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PHYLOGEOGRAPHY OF THE GAMBEL'S QUAIL (*CALLIPEPLA GAMBELII*) OF WESTERN NORTH AMERICA

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ABSTRACT.—We conducted a phylogeographic study of the Gambel's Quail (*Callipepla gambelii*) using sequences of the mitochondrial control region and NADH dehydrogenase subunit 2 (ND2) obtained from 167 specimens including hunter–harvested wings and museum study skins. The Gambel's Quail exhibited strong phylogeographic structure, large genetic gaps, and relatively high levels of haplotype ($Hd = 0.79$) and nucleotide diversity ($\pi = 0.01$). Thirty-four Gambel's Quail haplotypes clustered into two distinct haplogroups, with two additional highly divergent haplotypes. Distribution of the haplogroups was not concordant with sampled subspecies or ecogeographic regions; however, the overall distribution of the two haplogroups suggests that the Gambel's Quail may have been isolated in separate refugia of the Chihuahuan and Sonoran deserts during the Pleistocene. Both haplogroups appear to have undergone recent demographic expansion, possibly related to climatic changes associated with the onset of drier conditions in southwestern North America following the end of the Last Glacial Maximum. *Received 17 July 2013. Accepted 7 December 2013.*

Key words: *Callipepla gambelii*, Gambel's Quail, mitochondrial DNA, phylogeography, population structure.

Gambel's Quail (*Callipepla gambelii*) is a common resident of brushland and thorn-scrub habitats of southwestern North America. The geographic range of Gambel's Quail is centered in the Sonoran Desert and extends to adjacent parts of the Mojave and Chihuahuan deserts and southwestern California and Baja California (Brown et al. 1998, Fig. 1). The California Quail (*C. californica*) is regarded as a sister species of the Gambel's Quail, and both species have highly similar plumages. Gambel's and California quails are part of the crested quail complex that also includes the Elegant Quail (*C. douglasii*) and Scaled Quail (*C. squamata*).

The seven subspecies of Gambel's Quail were described based on slight variations of male plumage coloration, differences in body size, and geography. However, the taxonomic validity of some subspecies has been questioned (Hellmayr and Conover 1942, Ridgway and Friedmann 1946, Pitelka 1948, Madge and McGowan 2002). *Callipepla g. gambelii* has the widest range, occurring throughout the Sonoran Desert and adjacent areas, whereas the other subspecies have more limited ranges. *C. g. ignoscens* occurs in southeastern New Mexico and western Texas (Chihuahuan Desert), while the ranges of three other subspecies (*fulvipectus*, *friedmanni*, and *stephensi*) are restricted to parts of Sonora. *C. g. pembertoni* is found only on Tiburón Island in the Gulf of California off the coast of Sonora. The allopatric *C. g. sana* is endemic to southwestern Colorado.

The taxonomy and biogeography of the Gambel's Quail has received little recent attention, and genetic studies of this species are limited. Gambel's, California, and Elegant quails were formerly placed in the genus *Lophortyx*, but were considered to be the closest living relatives of the Scaled Quail (Holman 1961). Electrophoretic data (Gutíerrez et al. 1983) and mitochondrial DNA (mtDNA) sequences (Zink and Blackwell 1998) confirmed that the Gambel's, California, Elegant, and Scaled quails were more closely related to one another than to other New World quails. All four *Callipepla* species are thought to have diverged during the late Cenozoic (Hubbard 1973, Gutíerrez et al. 1983, Zink et al. 1987, Zink and Blackwell 1998).

A species that is characterized by low dispersal (<2 km), short–life span, and high population turnover such as the Gambel's Quail (Brown et al. 1998) should display high degree of phylogenetic structure throughout its range (Harrison and Hastings 1996, Avise 2000). Therefore, one major prediction would be that patterns of mtDNA variation in Gambel's Quail should show consid-

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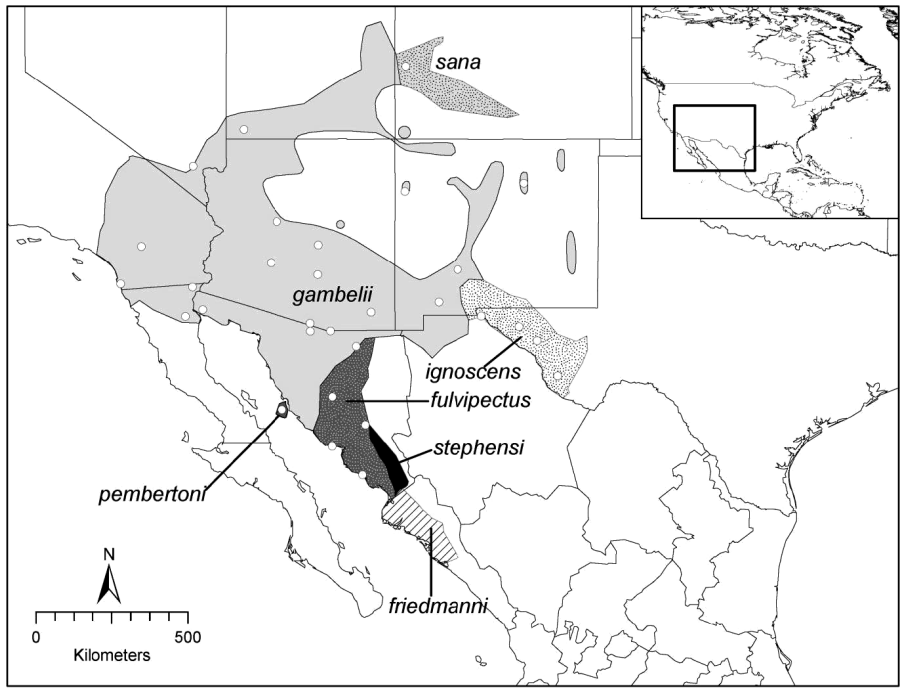

FIG. 1. Geographic distribution of the Gambel's Quail and its subspecies. Geographic distributions of each subspecies were based on modified ARCMAP shapefile of the range of the Gambel's Quail provided by Ridgely (2007). Subspecies boundaries were determined by data from Texas Game, Fish and Oyster Commission (1945), Ridgway and Friedmann (1946), Johnsgard (1988), and Madge and McGowan (2002).

erable phylogeographic structure. Our specific objectives were to: 1) test for patterns of phylogeographic structure throughout the range of the Gambel's Quail, 2) explore its demographic history, and 3) assess the genetic distinctiveness of the various subspecies of the Gambel's Quail.

METHODS

Sample Collection.—Between 2008–2010, we obtained contemporary samples (wings or feathers) of hunter–harvested Gambel's Quail from Arizona (Gila County, $n = 11$; Pinal County, $n = 39$), Utah (Washington County, $n = 17$), and New Mexico (Luna County, $n = 7$; Sierra County, $n = 2$). We also included contemporary samples of Gambel's Quail from Texas (Hudspeth County, $n = 19$; Presidio County, $n = 18$; Culberson County, $n = 9$) that had been collected 2002–2004 by Sullins (2006), and DNA extracted from blood from Gambel's Quail trapped at the Buenos Aires National Wildlife Refuge, Arizona ($n = 7$) in 2004. We supplemented the contemporary samples with tissues (toepad skin or feathers) obtained from museum specimens (online Supplemental Materials). Toepad samples consisted of small ($3 \times 1 \times 1$ mm) pieces of skin excised from the ventral side of the foot using a sterile razor or scalpel blade (changed for each specimen) (Mundy et al. 1997). Each sample was placed in either a sterile, 2.0–mL tube with a screw cap or a small Ziploc bag and stored at room temperature. We obtained tissue samples of California Quail, including hunter–harvested birds from Lake County, Oregon ($n = 6$) and Franklin ($n = 8$), Okanagan ($n = 8$), Walla Walla ($n = 5$), Yakima ($n = 8$) counties, and museum specimens (online Supplemental Materials), for use as outgroups and to detect hybridization where the ranges of California and Gambel's quails overlap. We also acquired tissue samples of three additional species of New World quails for use as outgroups in phylogenetic analysis, including a museum specimen of Elegant Quail (online Supplemental Materials) and hunter–harvested specimens of a Scaled Quail from Culberson County, Texas and a Northern Bobwhite (*Colinus virginianus*) from Goliad County, Texas.

Extraction.—Contamination represents a serious problem when working with DNA extracted

from museum specimens (Mulligan 2005). We developed an anti–contamination protocol based on guidelines from Hummel (2003), Pääbo et al. (2004), and Mulligan (2005). All DNA extractions from museum specimens and pre–amplification set–up procedures for those samples were carried out in a separate laboratory where no previous work on contemporary galliforms had been conducted. Separate equipment and supplies were used for museum and contemporary samples. Equipment and benchtops were sterilized with 20% bleach solution or DNA Away (Molecular BioProducts, San Diego, California) prior to use. Small equipment and supplies were sterilized with ultraviolet light for 1 hr. We adopted a ''one-way traffic'' protocol that prevented entry into the museum–specimen laboratory on the same day after conducting work in the ''contemporary'' DNA lab. We extracted DNA from ≤10 museum specimen samples at a time to minimize the risk of mistakes. We used 1–2 negative extraction controls for each round of extraction, and 1–3 negative controls each round of amplification, with polymerase chain reaction (PCR).

We extracted genomic DNA using the DNeasy® Blood & Tissue Kit and tissue protocol (Qiagen, Valencia, California). We altered the Tissue Protocol to maximize the amount of DNA obtained from historical samples. We increased the amount of proteinase K added to each sample from 20 μL to 30 μL. To increase the yield of DNA, we conducted the final elution step using a solution of diluted elution buffer (1 part buffer, 2 parts double–deionized water) to obtain a volume of 150 μL. Final elutions were then vacuum–centrifuged at 30 °C to a final volume of 50 μL to increase DNA concentration.

Amplification and Sequencing.—Because of its small effective population size and fast coalescence time, mtDNA is the leading indicator of intraspecific lineage divergence (Avise 2000, Zink and Barrowclough 2008, Barrowclough and Zink 2009). The mitochondrial control region (Vigilant et al. 1991, Wenink et al. 1994) and NADH dehydrogenase subunit 2 (ND2; Hackett 1996, Omland et al. 1999) display high levels of variability, making both useful as genetic markers for studies of recently diverged populations. Primers L16755 (5′ TAC GGC TTG AAA AGC CAT TG 3′; Nedbal et al. 1997) and OSU7713 (5′ CCT GAC CGA GGA ACC AGA GGC GC 3′; Van Den Bussche et al. 2003) were used to amplify 500 bp of the highly variable 5′ region I of the control region. However, these primers performed poorly on tissue samples from museum study skins. Therefore, we designed a pair of internal primers, NBW222F (5′ CTA AGC CCA TTG TAT GTA CAC GGA 3′) and NBW222R (5′ GGG TAC GAC CAA TAA ATC CAT CTG 3′). These primers were originally designed for museum specimens of bobwhites (*Colinus* spp.), but performed equally well on Gambel's, California, and Elegant quails. We used these internal primers to amplify a ~224-bp fragment of the most highly variable portion of the segment amplified by L16755 and OSU7713. Primers H5578 (5′ CCT TGA AGC ACT TCT GGG AAT CAG A 3′) and L5215 (5′ TAT CGG GCC CAT ACC CCG AAA AT 3′; Hackett 1996) were used to amplify 360 bp of the ND2 gene of all specimens.

Polymerase chain reactions were conducted in 25–μL reaction volumes containing 1.5–3.0 μL of total genomic DNA, 12.5 μL of AmpliTaq® Gold PCR Master Mix (Applied Biosystems, Forest City, California), 10.0 pmol of each primer, 1.0 μL of bovine serum albumin (2 mg/mL), 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 sec, annealing at 61 °C for 1.0 min, extension at 72.0 °C for 2.0 min, and a final extension at 72.0 °C for 30.0 min.

All PCR products were electrophoresed on a 1% agarose gel containing ethidium bromide and viewed under UV light to verify successful amplification. Amplification products were purified by an enzymatic method (ExoSAP–IT™, USB Corporation, and Wilmington, Maryland) and sequenced using the ABI BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems). Dye terminators were removed with the DyeEx 2.0 Spin Kit and protocol (Qiagen). Sequence reaction products were loaded on an ABI Prism 3130 Genetic Analyzer (Applied Biosystems) for separation and detection. Mitochondrial DNA sequences were edited in SEQUENCHER Version 4.6 (GeneCodes, Ann Arbor, Michigan), and aligned using CLUSTAL X Version 2.1 (Larkin et al. 2007). We trimmed control region sequences from contemporary specimens to the same length as those from museum specimens. Control region and ND2 sequences were concatenated using the program SEQUENCEMATRIX 1.7.8 (Vaidya

et al. 2011). Mitochondrial DNA sequences of Gambel's and California quail and outgroup taxa were deposited in GenBank as accession numbers KC556212–KC556268, KC556326–KC556486, KC556166, KC556168, KC556741 (control region); and KC556269–KC556325, KC556576–KC556736, KC556191, KC556204, and KC556747 (ND2).

Data Analysis.—We grouped samples from Gambel's Quail by county (United States) or municipality (Mexico). Samples were then subdivided into subspecies based on geographic data and the range of each subspecies (Ridgway and Friedmann 1946, Brown et al. 1998, Madge and McGowan 2002). We computed haplotype diversity (*Hd*) and nucleotide diversity (π, Nei 1987) for each subspecies and states from which specimens were collected with the computer program DNASP Version 5.10.01 (Librado and Rozas 2009). We also computed haplotype and nucleotide diversity for museum and contemporary samples and for any distinct groups of haplotypes observed. We examined relationships among the haplotypes by constructing a minimum spanning tree (Kruskal 1956, Prim 1957) in ARLEQUIN Version 3.5 (Excoffier and Lischer 2010). ARLEQUIN computes the minimum spanning tree from a matrix of the absolute number of differences (i.e., substitutions; Excoffier and Smouse 1994), calculated among all pairs of haplotypes using a modification the algorithm described in Rohlf (1973). We used the program HAPSTAR Version 0.6 (Teacher and Griffiths 2011) to draw the minimum spanning tree. The minimum spanning tree was edited in the program INKSCAPE Version 0.48 (www.inkscape.org). We also analyzed the relationships among haplotypes by constructing a phylogenetic tree using maximum likelihood in the computer program MEGA5 (Tamura et al. 2011). The phylogenetic analysis was based on the HKY (Hasegawa et al. 1985) model with a gamma distribution and invariant sites, which was selected as the best model of nucleotide substitution based on the Akaike's (1974) information criterion (AIC) and phyML (Guindon and Gascuel 2003) maximum likelihood calculations implemented in the computer program jMODELTEST Version 2.0 (Darriba et al. 2012). The maximum likelihood analysis was conducted using the subtree–pruning–regrafting heuristic search method as implemented in MEGA5, and the reliability of the inferred relationships was assessed using 1,000 bootstrap (Felsenstein 1985) replicates.

We tested the null hypothesis that the Gambel's Quail is a panmictic species using analysis of molecular variance (AMOVA; Excoffier et al. 1992) in the computer program ARLEQUIN. Panmixia is the lack of population structure. If the Gambel's Quail is a panmictic species, all measures of genetic differentiation among populations and groups of populations will be essentially zero. We performed two separate AMOVAs with different grouping schemes. First, we grouped populations as subspecies to determine how much genetic variation was because of subspecies taxonomy. Secondly, we grouped populations as geographic groups (Texas, Arizona, Sonora, California–Baja California, New Mexico–Colorado, and Utah–Nevada) to determine how much genetic variation was because of geography. Variance components were used to calculate phi–statistics (Φ, analogous to Wright's *F* statistics; Excoffier et al. 1992), which summarize the degree of differentiation among groups (Φ_{CT}), among populations within groups (Φ_{SC}), and within populations (Φ_{ST}). Statistical significance of the variance components were assessed using 16,000 bootstrap replicates.

Genetic structure is often a consequence of isolation by distance, in which the degree of genetic similarity declines with increasing geographic distance. We investigated the relationship between geographic and genetic distances using Mantel (1967) tests implemented in the Isolation by Distance web service (IBDWS Version 3.23, ibdws.sdsu.edu; Jensen et al. 2005, Ngan 2006). The Mantel test examines whether a matrix of pairwise genetic distances is correlated with a matrix of pairwise geographic distances. We performed separate Mantel tests using F_{ST} and Φ_{ST} as the measure of genetic distance. We excluded all populations represented by only a single individual (IBDWS allows only one population to be represented by a single individual). The shortest distances (km) between populations were calculated from decimal degrees using a modified version of the haversine formula (Sinnott 1984) implemented in the computer program GenAlex Version 6.5 (Peakall and Smouse 2012). Negative genetic distances were set to zero as were the genetic distances between populations fixed for the same haplotype. We assessed the statistical significance of the Mantel tests using 16,000 bootstrap replicates.

Historical demography of a species can be inferred by computing the distribution of observed pairwise nucleotide differences or mismatch distributions of mtDNA haplotypes (Rogers and Harpending 1992). A unimodal observed mismatch distribution is an indication of sudden demographic expansion, whereas bimodal or multimodal mismatch distributions imply demographic equilibrium or decline (Rogers and Harpending 1992). 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 distributions for major intraspecific groups of the Gambel's Quail. We evaluated the statistical support for demographic expansion or stability using the mean absolute error (MAE, Rogers et al. 1996) and the raggedness index (*r*, Harpending 1994). The mean absolute error is the goodness of fit between the observed and the expected mismatch distributions, where MAE approaches zero as the observed distribution approaches the expected distribution. The raggedness index quantifies the smoothness of the observed mismatch distribution, where small *r* values are typical of expanding populations; stationary or declining populations exhibit higher values.

We also evaluated the population history of the Gambel's Quail by computing Fu's (1997) F_S, which is the most powerful test statistic for detecting population growth in large samples and non–recombining DNA sequences (Ramos–Onsins and Rozas 2002, Ramírez–Soriano et al. 2008). This test statistic is based on the probability that of the number of haplotypes in a random sample is greater than the observed number of samples drawn from a constant–sized population. Large negative values of Fu's F_S indicate an excess of haplotypes as would be expected from demographic expansion. Positive values of Fu's F_S may be caused by demographic contractions, bottlenecks, or population subdivision (Fu 1997, Ramírez–Soriano et al. 2008).

Fu's F_S, MAE, and *r* statistics were performed using DNASP. We tested the null hypothesis of selective neutrality by constructing 95% confidence intervals for *r* and Fu's F_S. Demographic expansion was supported when selective neutrality was rejected by significant small values of *r* ($P < 0.05$) and significant negative values of 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). Confidence intervals were obtained with 1,000 coalescent simulations based on a neutral infinite–sites model, assuming large constant population size (Hudson 1990). Coalescent simulations were performed using the coalescent sampler implemented in DNASP. The coalescent sampler uses the *ran1* routine (Press et al. 1992) as a random number generator and a modified version of the *make_tree* algorithm (Hudson 1990) to generate a genealogy of haplotypes.

RESULTS

We obtained 219 bp of the control region and 345 bp of ND2 for 167 Gambel's Quail, and we successfully sequenced 40 of 48 museum specimens of Gambel's Quail. No contamination was detected in any of the negative extractions or PCR controls. The final concatenated set of control region and ND2 sequences was 564 bp.

We found 48 haplotypes, 36 of which occurred in Gambel's Quail (Fig. 2). Gambel's Quail exhibited moderately high haplotype diversity ($Hd = 0.79$) and low nucleotide diversity ($\pi = 0.01$; Table 1). Haplotype and nucleotide diversity of *C. g. gambelii* and *C. g. fulvipectus* were also relatively high ($Hd \geq 0.6$, $\pi \geq 0.01$), whereas *C. g. ignoscens* (western Texas) exhibited low levels of genetic diversity ($Hd = 0.32$, $\pi < 0.001$). California and Baja California also displayed low haplotype diversity ($Hd = 0.42$), although nucleotide diversity ($\pi = 0.01$) was higher than in Texas. The highest values of haplotype and nucleotide diversity ($Hd \geq 0.70$, $\pi = 0.01$) were found in Arizona, Utah–Nevada, and New Mexico–Colorado.

There were two distinct groups of Gambel's Quail haplotypes (Haplogroups 1 and 2) and two outlier haplotypes (AA and DD; Figs. 2, 3). Seven to eight mutational steps separated the haplogroups and outlier haplotypes from one another, and eight mutational steps separated the Gambel's Quail from the California Quail. More haplotypes occurred in Haplogroup 1 ($n = 24$) than Haplogroup 2 ($n = 12$). Haplotype and nucleotide diversity was slightly greater in Haplogroup 2 ($Hd = 0.942 \pm 0.037$ SD, $\pi = 0.005 \pm 0.0005$ SD) than in Haplogroup 1 ($Hd = 0.732 \pm 0.035$ SD, $\pi = 0.003 \pm 0.0002$ SD). Neither of these haplogroups was concordant with subspecies ranges or biogeographic barriers; however, Haplogroup 2 was concentrated in the western portion

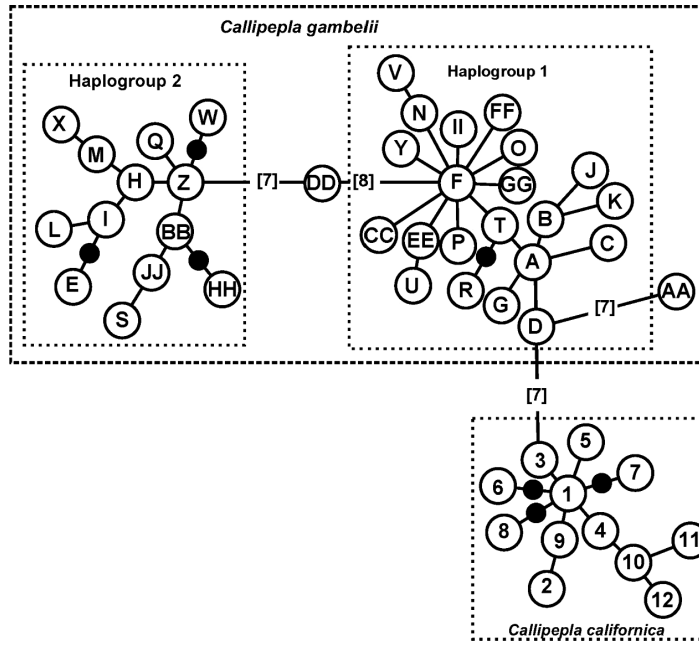

FIG. 2. Minimum spanning tree representing the phylogeographic structure of the Gambel's Quail in relation to the California Quail based on 218 concatenated sequences of the mitochondrial control region and ND2 (564 bp). Each white circle represents a unique haplotype, each line represents a single base substitution, and solid black circles are inferred missing haplotypes.

of the range, while the eastern edge of the range was dominated by Haplogroup 1 (Fig. 4). The two haplogroups of the Gambel's Quail formed star–like clusters of closely related haplotypes, with most haplotypes separated from their nearest neighbors by a single mutational step. ''Private'' haplotypes, those unique to specific subspecies, occurred in *C. g. gambelii* ($n = 27$) and *C. g. fulvipectus* ($n = 5$, Table 2). *Callipepla g. sana* possessed a unique haplotype. The *C. g. ignoscens* and *C. g. pembertoni* lacked unique haplotypes. Haplotype F was the most abundant and occurred in 71 individuals. Haplotype A, the second most common haplotype ($n = 22$), was more widespread geographically, and occurred in 14 localities (Table 3). Haplotype 4 occurred in all sampled subspecies ranges except *C. g. sana*, while haplotype F occurred in only *C. g. gambelii*

TABLE 1. Estimates of genetic diversity of the Gambel's Quail. Data are provided for sampled subspecies ranges and states including number of individual sequences (*n*), number of haplotypes (*h*), number of variable sites (*S*), haplotype diversity (*Hd*, with standard deviation), and nucleotide diversity (π with standard deviation).

	n	*h*	*S*	*Hd* ± SD	π ± SD
Callipepla gambelii	167	36	35	0.794 ± 0.029	0.009 ± 0.0011
C. g. gambelii	108	30	33	0.883 ± 0.022	0.011 ± 0.0014
C. g. fulvipectus	6	6	19	1.000 ± 0.096	0.016 ± 0.0043
C. g. ignoscens (Texas)	49	3	2	0.321 ± 0.079	0.001 ± 0.0002
C. g. pembertoni	3	2	1	0.667 ± 0.314	0.001 ± 0.0006
C. g. sana	1	1			
Arizona	65	22	28	0.792 ± 0.051	0.010 ± 0.0018
Utah-Nevada	19	6	22	0.737 ± 0.083	0.015 ± 0.0027
California-Baja California	9	3	18	0.417 ± 0.191	0.007 ± 0.0046
New Mexico-Colorado	12	7	23	0.909 ± 0.056	0.013 ± 0.0044
Sonora	13	9	21	0.872 ± 0.091	0.009 ± 0.0037

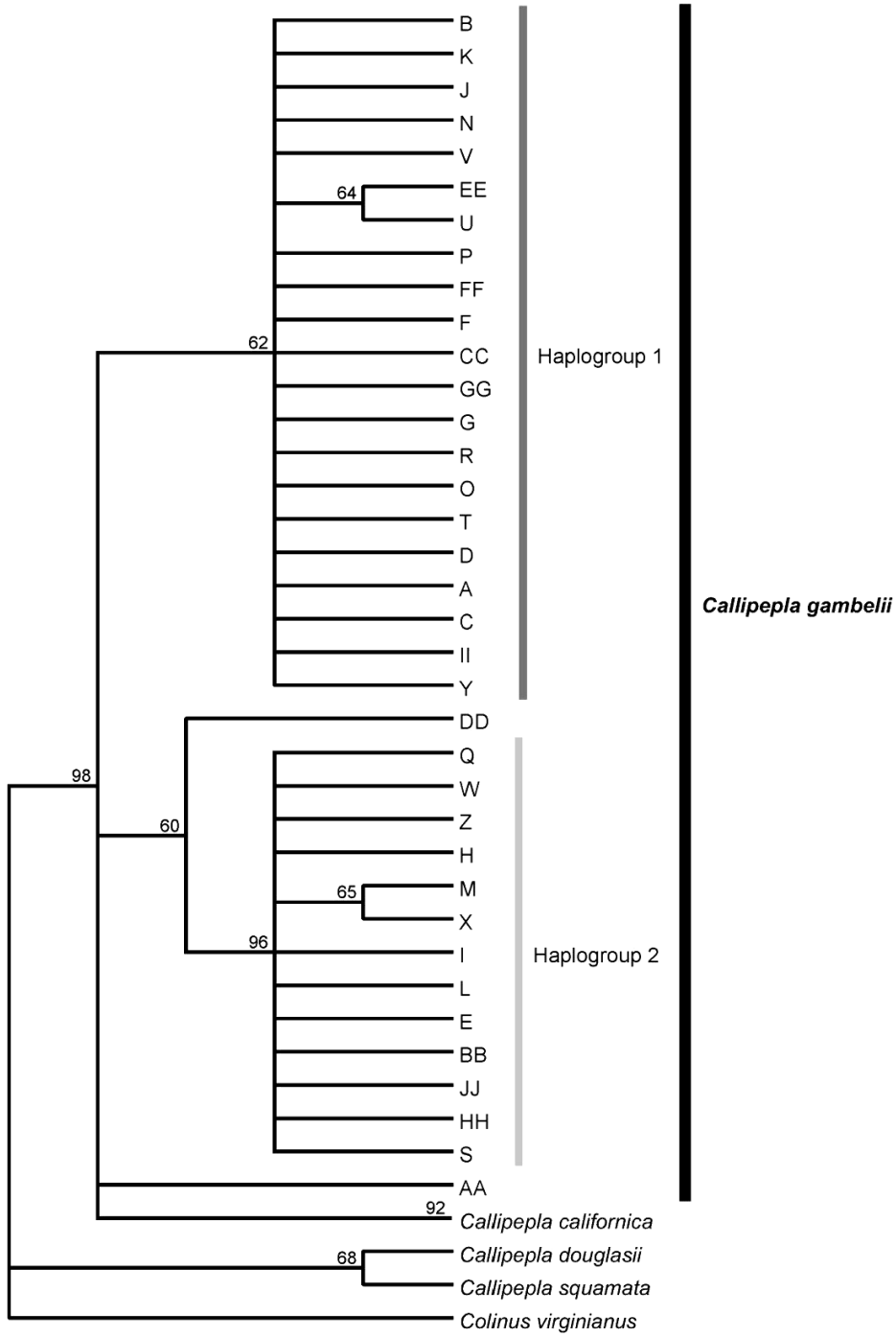

FIG. 3. Phylogenetic relationships of haplotypes of the Gambel's Quail as indicated by maximum likelihood analysis of concatenated mitochondrial control region and ND2 sequences (564 bp). Numbers represent bootstrap values (%) of adjacent nodes. Only nodes with posterior probability support ≥50% are labeled.

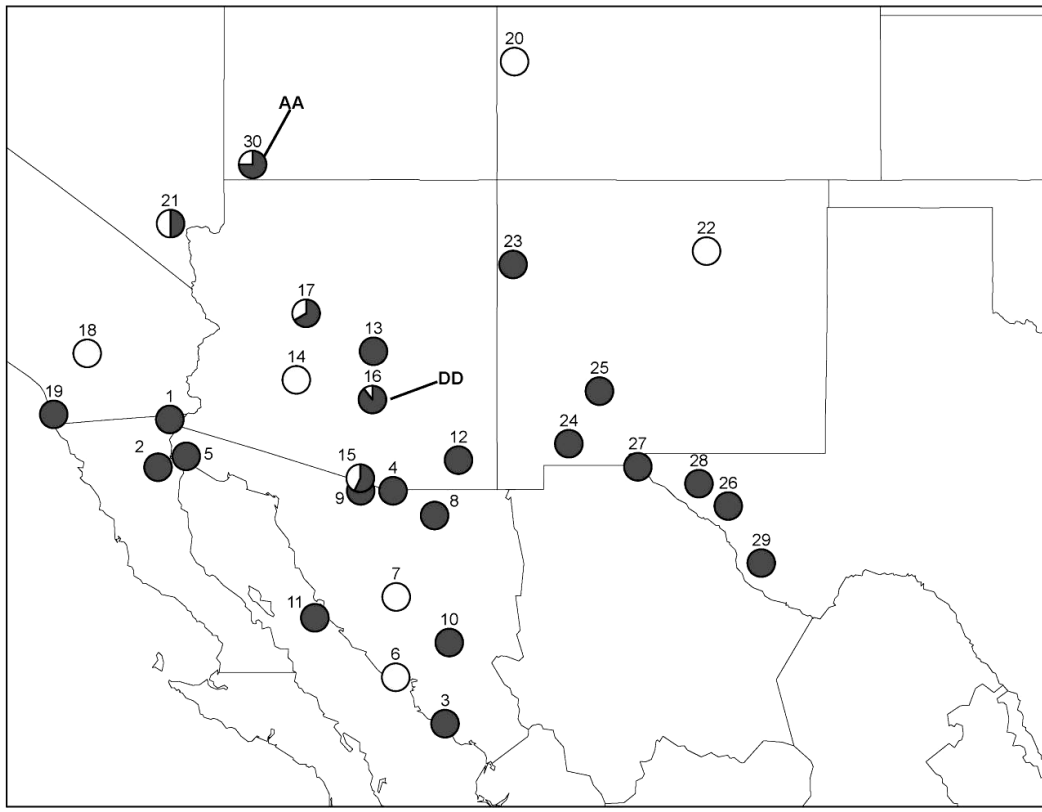

FIG. 4. Geographic distribution of Haplogroup 1 (dark gray) and Haplogroup 2 (light gray) throughout the range of Gambel's Quail. Numbers represent individual populations (Table 3), and size of pie slices indicate relative frequency of each haplogroup at each locality. Bold-faced letters represent the outlier haplotypes, AA and DD, with leader lines indicating in which locality each was found.

and *C. g. ignoscens*. The two outlier haplotypes, AA and DD, occurred only in *C. g. gambelii* and each was carried by a single individual. Haplotype AA occurred in Washington County, Utah, and haplotype DD occurred in Pinal County, Arizona.

Among the Gambel's Quail, 21 haplotypes occurred among the 127 contemporary samples collected 1999–2010 and 17 haplotypes among the 40 museum samples collected 1884–1953. Only two haplotypes (F and T) were shared among the museum and contemporary samples. Haplotypes F and T were each carried by a single museum specimen, while 70 contemporary specimens carried haplotype F and 10 contemporary specimens carried haplotype T. Haplotype and nucleotide diversity were similar among the

TABLE 2. Distribution of haplotypes among Gambel's Quail subspecies including the number of shared haplotypes (haplotypes found in >1 subspecies) and private haplotypes (haplotypes found in only 1 subspecies) for each subspecies.

Subspecies	Shared haplotypes (*n*)	Private haplotypes (*n*)
fulvipectus	A (1)	C (1), D (1), J (1), M (1), X (1)
gambelii	A (16), F (31), G (1), T (5)	B (2), E (1), H (1), I (1), K (1), L (1), N (5), O (6), P (1), Q (2), R (2), S (3), U (4), V (1), W (1), Y (9), Z (4), AA (1), BB (1), CC (2), DD (1), EE (1), FF (1), GG (1), H (1), II (1)
ignoscens	A (3), F (40), T (6)	
pembertoni	A (2), G (1)	
sana		JJ (1)

TABLE 3. Geographic distribution of Gambel's Quail haplotypes. Numbers in parentheses represent the number of individuals in each population that carried each haplotype.

Pop. no.	Locality	Haplogroup 1	Haplogroup 2	Outliers
1	Mexico: Baja California (Gardner's Lagoon and Seven Wells)	A (2), K (1)		
2	Mexico: Baja California (Mexicali)	A (4)		
3	Mexico: Sonora (Bahia Tobari)	A (1)		
4	Mexico: Sonora (Cabrero)	C (1), D (1)		
5	Mexico: Sonora (El Doctor)	T (1)		
6	Mexico: Sonora (Guaymas)		M (1)	
7	Mexico: Sonora (Pesqueira)		X (1)	
8	Mexico: Sonora (Santa Bárbara)	J (1)		
9	Mexico: Sonora (Sasabe)	A (1), B (1)		
10	Mexico: Sonora (Tecoripa)	A (1)		
11	Mexico: Sonora (Tiburon Island)	A (2), G (1)		
12	USA: Arizona (Cochise County)	A (2), B (1), F (1)		
13	USA: Arizona (Gila County)	F (8), O (1), T (1), V (1)		
14	USA: Arizona (Maricopa County)	E (1)		
15	USA: Arizona (Pima County)	F (1), G (1), R (2), S (3)		
16	USA: Arizona (Pinal County)	F (19), N (5), O (2), P (1), T (1), CC (2), EE (1), FF (1), GG (1), II (1)	Q (2), BB (1), II (2)	DD (1)
17	USA: Arizona (Yavapai County)	A (2)	I (2)	
18	USA: California (Riverside County)		H (1)	
19	USA: California (San Diego County)	A (1)		
20	USA: Colorado (Mesa County)		JJ (1)	
21	USA: Nevada (Clark County)	A (1)	W (1)	
22	USA: New Mexico (Catron County)		L (1)	
23	USA: New Mexico (Grant County)	A (2)		
24	USA: New Mexico (Luna County)	F (1), T (1), U (4)		
25	USA: New Mexico (Sierra County)	F (1), T (1)		
26	USA: Texas (Culberson County)	F (9)		
27	USA: Texas (El Paso County)	A (1)		
28	USA: Texas (Hudspeth County)	A (2), F (15), T (4)		
29	USA: Texas (Presidio County)	F (16), T (2)		
30	USA: Utah (Washington County)	O (3), Y (9)	Z (4)	AA (1)

museum ($Hd = 0.70$, $\pi = 0.01$) and contemporary samples ($Hd = 0.68$, $\pi = 0.01$). The outlier haplotypes occurred in contemporary samples. Overall, the museum and contemporary samples exhibited genetic structures similar to the full data set, with two large haplogroups separated by ≥7 mutational steps.

Panmixia was rejected by the AMOVA analysis, which showed that substantial genetic variation occurred among populations within groups (30–35%, $\Phi_{SC} = 0.33–0.36$, $P < 0.001$; Table 4). Most genetic variation occurred within populations (62–64%, $\Phi_{ST} = 0.36–0.39$, $P < 0.001$). A statistically significant relationship between geographic distance and genetic distance was revealed by the Mantel test (F_{ST}: $r = 0.42$, $P < 0.001$, $R^2 = 0.179$; Φ_{ST}: $r = 0.35$, $P = 0.002$, $R^2 = 0.120$; Fig. 5).

Unimodal mismatch distributions of both Gambel's Quail haplogroups indicated that each group has undergone recent demographic expansion (Fig. 6). Demographic expansion was further supported by statistically significant and negative values of Fu's F_S (Haplogroups 1 and 2, Table 5).

DISCUSSION

The Gambel's Quail exhibited strong phylogeographic structure as do many arid–adapted taxa of southwestern North America (Riddle et al. 2000, Zink et al. 2001, Zink 2002, Riddle and Hafner 2006). Phylogenetic divergence associated with the Sonoran and Chihuahuan deserts is a frequently observed phenomenon in desert taxa (Riddle and Hafner 2006, Pyron and Burbrink 2010). Deeper, older Chihuahuan–Sonoran diver-

TABLE 4. Analysis of molecular variance results for sampled Gambel's Quail based on different groupings of populations. Shown are degrees of freedom (*df*), sum of squares (*SS*), variance of components (φ), percentage of variance (%), fixation indices (Φ) and *P*-values of the significance of covariance of φ and Φ.

Source of variation	*df*	*SS*	φ	%	Φ	*P*-value
Populations grouped as subspecies						
Among groups	4	44.169	0.213	8.11	$\Phi_{CT} = 0.081$	0.065
Among populations within groups	26	141.819	0.799	30.37	$\Phi_{SC} = 0.331$	<0.001
Within populations	136	220.120	1.619	61.52	$\Phi_{ST} = 0.385$	<0.001
Total	166	406.108	2.631			
Populations grouped geographically						
Among groups	5	56.132	0.029	1.14	$\Phi_{CT} = 0.011$	0.085
Among populations within groups	25	129.856	0.896	35.24	$\Phi_{SC} = 0.356$	<0.001
Within populations	136	220.120	1.619	63.62	$\Phi_{ST} = 0.364$	<0.001
Total	166	406.108	2.544			

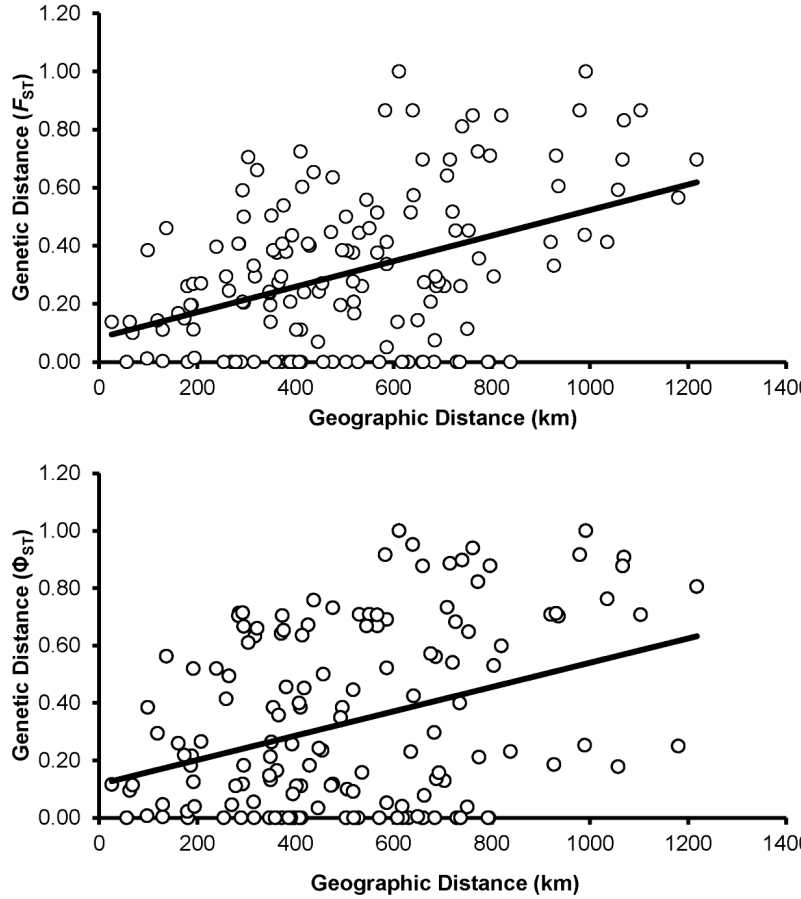

FIG. 5. Mantel test of the degree of genetic isolation of Gambel's Quail based on concatenated mitochondrial control region and ND2 sequences (564 bp) with increasing geographic distance. The y–axis is the genetic distance (either Φ_{ST} or F_{ST}), and the x–axis is the geographic distance in km. Regression equations and coefficients are as follows: Φ_{ST}: $y = 1.219 \times 10^{-3}x - 0.289$; $R^2 = 0.120$; $r = 0.35$, $P < 0.01$; F_{ST}: $y = 1.039 \times 10^{-3}x - 0.223$; $R^2 = 0.179$; $r = 0.42$, $P < 0.001$.

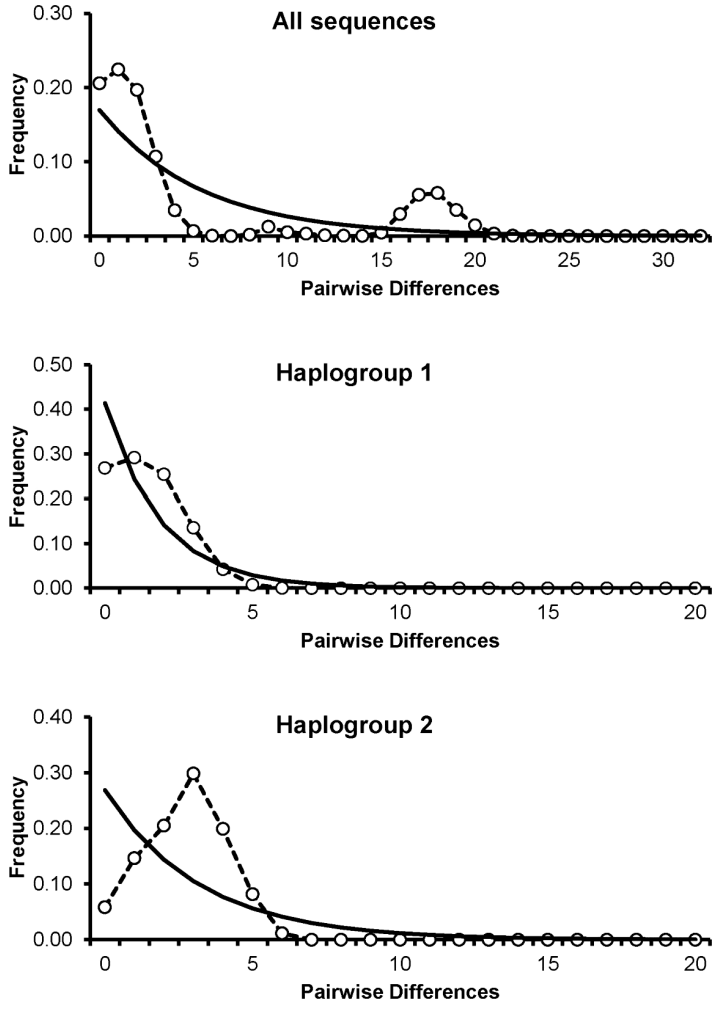

FIG. 6. Observed (solid line) and expected (dashed line) mismatch distributions of the Gambel's Quail (all sequences, $n = 167$) and the two haplogroups, Haplogroups 1 and Haplogroup 2, based on concatenated sequences of the mitochondrial control region and ND2. Mismatch distributions for the two haplogroups excluded outlier haplotypes AA and DD. The x–axis is the number of pairwise differences and the y–axis represents the frequency differences.

gences were probably a result of the Miocene (23.03–5.33 million years ago) uplift of the Sierra Madre Occidental (Riddle 1995, Riddle et al. 2000, Jaeger et al. 2005), whereas, shallower divergences have been attributed to the isolation of populations in separate refugia as a result of Pleistocene climatic oscillations (Riddle and Hafner 2006, Pyron and Burbrink 2010, Bryson et al. 2012).

TABLE 5. Values of mean absolute error (MAE), raggedness (r), and Fu's F_S for the Gambel's Quail and its two haplogroups. Sample size (n) and P-values of raggedness and Fu's F_S are also provided.

	n	S	MAE	r	Fu's F_S
All sequences	167	35	0.741	0.018 ($P = 0.098$)	−12.371 ($P = 0.002$)
Haplogroup 1	146	13	0.438	0.054 ($P = 0.013$)	−14.357 ($P < 0.001$)
Haplogroup 2	19	12	0.803	0.049 ($P = 0.157$)	−7.849 ($P < 0.001$)

Hubbard (1973) hypothesized that the four extant species of *Callipepla* originated when the range of a widespread ancestral species became fragmented during the Illinoian Glaciation (191,000–130,000 years ago). Climate and environmental changes isolated the ancestral species in three separate refugia, including a ''pre–*doulgasii*'' in Sonora and Sinaloa, ''pre–*squamata*'' in Chihuahua, and a ''pre–*californica/gambelii*'' in California (Hubbard 1973, p. 175). The pre–*californica/gambelii* isolate may have expanded into the southwestern United States and northwestern Mexico during the Sangamon Interglacial (125,000–75,000 years ago). During the Wisconsin Glaciation (75,000–19,000 years ago), eastern populations of the pre–*californica/gambelii* isolate became separated in a refugium in the Sonoran Desert, evolving into the modern Gambel's Quail (Hubbard 1973). Zink and Blackwell (1998) estimated that Gambel's and California quails diverged from their common ancestor about 1 million years ago. The genetic break between the haplogroups and outlier haplotypes of the Gambel's Quail are as large as the break between the Haplogroup 1 and California Quail, which suggests that the divergence of these lineages occurred at approximately the same time.

The distribution of the two haplogroups of Gambel's Quail is suggestive of a former Chihuahuan–Sonoran Desert split; however, this inference is obscured by the extensive geographic overlap of the two groups. Secondary contact among divergent Chihuahuan–Sonoran lineages because of post–Pleistocene expansion has been inferred for other non–volant, aridland taxa such as the western diamond-backed rattlesnake (*Crotalus atrox*, Castoe et al. 2007) and the red–spotted toad (*Anaxyrus punctatus*, Jaeger et al. 2005). Admixture observed in the western diamond-backed rattlesnake has resulted from eastward expansion of western lineages (Castoe et al. 2007), and secondary contact among eastern and western lineages of the red–spotted toad has been driven by the westward expansion of eastern lineages from the Chihuahuan Desert (Jaeger et al. 2005). It is not possible to conclusively determine the direction of range expansion of either of the two haplogroups of Gambel's Quail although much of the expansion appears to have been a westward expansion by Haplogroup 1.

Haplotypes AA and DD were genetically differentiated from the main haplogroups, and may be remnants of formerly common lineages. Alternatively, the genetic gaps separating these haplotypes, and the overall genetic structure observed in the Gambel's Quail, may reflect insufficient geographic sampling and sample size. Although the Gambel's and California quails frequently hybridize in parts of southern California where their respective ranges overlap (Grinnell 1915; Johnsgard 1988; Gee 2003, 2004), we detected no obvious evidence of hybridization or mitochondrial introgression among any of the samples. However few Gambel's Quail ($n = 2$) were sampled in this region. The maximum likelihood tree indicated that haplotype AA was the most divergent lineage but clustered with other Gambel's Quail haplotypes although bootstrap support was weak ($<50\%$).

Both haplogroups of Gambel's Quail exhibited star–like phylogenies, with Haplogroup 1 displaying a more typical star network with various haplotypes radiating from a central ancestral haplotype (Fig. 2). Star phylogenies are suggestive of recent, rapid demographic expansions (Slatkin and Hudson 1991), although star–like haplotype networks can also result from population bottlenecks (Depaulis et al. 2003). However, other analyses employed in this study supported demographic expansion as a more plausible explanation. These include the unimodal mismatch distributions, significant, negative values of Fu's F_S, and statistically significant Mantel tests indicating a pattern of isolation by distance, which suggests a geographic gradient of haplotypes potentially caused by range expansion (Excoffier et al. 2009). The lack of large genetic gaps within either haplogroups could indicate that expansion was a relatively recent event. Historically high levels of gene flow among populations may also account for the lack of genetic structure within both haplogroups (Avise 2000).

Assuming that range expansion occurred, how might one explain the events precipitating an expansion of the Gambel's Quail? The expansion of the two haplogroups was most likely driven by climatic changes associated with the end of the Wisconsin Glaciation. Analyses of sediment cores from caves, lakes, and streams (Metcalfe et al. 2002, Pigati et al. 2009, Brook et al. 2006, Chávez–Lara et al. 2012) and pollen and plant macrofossils found in middens constructed by packrats (*Neotoma* spp.; McAuliffe and Van Devender 1998; Metcalfe et al. 2000; Thompson and Anderson 2000; Holmgren et al. 2003, 2011) indicate that the climate of the Chihuahuan and

Sonoran deserts was cooler and wetter during the last glacial period. Cooler, wetter conditions supported open coniferous woodlands in lowlands, while thorn–scrub and shrublands appear to have been absent from the Chihuahuan and Sonoran deserts during the Late Pleistocene (Metcalfe et al. 2000). Modern precipitation regimes and desert shrub communities of southwestern North America were not established until 9,000–4,000 years ago (McAuliffe et al. 1998; Metcalfe et al. 2000; Holmgren et al. 2007, 2011). The distribution of the Gambel's Quail may have been highly restricted during cooler, wetter conditions of the Wisconsin Glaciation. The Gambel's Quail may have undergone rapid expansion during the Holocene as modern desert habitats expanded in southwestern North America. Post–Pleistocene expansions have also been inferred for several desert-adapted animal species (Ayoub and Reichert 2004, Jaeger et al. 2005, Douglas et al. 2006, Castoe et al. 2007, Wilson and Pitts 2012).

The presence of both haplogroups in Sonora, Arizona, Utah–Nevada, and New Mexico–Colorado partly accounts for the high haplotype and nucleotide diversity in these regions, and may represent areas of secondary contact. The higher haplotype and nucleotide diversity in Sonora, Arizona, Utah–Nevada, and New Mexico–Colorado is probably because of the fact this represents the center of the species' range. Western Texas and California–Baja California (where both haplogroups were present) are the respective eastern and western edges of the Gambel's Quails' range, and exhibit the lowest genetic diversity, which is consistent with range expansion, as genetic diversity is usually lower at the edge of a range expansion (Excoffier et al. 2009). Genetic diversity of marginal populations may also be lower because of repeated population crashes caused by suboptimal conditions (Sjörgren 1991), reduced dispersal and immigration (Yamashita and Polis 1995), smaller effective size and genetic drift (Soulé 1973).

The haplogroups identified for Gambel's Quail were not concordant with any of the subspecies, and the AMOVA results suggest that most (>90%) genetic variation occurs among and within populations rather than among subspecies. Our results suggest that the subspecies of Gambel's Quail do not reflect natural groups. The subspecies of Gambel's Quail were based largely on minor variations in plumage coloration and geography (Madge and McGowan 2002) and likely represent sporadic sampling of smooth clinal variation rather than distinct, phenotypically diagnosable units. A lack of concordance between phylogeographic structure and subspecies, however, can result from poor delineation of subspecies boundaries or inaccurate taxonomy (Winker 2010). In fact, many subspecies of North American birds were described on the basis of small samples, individuals collected outside of the breeding season, and non–rigorous descriptions (Rising 2007). For example, Figgins (1913, 1914) argued that Gambel's Quail in southwestern Colorado are descended from Gambel's Quail introduced into Colorado from California in 1885. Mearns (1914) described the subspecies *Callipepla* (*Lophortyx*) *gambelii sana* (*sanus*) on the basis of four specimens from this isolated population that had been provided to the United States National Museum by Figgins (1913). A unique haplotype (JJ) occurred in the single specimen of *C. g. sana* included in this study, but it was embedded within Haplogroup 2 and was most closely related to haplotypes S and BB (haplotypes unique *C. g. gambelii*).

Comparisons of genetic data from historical and contemporary specimens have revealed substantial changes in population structure and diversity in many species as a result of habitat fragmentation, mixing distinct populations, exploitation, or persecution by humans (Roy et al. 1994, Wandeler et al. 2007, Leonard 2008). The difference in the frequency of haplotypes among contemporary and museum specimens suggests that haplotype frequency has changed within ≥100 years. Most museum and contemporary specimens were collected from different localities, which prevented a direct test of changes in haplotype frequencies. The similarity in haplotype and nucleotide diversity among museum and contemporary samples suggests genetic diversity has remained relatively constant for 127 years.

Past attempts to establish game species outside of their native ranges as well as translocations conducted as part of restocking programs are problematic for phylogeographic studies of game species such as Gambel's Quail. Translocations can either obscure or erase the phylogenetic history of populations (Avise 2004). Most attempts at introducing Gambel's Quail outside of their native range have failed (Guillon 1960), with some exceptions such as in Colorado (Figgins 1913, 1914). Tiburón Island populations of the

Gambel's Quail (*C. g. pembertoni*) may also be the result of introductions of this species by humans (Rojas–Soto et al. 2010). Rojas–Soto et al. (2010) found a single ND2 haplotype and two cytochrome *b* (cty*b*) haplotypes among five specimens of *C. g. pembertoni*. The ND2 haplotype and one of the cyt*b* haplotypes was shared by Gambel's Quail from New Mexico, while another cytochrome *b* haplotype was unique to the *C. g. pembertoni* (Rojas–Soto et al. 2010). Similarly, we found only two concatenated ND2–control region haplotypes among three Tiburón Island specimens, but neither of these haplotypes was unique to the island. Human–mediated movements and introductions have also been proposed as explanations for the existence of populations of the California Quail on Santa Catalina Island (Johnson 1972) and populations of Northern Bobwhites on Caribbean Islands (Brennan 1999). Most island populations of New World quails are probably the result of human–mediated introductions, since New World quails are weak fliers, and are largely terrestrial.

It is also possible that selective sweeps or other forms of selection are the cause of the patterns of mtDNA variation we found in Gambel's Quail. The use of mtDNA in phylogeography has been criticized recently because of concerns that selective sweeps may obscure or erase phylogenetic and phylogeographic signals (Ballard and Whitlock 2004, Hurst and Jiggins 2005, Ilves et al. 2010). We cannot conclusively rule out the possibility that selection is responsible for the genetic structure observed, but we consider this to be unlikely for several reasons. First, the Gambel's Quail exhibited a phylogeographic structure similar to other co–distributed desert taxa (Riddle et al. 2000, Zink et al. 2001, Jaeger et al. 2005, Castoe et al. 2007, Wilson et al. 2010). The overall high diversity of mtDNA in natural populations suggests that selective sweeps are rare (Karl et al. 2012), with little evidence that selection has influenced patterns of mtDNA diversity in vertebrates (Nabholz et al. 2008, 2009; McCusker and Bentzen 2010). Selection does not appear to invalidate intraspecific phylogenetic inferences from mtDNA (Zink 2005, Kivisild et al. 2006, Zink et al. 2006). Lastly, numerous phylogeographic studies of birds have reported congruent histories between mitochondrial and nuclear genes (Hung et al. 2013 and references therein), which suggests that mtDNA phylogeographic patterns in most avian species are because of gene flow and genetic drift rather than natural selection (Drovetski et al. 2009; Hung et al. 2012, 2013; Zink et al. 2013).

Although this study suggests little differentiation exists among subspecies of Gambel's Quail, sequence data from *C. g. friedmanni* and *C. g. stephensi* and more intensive sampling of Mexico, California, Nevada, and Colorado will be needed to adequately explore the intraspecific phylogenetics of the Gambel's Quail. Future phylogeographic studies of the Gambel's Quail should include sequences from nuclear loci to evaluate the conclusions of this study, estimate divergence times and effective population size, and provide solid tests of neutrality of mtDNA sequences (Huang et al. 2012, Zink et al. 2013). Finally, a thorough literature review of the original descriptions of all seven subspecies of the Gambel's Quail would also make an important contribution to resolving the species' intraspecific phylogeny (Gutíerrez 1993).

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