PHYLOGEOGRAPHY OF THE SCALED QUAIL IN THE AMERICAN SOUTHWEST

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ABSTRACT.—We used sequences from the mitochondrial control region to examine the phylogeography and historical demography of the Scaled Quail and to determine whether the geographic distributions of mtDNA genealogy were concordant with the distribution of the subspecies. Overall, the Scaled Quail exhibited lower haplotype and nucleotide diversity than other quail species. The highest levels of haplotype diversity were found in 3 Texas counties: Dimmit, La Salle, and Hudspeth. The Scaled Quail exhibited no phylogeographic structure among its 16 haplotypes, and the patterns of genetic variation were not congruent with potential geographic barriers or current subspecies taxonomy. The geographic distribution of haplotypes and partitioning of molecular variation suggested slight genetic differentiation between the chestnut-bellied subspecies of Scaled Quail and the 3 western subspecies. The low levels of genetic diversity and the evidence of demographic expansion suggest that the Scaled Quail has undergone population and range expansion from a Pleistocene refugium. The greater genetic diversity within the Chestnut-bellied Quail may indicate the geographic location of the refugium.

RESUMEN.—En este estudio se utilizaron secuencias de mitocondrias de control regional para examinar la filogeografía y la demografía histórica de la codorniz escamosa para determinar si las distribuciones geográficas de la genealogía ADN mitocondrial eran concordantes con la distribución de las subespecies. En general, la codorniz escamosa mostró menores haplotipos y diversidad de nucleótidos que otras especies de codornices. Los niveles más altos de diversidad de haplotipos se encontraron en tres condados de Texas: Dimmit, La Salle, y Hudspeth. La codorniz escamosa no mostró estructura filo-geográfica entre sus 16 haplotipos y los patrones de variación genética no fueron congruentes con las posibles barreras geográficas o con la taxonomía de subespecies actuales. La distribución geográfica de los haplotipos y la partición de la variación molecular sugiere una ligera diferenciación genética entre las subespecies de codorniz escamosa de vientre castaño y las 3 subespecies occidentales. Los bajos niveles de diversidad genética y la evidencia de la expansión demográfica sugieren que la codorniz escamosa ha experimentado una expansión tanto de su población como de su rango que provienen de un refugio del Pleistoceno. La mayor diversidad genética en la codorniz escamosa de vientre castaño posiblemente indique que la distribución de esta subespecie sea la ubicación geográfica del refugio.

The Scaled Quail (*Callipepla squamata*) is a small gallinaceous bird (Odontophoridae) distributed across arid grasslands, brushlands, and desert scrub of northwestern Mexico and the southwestern United States (Fig. 1). Despite the species' restricted distribution, 4 subspecies have been described. *Callipepla s. pallida* has the widest distribution and occurs throughout most of the Chihuahuan Desert from southeastern Arizona to western Texas and northern Mexico (Sonora and Chihuahua). *Callipepla s. castanogastris* occurs in the Tamaulipan Brushlands of southern Texas, Nuevo Leon, and Tamaulipas; *C. s. squamata* occurs throughout the Mexican Central Plateau from southern Sonora to the Valley of Mexico; and *C. s. hargravei* comprises populations in the

Southern High Plains and Colorado Plateau (Dabbert et al. 2009). *Callipepla s. hargravei*, *C. s. pallida*, and *C. s. squamata* are designated on the basis of geography and subtle differences in the tone of bluish gray plumage, whereas *C. s. castanogastris* is distinguished from the other 3 subspecies by its chestnut or copper-colored belly.

Callipepla was formerly considered a monotypic genus. However, many ornithologists suggested a close relationship between the Scaled Quail and the 3 species formerly placed in the genus *Lophortyx*: Elegant Quail (*C. douglasii*), California Quail (*C. californica*), and Gambel's Quail (*C. gambelii*) (Holman 1961, Mayr and Short 1970, Hubbard 1973). Molecular studies based on allozymes (Gutiérrez et al. 1983) and

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Fig. 1. Historical (light gray) and contemporary (dark gray) geographic ranges of the Scaled Quail (*Callipepla squamata*) and its subspecies. Contemporary distribution is based on Ridgely et al. (2007), and historic distribution is based on data from Texas Game, Fish and Oyster Commission (1945), Ridgway and Friedmann (1946), Schemnitz (1959), Rea (1973), and Dabbert et al. (2009). Black dots represent localities from which contemporary specimens (hunter-harvested or trapped birds) were acquired or collection localities of museum specimens.

mitochondrial DNA (mtDNA) sequences (Zink and Blackwell 1998) confirmed the close relationship among these 4 species. Hubbard (1973) proposed that the Scaled Quail, Elegant Quail, and a pre-*californica-gambelii* lineage split from a common ancestor during the Illinoian Stage of the Pleistocene (300–130 thousand years ago) in response to climatic and ecological changes. Estimates of divergence times based on molecular data indicate that *Callipepla* species originated during the Late Pliocene and Early Pleistocene, 2.8–1.0 million years ago (Gutierrez et al. 1983, Zink and Blackwell 1998).

The climate of southwestern North America changed dramatically throughout the Late Quaternary in response to Milankovitch cycles of cool 100,000-year glacial periods interrupted by warmer 10,000-year interglacials (Graham 2011). Arid- and desert-adapted taxa of the American Southwest typically exhibit strong phylogeographic structure due to isolation in multiple refugia during cooler, wetter glacials. However, many species also show evidence of post-Pleistocene expansion in some parts of their respective ranges (Zink et al. 2001, Zink 2002, Riddle and Hafner 2006). If the Scaled

TABLE 1. Collection localities of hunter-harvested and trapped specimens of Scaled Quail (*Callipepla squamata*) from the United States used in this study.

State	County	Number of samples	Year collected
Arizona	Pima	7	1999
Colorado	Lincoln	13	2009
Texas	Brewster	17	2008–2009
	Culberson	13	2008–2009
	Dimmit	26	2008–2009
	Fisher	10	2008–2009
	Hudspeth	13	2008–2009
	Jeff Davis	12	2009–2010
	La Salle	7	2011
	Midland	8	2008–2009
	Reagan	12	2008–2009
	Upton	14	2008–2009
New Mexico	Dona Ana	15	2009–2010
	Luna	15	2009–2010
	Sierra	3	2009–2010
Oklahoma	Beaver	1	2010
	Texas	4	2010

Quail was isolated in multiple refugia, the species should exhibit strong phylogeographic structure. To the contrary, if the Scaled Quail was isolated in a single refugium, it should exhibit little or no phylogeographic structure. We used sequences from the mitochondrial control region to examine the phylogeography and historical demography of the Scaled Quail and to determine whether the geographic distributions of mtDNA genealogy were concordant with the distributions of the 4 subspecies.

METHODS

Sample Collection and Laboratory Methods

We obtained 182 contemporary samples of Scaled Quail from individuals that were either harvested or trapped in Texas, New Mexico, Arizona, and Colorado during 2008–2010 (Table 1). Extracted DNA was also obtained from 8 Scaled Quail trapped in 2004 at the Buenos Aires National Wildlife Refuge, Arizona. We supplemented the contemporary samples with tissues obtained from museum specimens of Scaled Quail, including toepads or feathers (Table 2). Toepad samples consisted of small ($3 \times 1 \times 1$ mm) pieces of skin cut from the ventral side of the foot via a sterile razor or scalpel blade (changed for each specimen; Mundy et al. 1997). Each skin or feather sample was placed in either a sterile 2.0-mL tube with a screw cap or a small plastic Ziploc® bag, and all were stored at room temperature. We

extracted genomic DNA by using the DNeasy® Blood & Tissue Kit and tissue protocol (Qiagen, Valencia, CA). Because contamination represents one of the most serious problems during work with DNA extracted from museum specimens (Mulligan 2005), we used an anti-contamination protocol developed from the recommendations of Hummel (2003), Pääbo et al. (2004), and Mulligan (2005); specific details may be found in Williford (2013).

The mitochondrial control region displays high levels of variability, making it a useful genetic marker for studies of recently diverged populations (Vigilant et al. 1991, Wenink et al. 1994). Primers L16755 (5' TAC GGC TTG AAA AGC CAT TG 3') (Nedbal et al. 1997) and OSU7713 (5' CCT GAC CGA ACC AGA GGC GC 3') (Van Den Bussche et al. 2003) were used to amplify ~500 base pairs (bp) of the highly variable 5' region of the control region from contemporary Scaled Quail samples. We designed a pair of internal primers, SQCR026–F (5' CTC AAC TAC GGG AAC AGC AT 3') and SQCR301–R (5' GGT GTC TTG TCT GTT GTG TAG G 3') for use in museum specimens, and these primers amplified a ~300 bp fragment from the most variable portion of the fragment produced by the L16755 and OSU7713 primer pair.

Polymerase chain reactions were conducted in 25-μL volumes that contained 1.5–3.0 μL DNA extract, 12.5 μL AmpliTaq® Gold PCR Master Mix (Applied Biosystems, Foster City, CA), 10 pmol of each primer, 1.0 μL bovine serum albumin ($2 \text{ mg} \cdot \text{mL}^{-1}$), and sufficient double-deionized water to reach the final volume. Amplification was performed using an ABI 2720 thermal cycler (Applied Biosystems) with the following protocol: initial denaturation at 94.0 °C for 10.0 min, 35 cycles of denaturation at 94.0 °C for 50.0 s, annealing 61 °C for 1.0 min, extension at 72.0 °C for 2.0 min, and a final extension of 72.0 °C for 30.0 min. All PCR products were electrophoresed on a 1% agarose gel that contained ethidium bromide and were viewed under UV light to verify successful amplification. Amplification products from successful reactions were purified by an enzymatic method (ExoSAP-IT™, USB Corporation, Wilmington, MD), and sequenced using the ABI BigDye Terminator v1.1 Cycle Sequencing Kit. Dye terminators were removed with the DyeEx 2.0 Spin Kit (Qiagen). Sequence reaction products were then loaded

TABLE 2. Museum specimens of Scaled Quail (*Callipepla squamata*) from Mexico and the United States used in this study. Specimens were obtained from the American Museum of Natural History (AMNH), the Texas Cooperative Wildlife Collection (TCWC), and the University of Colorado Museum (UCM).

Institution–catalog no.	Locality	Year collected
AMNH–343908	Saltillo, Coahuila, Mexico	1946
AMNH–353956	Chiricahua Mountains, Cochise County, Arizona, USA	1991
AMNH–353962	Santa Rita Mountains, Pinal County, Arizona, USA	1907
AMNH–353971	Duval County, Texas, USA ^a	1912
AMNH–472432	Silver City, Grant County, New Mexico, USA	1884
AMNH–472434	San Antonio, Bexar County, Texas, USA	1889
AMNH–67977	El Paso, El Paso County, Texas, USA	1891
AMNH–70058	Santa Fe, Santa Fe County, Texas, USA	1893
AMNH–751324	Benson, Cochise County, Texas, USA	1907
AMNH–751326	San Fernando, Tamaulipas, Mexico	1909
AMNH–80260	Marfa, Presidio County, Texas, USA	1887
AMNH–80261	Chinati Mountains, Presidio County, Texas, USA	1887
AMNH–80283	Nuevo Leon, Mexico, Mexico ^b	1889
AMNH–80286	Nuevo Leon, Mexico ^b	1889
AMNH–80295	Rio Grande City, Starr County, Texas, USA	1886
AMNH–80333	Rio Grande City, Starr County, Texas, USA	1880
AMNH–80369	Laredo, Webb County, Texas, USA	1895
AMNH–826440	Grenville, Union County, New Mexico, USA	1989
AMNH–91978	Rancho Baillon, Guanacevi, Durango, Mexico	1903
AMNH–91981	Rancho Baillon, Guanacevi, Durango, Mexico	1903
AMNH–91982	Rancho Baillon, Guanacevi, Durango, Mexico	1903
AMNH–91983	Rancho Baillon, Guanacevi, Durango, Mexico	1903
AMNH–91984	Rancho Baillon, Guanacevi, Durango, Mexico	1903
TCWC–10562	Falcon Dam, Zapata County, Texas, USA	1978
TCWC–10934	Uvalde County, Texas, USA ^a	1977
TCWC–10935	Uvalde County, Texas, USA ^a	1977
TCWC–11577	Pandale Crossing, Val Verde County, Texas, USA	1986
TCWC–11855	San Benito, Cameron County, Texas, USA	1939
TCWC–12982	Marathon, Brewster County, Texas, USA	1992
TCWC–2003	Encinal, La Salle County, Texas, USA	1939
TCWC–2004	Encinal, La Salle County, Texas, USA	1939
TCWC–4385	Springfield, Baca County, Colorado, USA	1948
TCWC–5184	Artesia Wells, La Salle County, Texas, USA	1951
UCM–7979	Hoehne, Las Animas County, Colorado, USA	1908
UCM–7981	Buffalo Creek, Jefferson County, Colorado, USA	1905
UCM–7983	Colorado Springs, El Paso County, Colorado, USA	1908
UCM–7984	Colorado Springs, El Paso County, Colorado, USA	1908

^aSpecimen tag provided only county-level collection data.

^bSpecimen tag provided only state-level collection data.

on an ABI Prism 3130 Genetic Analyzer (Applied Biosystems) for separation and detection.

DNA Analysis

Mitochondrial DNA sequences were edited in the computer program Sequencher 4.6 (Gene Codes, Ann Arbor, MI) and aligned using the program ClustalX (Larkin et al. 2007). Preliminary analyses showed that the longer sequences from contemporary samples and the data set from museum specimens all exhibited similar genetic structure and diversity. Therefore, we used the combined set of shorter control region sequences from contemporary and museum specimens for all subsequent phylogeographic and demographic analyses.

We pooled samples by county (USA) and municipality (Mexico) and treated these as populations. Then we grouped the populations by the range of individual subspecies. We used DnaSP version 5.10.01 (Librado and Rozas 2009) to compute the number of haplotypes (h), haplotype diversity (Hd), and nucleotide diversity (π) (Nei 1987) for each subspecies and each county and municipality. We also computed the haplotype and nucleotide diversity for historical samples (museum specimens collected 1880–1951, $n = 30$) and recent samples (museum specimens and hunter-harvested or trapped birds collected between 1977–2010, $n = 197$). We examined relationships among haplotypes by constructing a

minimum spanning tree in Arlequin version 3.5 (Excoffier and Lischer 2010). The computer program HapStar version 0.6 (Teacher and Griffiths 2011) was used to draw the minimum spanning tree; the tree was further altered in the program Inkscape version 0.48 (Inkscape, <http://www.inkscape.org>).

We used analysis of molecular variance (AMOVA) as implemented in Arlequin to determine how genetic variation was apportioned among subspecies (Excoffier et al. 1992). We evaluated 5 different arrangements. First, we performed an AMOVA with Scaled Quail populations arranged into 4 groups corresponding to the 4 subspecies. Additional AMOVAs involved arrangements of populations in which we tested each subspecies against a group containing the other 3 subspecies.

Historical events, such as demographic expansion or relative stability, produce distinctive patterns of DNA polymorphisms (Rogers and Harpending 1992). We evaluated the demographic history of the Scaled Quail by computing the observed pairwise nucleotide differences or mismatch distributions. A unimodal observed mismatch distribution is an indication of sudden demographic expansion, whereas bimodal or multimodal mismatch distributions imply demographic equilibrium or decline. We used DnaSP to compute the expected mismatch distribution under a model of constant population size (Watterson 1975, Slatkin and Hudson 1991, Rogers and Harpending 1992) and the observed mismatch distribution of the Scaled Quail. We examined the fit of the observed mismatch distribution to the expected mismatch distribution by using mean absolute error (MAE—Rogers et al. 1996) and the raggedness index (r —Harpending 1994).

We performed several neutrality tests to further assess the demographic history of the Scaled Quail, including Tajima's D (Tajima 1989), Fu and Li's D^* and F^* (Fu and Li 1993), and Fu's F_S (Fu 1997). Negative values of Tajima's D , Fu and Li's D^* and F^* , and Fu's F_S indicate demographic expansion, whereas positive values may result from demographic contractions, bottlenecks, or population subdivision (Simonsen et al. 1995, Fay and Wu 1999, Depaulis et al. 2003, Ramírez-Soriano et al. 2008). We tested the null hypothesis of selective neutrality by constructing confidence intervals of 95% for raggedness, Tajima's D , Fu

and Li's D^* and F^* , and Fu's F_S . Demographic expansion was supported when selective neutrality was rejected by significant small values of raggedness ($P < 0.05$) and significant negative values of Tajima's D ($P < 0.05$), Fu and Li's D^* ($P < 0.05$) and F^* ($P < 0.05$), and Fu's F_S ($P < 0.02$; Fu's F_S is considered significant only if $P < 0.02$ since the 5% significance level of F_S corresponds to the lower second percentile of the empirical distribution; Fu 1997). All neutrality tests were performed in DnaSP and confidence intervals were obtained with 1000 coalescent simulations in DNASP's coalescent simulator.

RESULTS

We sequenced 409 bp of the mitochondrial control region from 190 contemporary specimens and 267 bp from 37 of 80 museum specimens of Scaled Quail. All sequences of haplotypes were submitted to GenBank as accession numbers KC495544–KC495559. We observed no contamination in any of the extraction or PCR negative controls. Fourteen control region haplotypes occurred in the contemporary samples. Two singleton haplotypes (one found in Upton County, TX, and the other in Jeff Davis County, TX) were collapsed into haplotype A (the most common haplotype) when the sequences were truncated to 267 bp to align with sequences from museum specimens. Four additional haplotypes occurred in the museum specimens.

Overall, the Scaled Quail exhibited low levels of haplotype and nucleotide diversity ($Hd = 0.39$, $\pi = 0.002$). Most populations and the subspecies ranges of *C. s. pallida*, *C. s. hargravei*, and *C. s. squamata* displayed low values of Hd (0.08–0.333) and π (≤ 0.001). *Callipepla s. castanogastris* exhibited higher levels of genetic diversity ($Hd = 0.776$, $\pi = 0.005$; Table 3). Three localities in Texas (Dimmit, La Salle, and Hudspeth counties) exhibited diversity values similar to that of *C. s. castanogastris* ($Hd \geq 0.664$, $\pi \geq 0.003$). Dimmit County, Texas, had the greatest number of haplotypes; however, 27 hunter-harvested samples were collected from this county—a larger sample size than from any other locality.

The minimum spanning tree (Fig. 2) of the Scaled Quail formed a star-like arrangement, where most haplotypes were connected to their nearest neighbors by a single base substitution.

TABLE 3. Mitochondrial control region (267 bp) genetic diversity indices of the Scaled Quail (*Callipepla squamata*), its subspecies, and sampled populations. Shown are sample size (n), number of haplotypes (h), haplotype diversity (Hd , with SD), and nucleotide diversity (π , with SD).

Subspecies and localities	n	h	Hd (SD)	π (SD)
All sequences	227	16	0.388 (0.041)	0.0020 (0.0003)
<i>C. s. castanogastris</i>	49	10	0.776 (0.049)	0.0049 (0.0006)
Nuevo Leon ^a	2	2	1.000 (0.500)	0.0074 (0.0038)
San Fernando, Tamaulipas	1	1		
Bexar County, Texas	1	1		
Cameron County, Texas	1	1		
Dimmit County, Texas	27	8	0.795 (0.061)	0.0050 (0.0007)
Duval County, Texas	1	1		
La Salle County, Texas	10	4	0.711 (0.117)	0.0049 (0.0014)
Starr County, Texas	2	2	1.000 (0.050)	0.0038 (0.0019)
Uvalde County, Texas	2	1		
Webb County, Texas	1	1		
Zapata County, Texas	1	1		
<i>C. s. squamata</i>	6	2	0.333 (0.215)	0.0013 (0.0008)
Saltillo, Coahuila	1	1		
Guanacevi, Durango	5	2	0.400 (0.237)	0.0015 (0.0009)
<i>C. s. hargravei</i>	24	2	0.083 (0.075)	0.0003 (0.0003)
Baca County, Colorado	1	1		
El Paso County, Colorado	2	1		
Jefferson County, Colorado	1	1		
Las Animas County, Colorado	1	1		
Lincoln County, Colorado	13	2	0.154 (0.126)	0.0006 (0.0005)
Santa Fe County, New Mexico	1	1		
Union County, New Mexico	1	1		
Beaver County, Oklahoma	1	1		
Texas County, Oklahoma	4	1		
<i>C. s. pallida</i>	148	11	0.250 (0.047)	0.0012 (0.0003)
Cochise County, Arizona	1	1		
Pima County, Arizona	8	2	0.250 (0.180)	0.0009 (0.0007)
Pinal County, Arizona	1	1		
Dona Ana County, New Mexico	15	3	0.257 (0.142)	0.0010 (0.0006)
Grant County, New Mexico	1	1		
Luna County, New Mexico	15	4	0.371 (0.153)	0.0015 (0.0007)
Sierra County, New Mexico	3	1		
Brewster County, Texas	18	2	0.111 (0.096)	0.0004 (0.0004)
Culberson County, Texas	12	1		
El Paso County, Texas	1	1		
Fisher County, Texas	9	2	0.389 (0.164)	0.0016 (0.0006)
Hudspeth County, Texas	13	6	0.641 (0.150)	0.0035 (0.0011)
Jeff Davis County, Texas	12	2	0.167 (0.134)	0.0006 (0.0005)
Midland County, Texas	8	1		
Presidio County, Texas	2	1		
Reagan County, Texas	12	1		
Upton County, Texas	14	3	0.275 (0.148)	0.0011 (0.0006)
Val Verde County, Texas	1	1		

^aDNA was obtained from 2 museum specimens collected from Nuevo Leon, Mexico; however, the specimen tags provided no additional locality information.

Haplotype A, the most common haplotype, occurred in all subspecies' ranges (Fig. 3) and in 32 (84%) of 38 populations. Two haplotypes occurred in *C. s. squamata* and *C. s. hargravei*, neither of which was unique to the range of either subspecies (Fig. 3). In contrast, 11 haplotypes occurred in *C. s. pallida*, of which 6 were unique, and 5 haplotypes were unique to *C. s. castanogastris*. Eight haplotypes (C, E, I, J, L, M, N, P) were "private haplotypes,"

with each restricted to a single population. The greatest diversity of haplotypes occurred within the range of *C. s. castanogastris* (Fig. 3, Table 3).

Fewer haplotypes ($h = 7$) occurred in the historical samples than in the recent samples ($h = 13$). Four haplotypes (C, E, J, and P) were unique to the historical samples, whereas 8 (B, H, I, K, L, M, N, and O) were unique to recent samples. The historical samples exhibited

Fig. 2. Minimum spanning tree of 227 Scaled Quail (*Callipepla squamata*) mitochondrial control region haplotypes (267 bp). Each white circle represents a unique haplotype, with circle size proportional to each haplotype's overall frequency. Boldface letters (A, B, C, etc.) designate individual haplotypes, with the number of individuals (if $n > 1$) possessing that haplotype in parentheses after the letter. Additional text inside or next to each haplotype represents the states of Mexico and the United States in which each haplotype occurred, and numbers in parentheses represent the frequency of occurrence. The single solid black circle represents an inferred missing haplotype. State codes are as follows: AZ = Arizona, CO = Colorado, NM = New Mexico, OK = Oklahoma, TX = Texas, CL = Coahuila, DG = Durango, NL = Nuevo León, TM = Tamaulipas.

slightly higher levels of haplotype and nucleotide diversity ($Hd = 0.510$, $SD\ 0.109$; $\pi = 0.003$, $SD\ 0.0008$) than the recent samples ($Hd = 0.369$, $SD\ 0.044$; $\pi = 0.002$, $SD\ 0.0003$).

The AMOVA results demonstrated that most genetic variation occurred within populations (80%–94%) regardless of how populations were grouped (Table 4). Among-group variation was consistently low. In terms of among-group differences, the arrangement of *C. s. castanogastri* versus a group composed of *C. s. pallida*, *C. s. squamata*, and *C. s. hargravei* (hereafter referred to as the “western group”) accounted for the greatest variation (12%, $P < 0.001$).

The observed mismatch distribution (Fig. 4) failed to reject the null hypothesis of constant population size, as the observed and expected distributions were nearly identical. The mean absolute error, however, was relatively large ($MAE = 0.128$), as was raggedness, but r was statistically insignificant ($r =$

0.154 , $P > 0.05$). Preliminary analysis showed that the observed mismatch distribution was also concordant with the expected distributions under models of sudden population expansion (Schneider and Excoffier 1999) and populations of fluctuating size (i.e., undergoing growth or decline; Rogers and Harpending 1992). The null hypothesis of demographic equilibrium was rejected by all neutrality tests, as Tajima's D ($D = -1.845$, $P < 0.001$), Fu and Li's D^* ($D^* = -2.590$, $P < 0.05$) and F^* ($F^* = -2.773$, $P = 0.05$), and Fu's F_S ($F_S = -16.838$, $P < 0.001$) were negative and statistically different from 0.

DISCUSSION

Many nonavian vertebrates of southwestern North America possess highly divergent lineages that predate the Last Glacial Maximum (26,500–19,000 years ago). These taxa often display strong phylogeographic structure

Fig. 3. Geographic distribution of 16 Scaled Quail (*Callipepla squamata*) control region haplotypes among 4 subspecies, 227 individuals, and 38 localities. Pie chart size reflects sample size, and slice size reflects the frequency of the corresponding haplotype.

associated with multiple Pleistocene refugia or ecogeographic barriers (Riddle and Hafner 2006, Pyron and Burbrink 2010). Scaled Quail are ground-dwelling birds and have a life history similar to rodents (i.e., largely sedentary, high reproductive rates, high mortality). Most species of small mammals in the North American arid regions exhibit deep divergence, a high degree of population structure, and deep phylogeographic breaks (Riddle et al. 2000, Neiswenter and Riddle 2010, 2011, Anderson and Light 2012). In contrast, we observed a lack of phylogeographic structure in the range of the Scaled Quail and found no empirical evidence of long-term historical isolation of populations in separate refugia. One explanation

may be that the Scaled Quail has colonized much of its current range relatively recently, coincident with the expansion of grasslands (Axelrod 1985, Hoyt 2000) and desert thornscrub (Metcalf 2006, Graham 2011) at the end of the Last Glacial Maximum. The Scaled Quail's distribution also has probably waxed and waned as grasslands and thornscrub habitats expanded and contracted due to Holocene climate fluctuations. Finally, the Scaled Quail may never have crossed any geographical barriers that would have reduced gene flow, or if such an event did occur, the populations did not persist.

Star-like haplotype networks or star phylogenies are characterized by an ancestral, common

Fig. 4. Observed (dashed line) and expected (solid line) mismatch distributions based on 227 Scaled Quail (*Callipepla squamata*) control region sequences (267 bp). The x-axis is the number of pairwise differences (mismatches) and the y-axis represents the frequency of differences.

TABLE 4. Analysis of molecular variance results for sampled Scaled Quail (*Callipepla squamata*) based on different groupings of populations. Shown are the source of variation, degrees of freedom (df), sum of squares (SS), variance of components (φ), percentage of variance (%), fixation index (Φ), and P value indicating the significance (95% confidence intervals) of the covariance of φ and Φ .

Source of variation	df	SS	φ	%	Φ	P
Populations grouped as 4 subspecies						
Among groups	3	3.421	0.016	5.99	$\Phi_{CT} = 0.060$	0.0061
Among populations within groups	36	13.338	0.027	10.00	$\Phi_{SC} = 0.106$	0.0259
Within populations	187	42.958	0.230	84.01	$\Phi_{ST} = 0.160$	0.0027
Total	226	59.718	0.273			
Combined <i>C. s. pallida</i> – <i>C. s. hargravei</i> – <i>C. s. squamata</i>						
Among groups	1	3.176	0.033	11.57	$\Phi_{CT} = 0.116$	0.0003
Among populations within groups	38	13.584	0.024	8.51	$\Phi_{SC} = 0.096$	0.0610
Within populations	187	42.958	0.230	79.92	$\Phi_{ST} = 0.201$	0.0032
Total	226	59.718	0.287			
Combined <i>C. s. pallida</i> – <i>C. s. squamata</i> – <i>C. s. castanogastris</i>						
Among groups	1	0.412	–0.003	–1.10	$\Phi_{CT} = -0.011$	0.4223
Among populations within groups	38	16.348	0.037	14.02	$\Phi_{SC} = 0.139$	0.0006
Within populations	187	42.958	0.230	87.08	$\Phi_{ST} = 0.129$	0.0034
Total	226	59.718	0.264			
Combined <i>C. s. squamata</i> – <i>C. s. hargravei</i> – <i>C. s. castanogastris</i>						
Among groups	1	1.258	0.006	2.13	$\Phi_{CT} = 0.021$	0.0260
Among populations within groups	38	15.501	0.034	12.50	$\Phi_{SC} = 0.128$	0.0596
Within populations	187	42.958	0.230	85.38	$\Phi_{ST} = 0.146$	0.0023
Total	226	59.718	0.269			
Combined <i>C. s. pallida</i> – <i>C. s. hargravei</i> – <i>C. s. castanogastris</i>						
Among groups	1	0.125	–0.024	–9.73	$\Phi_{CT} = -0.097$	0.7796
Among populations within groups	38	16.635	0.038	15.46	$\Phi_{SC} = 0.141$	0.0026
Within populations	187	42.958	0.230	94.27	$\Phi_{ST} = 0.057$	0.0031
Total	226	59.718	0.244			

haplotype at the center of the star, with descendant haplotypes connected to it by short branches (Slatkin and Hudson 1991). This type

of phylogeographic pattern usually results from recent, rapid demographic expansion (Slatkin and Hudson 1991). The star phylogeny and

shallow phylogeographic structure (as seen from AMOVA results) of the Scaled Quail are evidence for past demographic expansion from a compact refugium. This conclusion was supported by negative, statistically significant values of Tajima's D , Fu and Li's D^* and F^* , and Fu's F_S . The mismatch analysis proved uninformative probably due to the overall low variability of the control region sequences. Significant values of all neutrality tests are evidence against background selection. Genetic hitchhiking can also produce negative values of Fu's F_S and Tajima's D (Fu 1997). Genetic hitchhiking occurs when haplotypes of a specific gene become widespread in a population due to that gene's coincidental association with another locus that is under selection (Maynard Smith and Haigh 1974). Significant negative values of Fu and Li's D^* and F^* are evidence against genetic hitchhiking because these statistics are far less sensitive to genetic hitchhiking than Fu's F_S (Fu 1997). Therefore, we concluded that expansion from a single refugium, perhaps during the late Pleistocene or Holocene, best explained the phylogeographic structure of the Scaled Quail.

Late Quaternary glacial cycles and geographic barriers have had different effects on the vertebrate taxa of southwestern North America, where some species exhibit evidence of recent expansion from a single refugium (Zink et al. 2001, Zink 2002, Douglas et al. 2006, Riddle and Hafner 2006). The Scaled Quail is considered an aridland specialist (Silvy et al. 2007, Dabbert et al. 2009); therefore, its historical distribution has probably fluctuated repeatedly as periodic climatic changes resulted in expansion or contraction of preferred habitats. The climate of southwestern North America during the Last Glacial Maximum may have been as much as 5 °C cooler than today, with greater winter precipitation (Menking et al. 2004, Asmerom et al. 2010). Pollen and plant remains from woodrat (*Neotoma* spp.) middens indicate that woodlands of pine (*Pinus* spp.), juniper (*Juniperus* spp.), and oak (*Quercus* spp.) were widespread at lower elevations of the Chihuahuan Desert at this time (Wells 1966, Van Devender and Spaulding 1979, Metcalfe et al. 2002, Holmgren et al. 2007). Scaled Quail often occur in habitats with a high percentage of bare ground, and they tend to avoid areas of dense shrublands and grasslands (Schemnitz 1964, Wilson and

Crawford 1987, Saiwana et al. 1998, Joseph et al. 2003). This type of habitat may not have been widely distributed in southwestern North America until the onset of warmer, drier conditions in the region 15,000 years ago (Metcalfe et al. 2002, Holmgren et al. 2006, Pigati et al. 2009).

The distribution of haplotypes can provide qualitative insights into the location of Pleistocene refugia (Waltari et al. 2007, Provan and Bennett 2008, Wilson and Pitts 2012). The highest haplotype and nucleotide diversity occurred within the range of *castanogastris*, which encompasses most of the Tamaulipan Brushlands. This high diversity may indicate that the Scaled Quail was isolated in this region during part of the Pleistocene and that the rest of the Scaled Quail range resulted from recent colonization. Lower genetic diversity is expected at the periphery of the expansion due to the loss of haplotypes through a series of founder events and local bottlenecks (Austerlitz et al. 1997). Little is known about the Pleistocene environments of southern Texas due to poor preservation of Quaternary pollen and plant macrofossils (Bryant and Holloway 1985) and limited attempts at recovery of such data (Presley 2003). Modern plant communities were present in southern Texas by the Late Holocene (~6000 years ago), but similar vegetation may have been present in southern Texas as early as the Late Pleistocene (Bryant and Holloway 1985).

Most of the phylogeographic signal in the data is probably the result of demographic events over many thousands of years. However, Scaled Quail have undergone additional range contractions and population declines during the past 40 years (Church et al. 1993). Population declines have been attributed to the combined effects of overgrazing, invasion of exotic grasses, and brush encroachment (Rollins 2000, Engel-Wilson and Kuvlesky 2002, Brennan and Kuvlesky 2005). Nonetheless, recent declines do not seem to have greatly affected the mtDNA diversity of the Scaled Quail. Historical (1880–1951) and contemporary (1977–2011) groups share many of the same haplotypes, and the contemporary group possesses more unique haplotypes than the historical group. The greater number of unique haplotypes in the contemporary group was probably due to the group's larger sample size. The higher haplotype and nucleotide

diversity observed in the historical group was due to the group's higher relative frequencies of the haplotypes.

Genetic diversity in Scaled Quail was exceptionally low in comparison with its close relatives, Northern Bobwhite and Gambel's Quail. Eo et al. (2010) found 41 control region haplotypes and high levels of haplotype ($Hd = 0.89$) and nucleotide diversity ($\pi = 0.0034$) among bobwhites in the southeastern United States. A more geographically restricted study of Northern Bobwhites in southern Texas also showed higher levels of genetic diversity ($h = 42$, $Hd = 0.88$, $\pi = 0.008$; Wehland 2006). A rangewide study of the Gambel's Quail revealed a greater number of haplotypes ($n = 36$) and higher haplotype ($Hd = 0.79$) and nucleotide diversity ($\pi = 0.009$; Williford 2013). The low genetic diversity observed in the Scaled Quail may be due to either a lower long-term effective population size or insufficient geographic sampling in the Mexican portion of the Scaled Quail's range. Additional specimens from Mexico are needed to determine whether low genetic diversity is a characteristic of the Scaled Quail throughout its range.

Translocations and interbreeding among different populations represent a potential pitfall of phylogeographic studies of game species such as the Scaled Quail. Managers have repeatedly attempted to bolster local populations of Scaled Quail with translocated stock; however, most introductions of Scaled Quail within and outside of the species range have failed (Casto 2006, Dabbert et al. 2009). Infrequent occurrence of specimens of Scaled Quail with chestnut bellies in Arizona outside of the range of *C. s. castanogastris* has led some authorities to suggest that at least a few introductions have been successful (Brown 1989). If reproductively and ecologically compatible, translocated stock can increase local and regional genetic diversity (Ciborowski et al. 2007, Johnson et al. 2010, Herber et al. 2012). But translocations may also reduce diversity due to strong introgression of genes from introduced populations into native ones, founder effects and subsequent inbreeding depression in isolated populations, or outbreeding depression due to hybridization of ecologically incompatible stocks (Rhymer and Simberloff 1996, Avise 2004, Edmands 2007). The overall low levels of haplotype and nucleotide

diversity observed in the Scaled Quail could be viewed as evidence that successful translocations may have reduced genetic diversity in some parts of the species' range. Although Late Quaternary expansion is overall a more satisfactory explanation, we cannot exclude the possibility that translocation and restocking have also impacted the Scaled Quail's phylogeographic structure.

The lack of congruence between patterns of mtDNA variation and taxonomic designations of Scaled Quail subspecies brings into question the validity of retaining these currently recognized taxa. Incongruence between mtDNA variation and subspecies designations in birds is probably because many subspecies described in the early 20th century were based on one or a few arbitrarily chosen characters (Zink 2004). However, conflicts between subspecies taxonomy and mtDNA phylogeography may be the result of faulty delineation of subspecies boundaries, as most subspecies and populations have not been critically examined (Remsen 2010, Winker 2010). The largest study of geographic variation of plumage coloration and morphology within the Scaled Quail focused on the northwestern portion of the range (Rea 1973). Furthermore, discordance among mtDNA phylogeography and subspecies taxonomy is sometimes due to the different rates of evolution among mtDNA and nuclear genes that control phenotypic traits (Edwards et al. 2005, Mumme et al. 2006, Pérez-Emán et al. 2010). Many of the morphological traits may be of relatively recent origin; therefore, neutral markers may not reflect the divergence of and variation in adaptive loci of phenotypically diagnosable subspecies (Remsen 2010). A single gene will rarely capture the full evolutionary history of a species (Remsen 2010).

Analysis of molecular variance showed that approximately 12% of the genetic variation in the Scaled Quail was due to the differentiation between the western subspecies and *C. s. castanogastris*. The *castanogastris* subspecies is easily distinguished from the other subspecies by the chestnut patch on the belly, a discrete trait that might have a genetic basis. Historically, the geographic ranges of *pallida* and *castanogastris* were known to overlap in the Texas counties of Kinney, Uvalde, Real, and Bandera (Texas Game, Fish and Oyster Commission 1945). Anecdotal observations suggest

that *C. s. castanogastris* may be ecologically segregated from *C. s. pallida*, because *pallida* is most abundant in open grasslands with little (10%–15%) shrub cover, while *castanogastris* uses thorn-scrub vegetation (Silvy et al. 2007). If *pallida* and *castanogastris* are adapted to different habitats, then gene flow may be reduced or absent between these 2 subspecies. Comparative ecological studies have not been conducted in Kinney County, where these 2 subspecies may still coexist.

A rangewide study of geographic variation of phenotypic traits is needed to determine the diagnosability of the 4 subspecies of the Scaled Quail. Microsatellite data combined with comparative natural history studies could help to determine the level of ecological and genetic differentiation between *C. s. castanogastris* and western subspecies of the Scaled Quail. Additional sampling of the Mexican portion of the range would also help to further refine the phylogeographic and demographic history of the Scaled Quail.

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