which we took the smaller sample. If  $F_i$  indicates the frequency of a particular allele at a given locus over both populations, the probability that any given individual has no copy of the allele is  $M = (1 - F_i)^2$ . The probability that the allele will be totally missing in a sample of size  $n$  is  $M^n$ .

Population genetic differentiation was assessed with two types of measures. We used FSTAT (Goudet 1995) to calculate the  $F_{ST}$ -like measure theta (Weir and Cockerham 1984), which uses Kimura and Crow's (1964) assumption of an infinite-alleles mutational model, and we used RSTCALC (Goodman 1997) to assess rho, an unbiased estimator of Slatkin's  $R_{ST}$  (Slatkin 1995), which assumes a stepwise mutational model (SMM; Ohta and Kimura 1973). Goodman's rho is advantageous, in that it eliminates bias caused by unequal sample sizes and unequal variances across loci. Both theta and  $R_{ST}$  have been shown to perform well, as compared with other genetic measures, with microsatellite data (Ruzzante 1998). A bootstrap procedure was used in FSTAT to calculate a 95% confidence interval (CI) around theta estimates.

Genetic distances for all population pairs across all loci were calculated to determine whether a relationship existed between geographic and genetic distances. We used TFPGA to calculate Nei's unbiased genetic distance (Nei 1978), and the "GenDist" subroutine of PHYLIP (Felsenstein 1995) to calculate Cavalli-Sforza chord distances (Cavalli-Sforza and Edwards 1967). The latter measure is purely geometric, makes no assumptions about the forces that drive population genetic differentiation, and has been shown to provide accurate phylogeographic tree topologies, as compared with other measures (Takezaki and Nei 1996). We used the "Consense" and "Neighbor" subroutines of PHYLIP (Felsenstein 1995) to construct an unrooted neighbor-joining tree using 1,000

bootstrapped Cavalli-Sforza genetic distances. Populations with five or fewer individuals sampled were omitted. To further assess the strength of the geographic-genetic distance relationship, we used Paetkau et al.'s (1995) log-likelihood assignment test to determine the ability of genotypic data to assign individuals correctly to their home population. The assignment test was conducted for all 15 study populations and for the western form versus the Florida form.

A comparison of male and female population genetic parameters was done by grouping all males within subspecies and all females within subspecies so that two populations were analyzed for each sex. That was necessary to maintain sufficient sample sizes, particularly because samples of some populations, such as Idaho, comprised individuals of only one sex. For each sex, we calculated population and global estimates of theta, rho,  $F_{IS}$ , and relatedness (Queller and Goodnight 1989) using FSTAT.

The Bayesian approach of Pritchard et al. (2000) assesses whether the sampled genotypes are substructured into multiple ( $K > 1$ ) clusters or constitute a single Hardy-Weinberg population ( $K = 1$ ). Log-likelihood ratios from Monte Carlo Markov chain sampling provide the basis for deciding what number of clusters best fits the data. To compute the Bayesian clustering, we used the program STRUCTURE (see Acknowledgments).

We used two measures to test for deviation from demographic or evolutionary equilibrium that would result from a genetic bottleneck. First, we calculated Garza and Williamson's (2001)  $M$ , which is the ratio of the number of alleles to the range in allele size. As populations decline, numbers of alleles and the range in allele size are diminished, but, because of the typically chunky distribution of microsatellite alleles, those two variables do not usually decrease at equal rates. Low-frequency alleles often occur in the interior of the allele distribution, rather than strictly at the tails as expected under the SMM. Those uncommon alleles are most likely to be lost during genetic drift, decreasing total allele number while not necessarily decreasing the range in allele sizes immediately. Thus, a low  $M$  (i.e.  $< 0.68$ ) can be used to detect a recent reduction in population size. To calculate  $M$ , proportions of one-step mutations ( $p_s$ ), mean size of larger mutations ( $\Delta_s$ ), and pre-bottleneck theta ( $\theta_b = 4N_e\mu$ , where  $\mu$  is mutation

rate) were estimated.  $M$  was calculated for each subspecies, using the values of  $p_s = 90\%$  and  $\Delta_s = 3.5$ , which Garza and Williamson (2001) suggested as reasonable parameters for the model.  $M < 0.68$  was considered indicative of a genetic bottleneck. We also used the program BOTTLENECK, version 1.2.02 (Cornuet and Luikart 1996), which detects departure from mutation-drift equilibrium as assessed by heterozygosity excess or deficiency, to look for recent genetic bottlenecks at the levels of subspecies and populations.

Finally, we estimated relative effective population sizes ( $N_e$ ) using MISAT (Nielsen 1997), which yields a likelihood estimate of  $\theta_{N_e}$  for each population-locus combination. To obtain a single  $\theta_{N_e}$  estimate for each population, we log-transformed the estimates and performed a linear, multivariate regression. The regression coefficients were then back-transformed and confidence intervals calculated.

## RESULTS

Multilocus genotypes were obtained for 201 Burrowing Owls. The seven microsatellite loci were quite variable, with mean  $H_{exp}$  of 0.450 (range: 0.067–0.793) and a maximum of 11 alleles at a locus (Table 1). The relatively high variability at the seven sampled loci suggested they are useful for detecting potential population genetic differentiation and parentage. Analysis of six family groups with 10 juveniles indicated Mendelian inheritance, except for one individual at one locus (BUOW7), whose genotype at one of the seven loci was incompatible with that of its father. Individuals in that family group were genotyped three times to validate scores, and parents and offspring matched at all other loci, including several uncommon alleles that make it very unlikely that the mismatch was attributable to extra-pair fertilization. No evidence was found for null alleles through inbreeding coefficients or nonamplifying individuals, and we tentatively attributed the mismatch to a mutation event. Of the 105 Hardy-Weinberg population-locus tests for deviation from equilibrium expectations, six were significant at the 0.05 level. However, when a Bonferroni adjustment ( $\alpha = 0.05/105 = 0.0005$ ) was applied to minimize Type I errors that may occur when a large number of tests are conducted, only the Lemoore-BUOW7 test was

significant ( $P = 0.0001$ ), and that was attributable to an observed homozygote excess. In all populations,  $H_{obs}$  was less than or equal to  $H_{exp}$  (Table 2); however, differences were small and suggested only minor deviations from equilibrium expectations.

The Florida form had significantly fewer alleles per locus (95% CI, 2.4–3.2) than the western form (95% CI, 4.2–5.6; Table 2), and Florida alleles were generally a subset of those in the west. One Florida individual had an allele at the BUOW1 locus that was not found in any other population. The probability that the allele was present in the western form but not detected because of sample size was 0.67, which indicates that this was probably not an allele unique to the Florida form. Nineteen percent (8 of 42) of the population-locus combinations in the Florida form were fixed for a single allele, contrasting with 4.8% (3 of 63) fixation in the west. Locus BUOW6 was fixed in all Florida populations (Table 2). Expected heterozygosity

( $H_{exp}$ ) was significantly higher in the western form (95% CI, 0.507–0.547) than in the Florida form (95% CI, 0.289–0.354).  $F_{IS}$  was small but significantly greater than zero in the western form overall (0.061) and comparable in the two forms ( $\approx 0.062$ ), though significant only in the western form (Table 2). Inbreeding coefficients were more variable in Florida, and none differed significantly from zero.

Rho and theta indicated a small but significant degree of overall population genetic differentiation (Table 3). Rho, estimated over all 15 populations and 7 loci, was 0.014, and theta 0.051 (95% CI, 0.033–0.066). Because much of that subdivision may be attributable to differences between the western and Florida forms, genetic differentiation was also assessed within the forms separately. Subdivision within the forms was minimal, and although theta was greater in the Florida form (0.038) than in the western (0.014), confidence intervals overlapped substantially (Table 3).

TABLE 2. Measures of genetic variation and distance in 15 Burrowing Owl populations.

| Population                    | Mean sample size per locus (SD) | Number of alleles at seven loci <sup>a</sup> (SD) | $H_{obs}$ | $H_{exp}$ | $F_{IS}$ averaged over loci <sup>b</sup> |
|-------------------------------|---------------------------------|---|-----------|-----------|--|
| <i>Athene cunicularia</i>     |                                 |   | 0.514     | 0.515     | 0.064**                                  |
| <i>A. c. hypugaea</i>         |                                 |   |           |           |  |
| Casper, Wyoming               | 10.0 (0)                        | 7, 8, 2, 4, 5, 2, 4                               | 0.454     | 0.478     | 0.015                                    |
| Cheyenne, Wyoming             | 17.0 (0)                        | 8, 8, 2, 4, 11, 2, 4                              | 0.521     | 0.537     | 0.015                                    |
| Torrington, Wyoming           | 20.0 (0)                        | 8, 9, 3, 3, 10, 2, 3                              | 0.535     | 0.549     | 0.011                                    |
| Wheatland, Wyoming            | 5.0 (0)                         | 6, 5, 2, 3, 5, 1, 3                               | 0.517     | 0.575     | 0.329*                                   |
| Carrizo Plains, California    | 22.0 (0)                        | 8, 7, 2, 3, 7, 2, 3                               | 0.512     | 0.524     | -0.029                                   |
| Lemoore, California           | 37.0 (0)                        | 10, 9, 3, 4, 10, 3, 4                             | 0.556     | 0.564     | 0.091*                                   |
| Salton Sea, California        | 18.9 (0.4)                      | 9, 9, 3, 3, 8, 1, 3                               | 0.489     | 0.502     | 0.014                                    |
| Grand View, Idaho             | 20.0 (0)                        | 9, 8, 3, 4, 9, 1, 4                               | 0.491     | 0.503     | 0.137                                    |
| Cedar City, Utah              | 5.0 (0)                         | 5, 6, 2, 2, 5, 2, 2                               | 0.469     | 0.521     | 0.253*                                   |
| Overall                       |                                 | 4.9 (2.8)   | 0.537     | 0.539     | 0.061**                                  |
| <i>A. c. floridana</i>        |                                 |   |           |           |  |
| Cape Coral, Florida           | 8.9 (0.9)                       | 4, 7, 1, 3, 5, 1, 3                               | 0.323     | 0.346     | -0.069                                   |
| Eglin Air Force Base, Florida | 6.4 (0.5)                       | 3, 3, 1, 3, 4, 3, 3                               | 0.321     | 0.346     | 0.279*                                   |
| Gilchrist County, Florida     | 4.4 (0.8)                       | 3, 4, 1, 2, 4, 1, 2                               | 0.271     | 0.302     | -0.157                                   |
| Keys, Florida                 | 6.0 (0)                         | 3, 4, 1, 3, 2, 1, 2                               | 0.300     | 0.327     | 0.137                                    |
| Miami, Florida                | 11.1 (1.2)                      | 3, 7, 1, 4, 4, 2, 3                               | 0.337     | 0.353     | 0.018                                    |
| Tampa, Florida                | 5.7 (0.5)                       | 3, 5, 1, 2, 3, 1, 1                               | 0.226     | 0.249     | 0.215                                    |
| Overall                       |                                 | 2.9 (1.5)   | 0.337     | 0.341     | 0.063                                    |
| Males                         |                                 |   | 0.537     | 0.542     | 0.106*                                   |
| Females                       |                                 |   | 0.526     | 0.530     | 0.067**                                  |

<sup>a</sup>Loci are BUOW1, BUOW4, BUOW6, BUOW7, BUOW11, BUOW13, and BUOW16, respectively.

<sup>b</sup>Asterisks indicate estimates significantly different from zero: \*  $P < 0.05$ , \*\*  $P < 0.01$ .



TABLE 3. Genetic differentiation among all populations, within subspecies, and by sex.

|                           | $\theta$ | 95% CI      | $\rho$ |
|---------------------------|----------|-------------|--------|
| <i>Athene cunicularia</i> | 0.051    | 0.033–0.066 | 0.014  |
| <i>A. c. hypugaea</i>     | 0.014    | 0.007–0.022 | 0.009  |
| <i>A. c. floridana</i>    | 0.038    | 0.003–0.055 | 0.014  |
| Males                     | 0.084    | 0.033–0.144 | 0.039  |
| Females                   | 0.098    | 0.045–0.151 | 0.015  |

Bayesian clustering analysis (Pritchard et al. 2000) indicated the existence of five clusters in the absence of prior information on the sampled population. Because clusters 4 and 5 had no members with majority assignment to either cluster and because the first three clusters provided reasonably clear assignment to the three major geographic regions of occurrence for North American Burrowing Owls, we present the results for the first three clusters, corresponding to the Florida region (FL), the California region (CA), and the Mountain West (MW; samples from Wyoming [WY], Utah [UT], and Idaho [ID]). Figure 2 provides a graphic representation of the strength of assignment of individuals to the three clusters.

To test for the effects of migratory habit on genetic structure, theta was also calculated for resident and migratory western populations separately. Theta for resident California populations was 0.021 (95% CI, 0.016–0.024) and was 0.012 (95% CI, 0.000–0.029) for migratory populations. Theta for resident Florida populations was 0.038 (95% CI, 0.003–0.055). Although resident populations in California and Florida showed slightly greater genetic structure than migratory populations, confidence intervals overlapped widely.

Pairwise genetic distance measures revealed a split between the western and Florida forms, but there was no obvious pattern within subspecies. In the western form, Nei's genetic distance was greatest between the Salton Sea, California, and Lemoore, California, populations (0.029); whereas Cavalli-Sforza distance was greatest between the Carrizo Plains, California, and Casper, Wyoming, populations (0.049) (Wheatland and Idaho were excluded from this test because of small sample sizes). In the Florida form, Nei's genetic distance was greatest between Miami and the Keys, and Cavalli-Sforza genetic distance was greatest between Tampa and the Keys (0.053 and 0.064,

respectively; Gilchrist County was omitted because of small sample size). Mantel tests using Cavalli-Sforza genetic distances showed no significant relationship between genetic and geographic distances within subspecies ( $P > 0.10$ ). Indeed, of 5,000 randomized matrices, 89% showed a better correlation between geographic and genetic distance than we observed.

When all 15 populations were considered, probability of correct assignment of an individual to its home population was low, averaging 19.3% and ranging from 0 to 31.6% for Utah and Salton Sea, respectively. However, at a broader scale (Florida vs. the west), genotypic differences provided relatively accurate assignment of individuals to the correct form (Fig. 3). More than 95% of western and >93% of Florida birds were correctly assigned by form. Misassigned individuals were from sundry populations and tended to be individuals with one or a few alleles that were rare in their home form but common in the other.

A neighbor-joining tree based on Cavalli-Sforza distances further supported a strong split between the western and Florida forms, but revealed very little structure within forms (Fig. 4). Within Florida, there was 100% bootstrap support for separation of populations into southern (Keys, Miami, and Cape Coral) and northern (Tampa and Eglin Air Force Base) clades. In contrast, western populations showed no geographically meaningful groupings, with each containing populations from different states.

To obtain estimates of relative effective population size, all populations were compared with Casper, which was arbitrarily selected as the reference population. An  $N_e$  estimate with 95% confidence interval overlapping 1 indicated an  $N_e$  comparable to the Casper reference. Florida populations had roughly one-third the effective population size of Casper, and all Florida  $N_e$  estimates were significantly <1 ( $N_e$  ranged from 0.24 [95% CI, 0.10–0.54] for Tampa to 0.39 [95% CI, 0.20–0.74] for Eglin Air Force Base). By contrast,  $N_e$  confidence intervals for all western populations except Idaho overlapped 1, indicating  $N_e$  values similar to the Casper population. The estimate for Idaho, the only population with a significantly larger  $N_e$  estimate than Casper, was 2.03 (95% CI, 1.05–3.92).

The bottleneck index,  $M$ , was 0.88 and 0.82 for the western and Florida forms, respectively. Both values were much greater than 0.68, indicating no evidence of a genetic bottleneck.

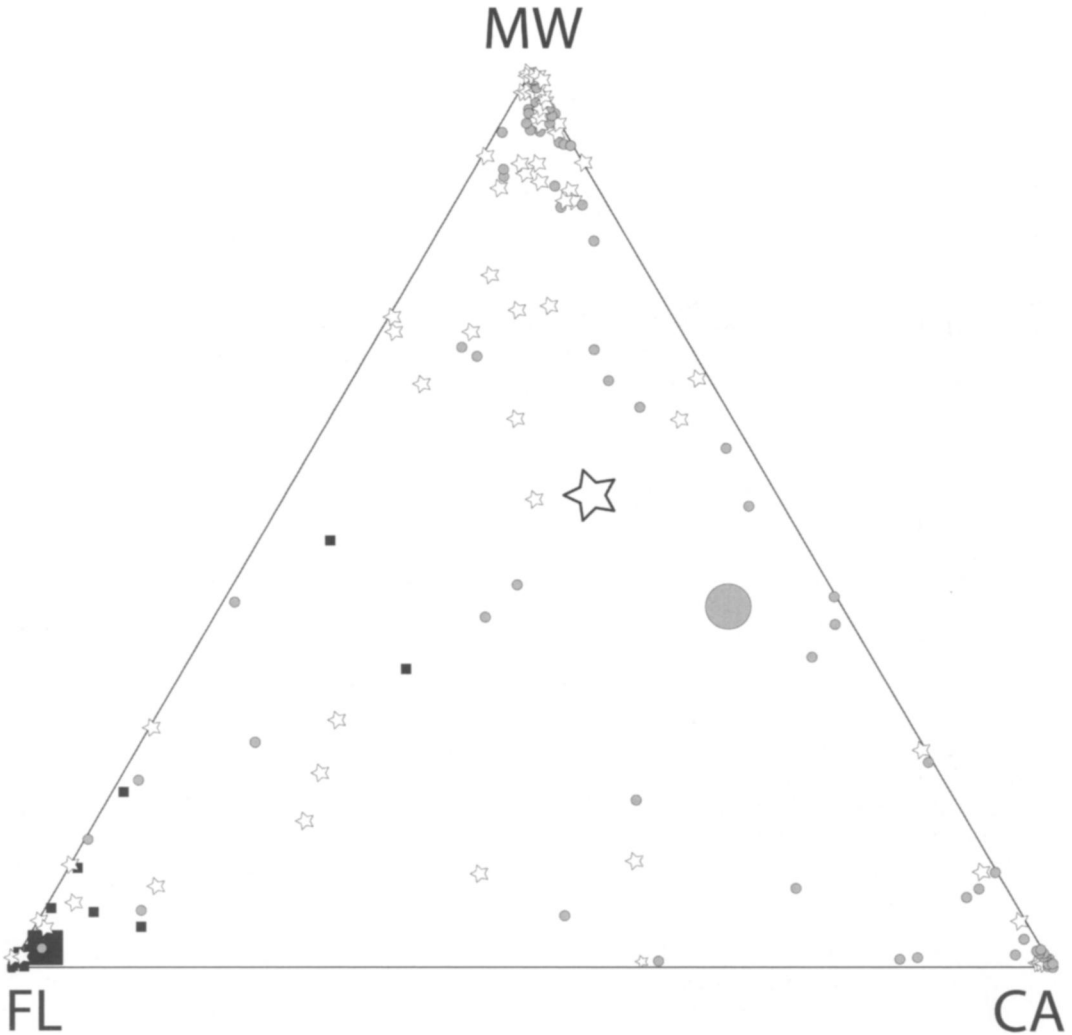


FIG. 2. Ternary diagram of cluster assignments from the Bayesian program STRUCTURE for three regions from which we sampled Burrowing Owls. Birds from Florida (FL) are indicated by black squares, birds from California (CA) are by gray circles, and birds from the Mountain West region (MW; samples from Idaho, Utah, and Wyoming) by stars. Larger symbols indicate the centroids for the respective populations. Labels at the three vertices represent the regional attribution for cluster assignments. Note that Florida birds (and their centroid) are closer to the Florida vertex than individuals from either of the two western regions are to their respective vertices. Each of the three Florida individuals most distant from the FL vertex had unusual genotypes. One had missing data at five of the eight loci, another had four alleles that were rare in FL and much more common in the west, and the third had two alleles that were rare in FL. The results suggest that Florida is much more distinct from the two western regions than the two western region are from each other.

Under assumptions of an infinite-alleles model (IAM), results from BOTTLENECK indicated no deviation from mutation-drift equilibrium with either the sign test for heterozygosity excess

or the two-tailed Wilcoxon test for heterozygote excess or deficiency in the western form ( $P = 0.313$ ;  $P = 0.375$ ) or the Florida form ( $P = 0.582$ ;  $P = 0.563$ ). Under the stepwise mutation

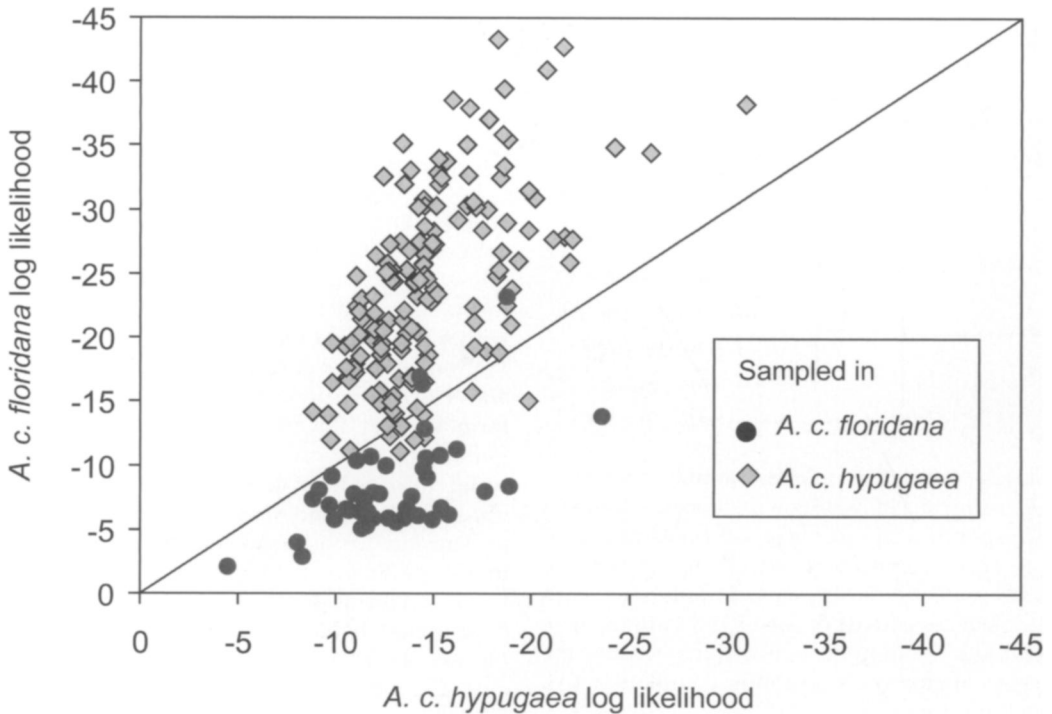


FIG. 3. Assignment test of individuals to subspecies by genotypic likelihood (Paetkau et al. 1995). An individual with a score above the diagonal line of equality has a genotype best assigned to *A. c. hypugaea*, whereas an individual below the diagonal has a genotype best assigned to *A. c. floridana*. Most individuals were correctly assignable subspecies. Only 5% (8 of 155) of *A. c. hypugaea* and 7% (3 of 46) of *A. c. floridana* individuals were misassigned. Note that all misassignments were relatively close to the line of equality.

model (SMM), results showed a heterozygote deficiency under the sign test and the two-tailed Wilcoxon test for both the western ( $P = 0.002$ ;  $P = 0.008$ ) and Florida ( $P = 0.052$ ;  $P = 0.031$ ) forms. Likewise, there was no evidence for genetic bottleneck within the 15 populations under either SMM or IAM assumptions.

Relatedness among males (0.142 [95% CI, 0.055–0.245]) did not differ significantly from that among females (0.169 [95% CI, 0.081–0.250]).  $F_{IS}$  estimates for males (0.106 [95% CI, 0.011–0.202]) and females (0.067 [95% CI, 0.027–0.113]) were significantly different from zero ( $P < 0.05$ ); but estimates were not significantly different from one another ( $P > 0.05$ ) (Table 2). Theta was not significantly ( $P > 0.05$ ) greater for females (0.098 [95% CI, 0.045–0.151]) than for males (0.084 [0.033–0.144]) (Table 3), given that 95% confidence intervals overlapped. Although  $F_{ST}$  (measured here by theta) can be a powerful

statistic for demonstrating sex-biased dispersal even with a biparentally inherited DNA marker, sex bias must be intense to be detected when there are <20 loci under consideration (Goudet et al. 2002). The seven loci assessed here may not have been sufficient to detect dispersal differences between sexes.

## DISCUSSION

### GENETIC DIFFERENTIATION BETWEEN WESTERN AND FLORIDA FORMS

Microsatellite DNA markers revealed substantial genetic differentiation between the western and Florida forms of Burrowing Owls, a result that is consistent with mitochondrial evidence from Desmond et al. (2001) showing 0.7% sequence divergence between the two forms. Allele frequency differences between the forms

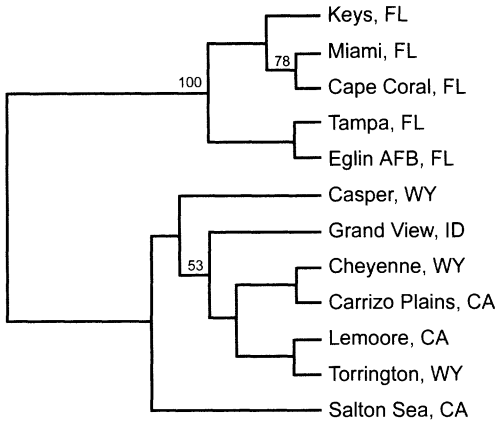


Fig. 4. Unrooted, neighbor-joining consensus tree using Cavalli-Sforza distances. Numbers correspond to percentage of bootstrap support (1,000 replicates), which are shown for nodes with >50% support. Populations with sample sizes of five or fewer individuals were excluded. Note that populations within the western region show an almost complete lack of geographic clustering.

were great enough to split the two unequivocally on a phylogenetic tree and to produce a high probability ( $\geq 93\%$ ) of assigning individuals to the correct form on the basis of genotype. By contrast, we found little or no structure within the large area of the western form.

#### GENETIC DIFFERENTIATION WITHIN FORMS

Contrary to our expectation, populations within subspecies were essentially panmictic, despite behaviors, such as semicolonial nesting and philopatry, that might be expected to limit dispersal. Overall patterns of genetic structure among populations were similar between the Florida and western forms, though the Florida form showed slightly greater differentiation among populations. Bayesian clustering analysis provided support for multiple clusters (substructure) in the absence of prior information on population origin. The first three clusters had a clearly interpretable geographic basis, with moderate separation between the mountain west and California and strong separation between those two clusters and the Florida form. The results corroborate those of the other analyses in suggesting that little structure is

evident in the western United States, but that Florida owls are reasonably distinct from western owls. Lack of structure within forms reveals a degree of population connectivity that was not previously appreciated from traditional mark-recapture studies.

Reduced genetic diversity ( $H_{exp}$ ) and low  $N_e$  within Florida probably resulted from genetic drift of a small founding population in Florida following a vicariant split from the western form. This pattern of lower genetic variation within the Florida form is similar to that seen in McDonald et al.'s (1999) comparison of Western and Florida scrub-jays, in which the Florida form had far fewer alleles than the western. Recent population declines or fluctuations could also reduce genetic diversity, but two different genetic bottleneck measures provided no support for a recent bottleneck hypothesis in Florida Burrowing Owls. In fact, Burrowing Owls in Florida have expanded recently from a restricted habitat in the prairies of central Florida to cleared sand habitats along the coasts, as well as to inland areas cleared for such land uses as airports and golf courses (Neill 1954, Ligon 1963, Courser 1970, Robertson and Woolfenden 1992). That pattern of recent expansion may result in more homogeneous populations that are relatively genetically depauperate overall. Lewis (1991), for example, found that a common, widespread but recently expanded *Polygonella* (*Polygonaceae*) found over much of North America was much less genetically variable than a rare scrub-endemic congener found only in Florida.

There are two alternatives for explaining the low differentiation among populations within each of the two forms—evolutionary disequilibrium or high historical gene flow. Our results strongly favor the high-gene-flow explanation. The first alternative, evolutionary disequilibrium, could result from insufficient time since colonization or possibly a population bottleneck. Nevertheless, recent colonization as a source of evolutionary disequilibrium can be ruled out, given a fossil record of the species distributed throughout North, Central, and South America and the West Indies since the late Pleistocene (Haug et al. 1993). Further, although evidence exists for recent population declines throughout much of the western Burrowing Owl range, bottleneck analyses indicated that population declines have not

been precipitous or recent enough to result in a detectable signal of bottlenecking. Finally, the recent range expansion in Florida and the lack of a genetic signature of bottlenecking there argue strongly against a bottleneck effect in the Florida subspecies.

The second alternative, high gene flow, seems a likely explanation for the observed lack of population genetic differentiation. The successful dispersal of one individual per generation between two populations is generally all that is required to prevent genetic differentiation (Slatkin 1987). It is clear that Burrowing Owls have the potential for long dispersal distances, as demonstrated by migratory habit and a few observed long-distance dispersal events (D. Rosenberg unpubl. data). A stable isotope study also suggested extensive movement of Burrowing Owls (Duxbury 2004). Long-distance dispersal events, despite the difficulty of observing them in the field (Koenig et al. 1996), may be sufficient to homogenize populations. Such dispersal may be relatively common; low band-return rates in traditional mark-recapture studies may actually represent dispersal rather than deaths. That interpretation is supported by low genetic differentiation, even between western populations separated by major geographic barriers or long distances. Gene flow among populations may be facilitated in other ways, as well. For instance, if migratory individuals occasionally breed in resident populations or form pair bonds with owls from other migratory populations on wintering grounds, that could be a major source of gene flow from other populations in western Burrowing Owls. Similarly, two populations may have no gene flow between them but may receive immigrants from the same source population, generating a low  $F_{ST}$  in the absence of direct gene flow (Whitlock and McCauley 1999).

#### EFFECTS OF MIGRATORY HABIT

Although differences between migratory and resident populations were slight, a pattern emerged of greater genetic differentiation among resident populations. When migratory and resident populations were analyzed separately, the 95% confidence interval for theta for migratory populations included zero, indicating no significant degree of genetic structure in the migratory component. By contrast, theta

estimates for resident California and Florida populations were significantly greater than zero ( $P < 0.05$ ) and were 2 $\times$  and 4 $\times$  greater, respectively, than the theta estimate for migratory populations; however, theta confidence intervals for migratory and resident populations overlapped, indicating a weak relationship. These results provide tentative evidence that lower dispersal distances observed in residents may be sufficient to generate genetic structure in this and other partially migratory species.

#### INBREEDING AND MANAGEMENT CONCERNS

In contrast to the minisatellite study of a Davis, California, population by Johnson (1997a), we found no evidence of significant homozygote excess. The Davis population, which went locally extinct three years after completion of the genetic sampling, may have been anomalous (Johnson 1997b). Small population size and limited gene flow with other populations likely caused the high homozygosity, which may also characterize other isolated, declining populations not represented in the present study. The problem of comparing the present study with Johnson's (1997a) minisatellite study is further compounded by choice of molecular marker, which might reveal different patterns of genetic structure given potential differences in mutation rates and other marker-specific factors.

Among the populations studied, high gene flow suffices to explain the minimal population structure. It is difficult, however, to discriminate between historical and contemporary gene flow using genetic data from a single time period. Although those data clearly suggest that high gene flow and connectivity have been historically characteristic of the populations studied here, very recent isolation of populations through habitat fragmentation and other means may not necessarily be detectable using genetic analyses. As Burrowing Owl habitat continues to be lost and fragmented, gene flow among populations may become limited, with the attendant consequences of increased exposure to genetic drift, demographic instability, and local inbreeding. That may explain the high homozygosity that preceded extinction in Johnson's Davis, California, population (Johnson 1997a, b). The high gene flow evident in populations studied here, however, provides tentative evidence that



the patchy nature of Burrowing Owl habitat (caused by discontinuous grassland habitat and the tendency for colonial nesting) does not, by itself, isolate populations. High dispersal rates may provide natural augmentation of declining populations and indicate, at least in theory, the ability of individuals to search for better habitat in the face of increasing human-caused habitat fragmentation.

Captive-rearing and reintroduction programs have been undertaken in Canada (Leupin and Low 2001) and Minnesota (Martell et al. 2001) and may become increasingly common as populations disappear at the edges of the Burrowing Owl range. Given the lack of observable genetic differentiation among populations, it may be tempting to translocate individuals among geographically distant populations. The relationship between genetic variation at non-coding DNA loci and selected fitness-related loci is, however, not well understood (Pearman 2001). Local adaptation can occur in the face of substantial gene flow among populations (e.g. Wade 1982, Sork et al. 1993), which warrants caution in moving individuals from disparate populations. Despite high potential gene flow among Burrowing Owl populations, selection at loci involved in migratory behavior or temperature tolerances, for example, cannot be ruled out. As a precautionary measure, Burrowing Owl translocations should occur within similar environments and migratory strategies. Given that provision, the potential genetic consequences of reintroduction may be minor while realizing the genetic benefits of maintenance of heterozygosity and allele number (Westemeier et al. 1998), as well as the obvious demographic benefit of augmenting declining populations.

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