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REGIONAL VARIATION IN MTDNA OF THE LESSER PRAIRIE-CHICKEN

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Abstract. Cumulative loss of habitat and long-term decline in the populations of the Lesser Prairie-Chicken (*Tympanuchus pallidicinctus*) have led to concerns for the species' viability throughout its range in the southern Great Plains. For more efficient conservation past and present distributions of genetic variation need to be understood. We examined the distribution of mitochondrial DNA (mtDNA) variation in the Lesser Prairie-Chicken across Kansas, Colorado, Oklahoma, and New Mexico. Throughout the range we found little genetic differentiation except for the population in New Mexico, which was significantly different from most other populations. We did, however, find significant isolation by distance at the rangewide scale ($r = 0.698$). We found no relationship between haplotype phylogeny and geography, and our analyses provide evidence for a post-glacial population expansion within the species that is consistent with the idea that speciation within *Tympanuchus* is recent. Conservation actions that increase the likelihood of genetically viable populations in the future should be evaluated for implementation.

Key words: DNA, genetic diversity, Lesser Prairie-Chicken, mitochondrial DNA, prairie grouse, *Tympanuchus pallidicinctus*.

Estructura Genética Regional del ADNmt en *Tympanuchus pallidicinctus*

Resumen. La pérdida de hábitat y la disminución poblacional de largo plazo sufrida por *Tympanuchus pallidicinctus* ha conducido a preocupaciones en torno a la viabilidad de la especie a lo largo de su distribución geográfica en la parte sur de las Grandes Planicies. Para que la conservación sea más eficiente, es necesario entender la distribución pasada y presente de la variación genética. Examinamos la distribución de la variación en el ADN mitocondrial (ADNmt) en *T. pallidicinctus* a través de Kansas, Colorado, Oklahoma y Nuevo México. Encontramos poca diferenciación genética a lo largo de la distribución, excepto para la población de Nuevo México que fue significativamente diferente de la mayoría de las otras poblaciones. Sin embargo, encontramos un patrón significativo de aislamiento por distancia a la escala de toda la distribución ($r = 0.698$). No encontramos relación entre la filogenia de haplotipos y la geografía. Además, nuestros análisis proveen evidencia de una expansión poblacional post-glacial de la especie, lo que concuerda con la idea de que la especiación en *Tympanuchus* es reciente. Es necesario evaluar la posible implementación de acciones de conservación que incrementen la probabilidad de contar con poblaciones genéticamente viables en el futuro.

INTRODUCTION

Throughout the Holarctic Region grouse have declined dramatically in recent years as a result of habitat loss, degradation, and fragmentation resulting from human land uses (e.g., silviculture, agriculture, recreation, urbanization, Storch 2007). Habitat modification and loss have affected dispersal and gene flow in several species of grouse (Bouzat et al. 1997, Segelbacher et al. 2003, Johnson et al. 2003, 2004, Oyler-McCance et al. 2005a), and population declines have led to

reduced egg fertility and productivity in at least two populations (Westemeier et al. 1998, Stiver et al. 2008).

The Lesser Prairie-Chicken (*Tympanuchus pallidicinctus*) is a lek-mating grouse occupying the southern Great Plains (including parts of Kansas, Colorado, Oklahoma, Texas, and New Mexico) vegetated primarily with sand sagebrush (*Artemisia filifolia*) and sand shinnery oak (*Quercus havardii*) (Hagen and Giesen 2005). Although the range of the Lesser Prairie-Chicken before European settlement was relatively large and contiguous, the current distribution of this

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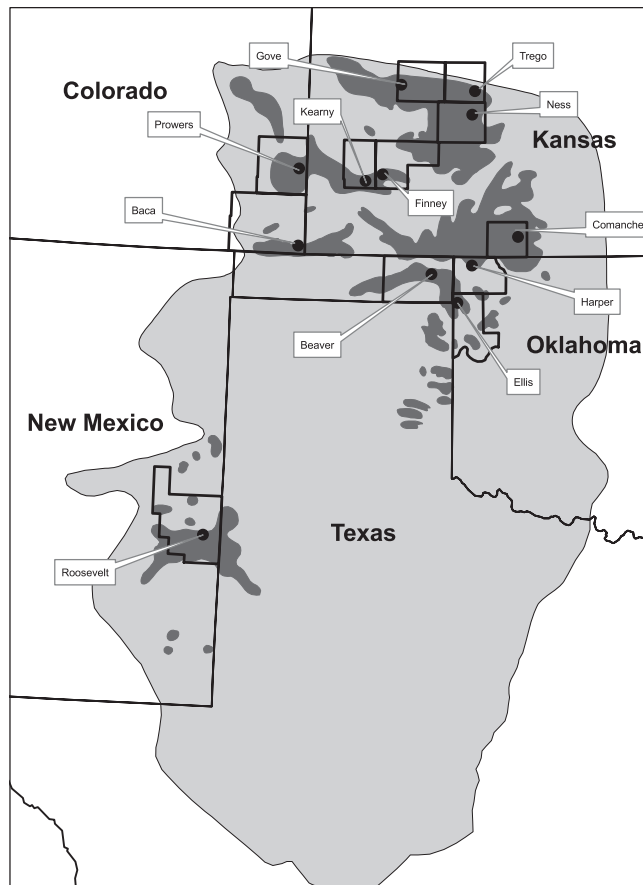


FIGURE 1. Current range (dark gray) and historic range (light gray) of the Lesser Prairie-Chicken (adapted from Hagen and Giesen 2005). Sampling locations (black dots) are depicted within county boundaries. Oklahoma and New Mexico sites are from Van den Bussche et al. (2003).

species has been reduced to <8% of the historic distribution and is highly fragmented because of agriculture and other human development (Fig. 1, Hagen and Giesen 2005). Much of the habitat loss has occurred in the center of the Lesser Prairie-Chicken's range, the Texas panhandle, with vast areas of prairie no longer suitable for the species (Fig. 1). The cumulative loss

of habitat, declining population trends (Table 1), and imminent threats led to a recent increase in priority ranking of the 1995 listing as "warranted but precluded" under the Endangered Species Act (USFWS 2008).

The divergence of *Tympanuchus* spp. and post-glacial expansion of their distribution help explain contemporary patterns of population genetic structuring (Johnson 2008). These patterns, combined with current knowledge of habitat fragmentation and movement corridors, can elucidate rates of gene flow and populations at risk of reductions in genetic diversity. The distribution of genetic variation and the structure of certain populations of the Lesser Prairie-Chicken have been described from Oklahoma and New Mexico by Van den Bussche et al. (2003) and Bouzat and Johnson (2004). Bouzat and Johnson (2004) investigated the Lesser Prairie-Chicken's genetic structure of at four leks in New Mexico and found no differentiation in mtDNA sequence. In a separate study, Van den Bussche et al. (2003) used mtDNA sequence data to document genetic variation at 20 leks in Oklahoma and New Mexico and found reasonably high levels of genetic variation. The latter study found some regional structuring among leks within each state and detected a high level of differentiation between populations from Oklahoma and New Mexico in both the mitochondrial and nuclear genomes, Van den Bussche et al. (2003) suggested that habitat fragmentation and loss may have contributed to the genetic structuring observed. Both studies provided important information about local distributions of genetic variation. A rangewide perspective of genetic variation, however, currently unknown for the Lesser Prairie-Chicken, is necessary for conservation decisions at the level of the species. Rangewide genetic analyses for related species such as the Greater (*Centrocercus urophasianus*) and Gunnison (*C. minimus*) Sage-Grouse (Oyler-McCance et al. 2005a, b).

Here, using mitochondrial DNA (mtDNA) sequence data, we greatly extend previous studies' range and density of sampling to address the distribution of genetic variation across most of the Lesser Prairie-Chicken's range. We build on the initial work of Van den Bussche et al. (2003) in Oklahoma and New Mexico to include samples from the remaining portion

TABLE 1. The Lesser Prairie-Chicken's population status (size and trend) in the five states that constitute the species' current range.

State	Breeding population status		References
	Size	Trend 1980–2008	
Colorado	<1000	Declining	Giesen (2000), Davis et al. (2008)
Kansas	18 000–29 000	Stable/increasing	Johnsgard (2002), Davis et al. (2008)
New Mexico	6000	Declining	Johnsgard (2002), Davis et al. (2008)
Oklahoma	<3000	Declining	Johnsgard (2002), Davis et al. (2008)
Texas	6000	Declining	Silvy et al. (2004), Davis et al. (2008)

of the species' range in Kansas and Colorado. The combined information will allow for the development of a more cohesive and efficient conservation strategy based on the rangewide distribution of genetic variation in the Lesser Prairie-Chicken.

METHODS

STUDY AREAS

In 2000 to 2002, we captured Lesser Prairie-Chickens and collected blood samples from them in Baca and Prowers counties in Colorado and six counties in Kansas, three north (Gove, Trego, and Ness) and three south of the Arkansas River (Comanche, Finney, Kearny; Fig. 1). Samples from Trego and Ness counties were combined because of their geographic proximity and small sample sizes from these areas. We compared the birds' mtDNA to that of populations previously sampled by Van den Bussche et al. (2003). We used mtDNA data from Van den Bussche et al. (2003) but made comparisons among counties instead of among leks and included Harper County (pooling Harper [$n = 8$], and Ellis [$n = 53$] counties) and Beaver County ($n = 27$), Oklahoma, and Roosevelt County, New Mexico ($n = 63$; hereafter Roosevelt). We lacked samples from populations in Texas but sampled adjacent populations in New Mexico and Oklahoma (Fig. 1).

TISSUE COLLECTION AND DNA EXTRACTION

We sampled blood from 161 Lesser Prairie-Chickens (144 males, 17 females), captured during the spring and fall of 2000 to 2002 in funnel traps in Kansas and Colorado (Haukoos et al. 1990). Blood samples were obtained by clipping a toenail of each prairie-chicken and collecting two or three drops of blood into a microfuge tube previously coated with EDTA (Brinkman). All blood samples were stored frozen at -20°C . DNA was extracted from blood by either a phenol-chloroform method (Kahn et al. 1999) or the Wizard Genomic DNA Purification System (Promega), according to the manufacturer's instructions.

DNA SEQUENCING

Following Quinn (1992), we amplified a portion of the mtDNA control region in 25- μL reactions with the primers L16755 (Nedbal et al. 1997) and OSU7713 (Van den Bussche et al. 2003). The procedure consisted of preheating at 94°C for 2 min followed by 35 cycles of denaturation at 94°C for 40 sec, annealing at 55°C for 60 sec, and extension at 72°C for 2 min. PCR products were cleaned with shrimp alkaline phosphatase and exonuclease 1 (USB). Dye terminator cycle sequencing reactions were performed with the Beckman Coulter Quick Start Sequencing Kit according to the manufacturer's protocol. The products were precipitated according to the manufacturer's specifications, resuspended in 30 μL of formamide, and run on a Beckman Coulter CEQ 8000 XL Data Analysis System by method LFR-b. Sequences (466 base pairs) were

aligned with Sequencher 4.2 (GeneCodes) and compared to the sequences previously defined by Van den Bussche et al. (2003).

DATA ANALYSES

We calculated haplotype diversity (h) and nucleotide diversity (π) of mtDNA for each population with the program ARLEQUIN (version 2.000; Schneider et al. 2000). We examined population structure with an analysis of molecular variance (AMOVA) by investigating how much variation was explained by the separation of populations into four geographic regions, sand sagebrush prairie (Finney, Kearny, Prowers and Baca), mixed-grass prairie (Gove and Ness), mixed shrub (Comanche, Beaver, and Harper), and sand shinnery oak (Roosevelt). We used a Kimura (1980) two-parameter nucleotide-substitution model for all calculations. Pairwise Φ_{ST} values, measures of genetic differentiation between each group, were calculated, and their significance was determined by random permutations. Pairwise Φ_{ST} values among 10 populations were considered to be significant if their Bonferroni-corrected P -value was <0.001 . We examined the relationship between geographic and genetic distance with a Mantel (1967) test. We calculated probabilities for the Mantel test by using methods of Smouse et al. (1986).

To investigate the relationship among haplotypes we generated an unrooted haplotype network with the statistical parsimony software TCS version 1.13 (Clement et al. 2000). We used the algorithm of Templeton et al. (1992) to construct the network.

We investigated the possibility of a post-glacial species-wide population expansion by means of a mismatch distribution of pairwise genetic differences in programs ARLEQUIN (Schneider et al. 2000) and DnaSP (version 3.4, Rozas and Rozas 1999). DnaSP graphically compares the observed and expected distributions of populations at equilibrium and expansions by using Rogers' (1995) method of moments. We examined the mismatch distribution in three separate analyses: the entire sample of birds, all samples but excluding New Mexico, and New Mexico separately. It was important to separate the New Mexico population because of its genetic differences from Oklahoma populations and its disjunct range (Van den Bussche et al. 2003).

RESULTS

The mtDNA haplotypes originally defined by Van den Bussche et al. (2003) included a run of either seven or eight Ts at the beginning of the sequence. Because of ambiguity of where to place the deletion, we truncated the sequence by one base pair. This deletion resulted in our classifying as identical two haplotypes (J and Z) originally defined as distinct by Van den Bussche et al. (2003). Thus we refer to J and Z as a single haplotype (J) (Table 2). From individuals of the four states combined ($n = 278$), we identified 43 haplotypes, 30 of them

TABLE 2. Haplotypes and their frequency in 10 populations from 4 regions throughout the range of the Lesser Prairie-Chicken. Haplotypes A through EE previously described by Van den Busche et al. (2003).

Haplotype	Gove, KS	Ness, KS	Finney, KS	Kearny, KS	Prowers, CO	Baca, CO	Comanche, KS	Harper, OK	Beaver, OK	Roosevelt, NM	Frequency
A	1		1					3	2	20	27
B		1	2	2			2	5	4	10	26
C			1	4		2	1	6	5	12	31
D									3		3
E	2		1				1	8	1		13
F								7			7
G	1	1					2	2	5		11
H			1				1	5	1		8
I	1				2		4			6	13
J	1		6	7		1	1	2	2	2	22
K			1							5	6
L										4	4
M								4			4
N			4	1				2	1		8
O	1		5	1	1			3			11
P								3			3
Q										3	3
R								2			2
S					1	1		1			3
T								1	1		2
U										1	1
V								1			1
W								1			1
X	1		1	1	2			1			6
Y									1		1
AA									1		1
BB	1		5	2	2			1			11
CC								1			1
DD								1			1
EE								1			1
FF		1									1
GG	5	2	3	1		1	1				13
HH		1									1
JJ	1	1									2
KK	2	1	1		1						5
LL	1	1									2
OO	2	1		1							4
PP	1										1
SS	2		3		1						6
UU	2										2
VV	4										4
XX				4							4
ZZ							1				1
Total	29	10	35	24	10	5	14	61	27	63	278

had previously described by Van den Bussche et al. (2003) (Table 2). Sequences of the 43 haplotypes have been deposited in Genbank (accession numbers GU269158–GU269200). Haplotypes of mtDNA were characterized by 39 polymorphic sites consisting of 32 transitions, 3 transversions, 3 polymorphic sites containing both transitions and insertion/deletions, and 1 polymorphic site with both a transition and a transversion.

According to statistical parsimony, the 95% plausible set of the network comprised many haplotypes and contained

several ambiguous connections resolved by the frequency and topology criterion (Fig. 2). There was no relationship between haplotypes and geography, and all haplotype relationships were relatively shallow. Haplotypes C, A, B, and J were the most common (found in 52% of individuals sequenced) with J being the most widespread. Haplotypes E, I, and GG were also relatively common (Fig. 3). Haplotype C was found in all populations except Gove, Ness, and Prowers, while haplotype B was found everywhere birds were sampled except

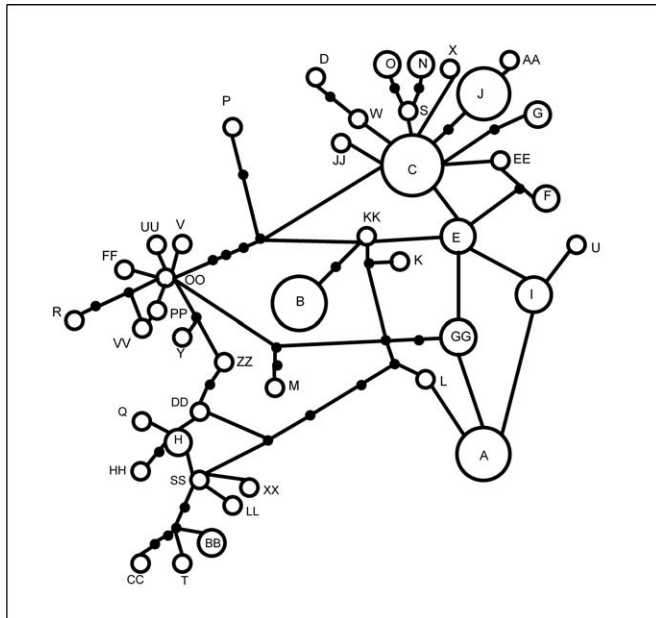


FIGURE 2. Unrooted estimated 95% parsimony cladogram of 43 haplotypes detected in the Lesser Prairie-Chickens. Haplotypes are represented by letters. Lines represent single mutations; dots represent intermediate haplotypes not found in our sample but necessary to link the haplotypes observed.

Gove, Prowers, and Baca (Fig. 3). Haplotype A was found in high frequency in Roosevelt and was absent from Ness, Prowers, Baca, and Comanche, while haplotype J was found everywhere except Ness and Prowers (Fig. 3).

Within-population haplotype diversity (h), which represents the number and frequency of haplotypes, was on average 0.916 (SE = 0.04), but Roosevelt, New Mexico, had the lowest

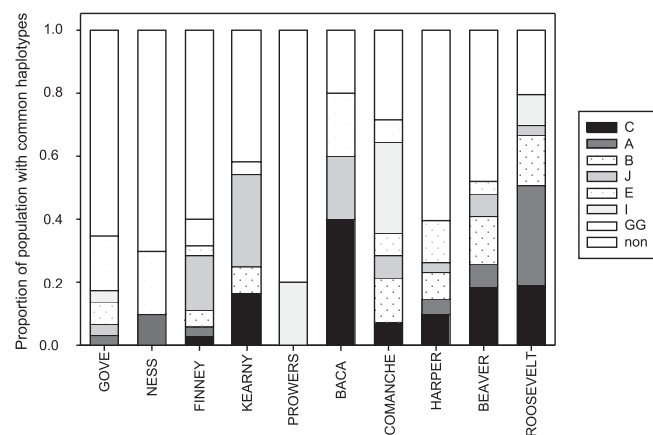


FIGURE 3. Proportion of individuals in each population with common haplotypes; “non” represents the number of uncommon haplotypes. Each bar represents the proportion of individuals in each population with these common haplotypes. The prevalence of haplotypes is ranked from greatest to least as C, A, B, J, I, E, and GG, the rank of the last three being equal.

TABLE 3. Summary statistics of mtDNA diversity across the range of the Lesser Prairie-Chicken.

Population	n^a	A^b	h^c (SE)	π^d (SE)
Gove, KS	29	17	0.948 (0.023)	0.011 (0.006)
Ness, KS	10	9	0.978 (0.054)	0.013 (0.008)
Finney, KS	35	14	0.919 (0.021)	0.013 (0.007)
Kearny, KS	24	11	0.887 (0.045)	0.012 (0.007)
Prowers, CO	10	7	0.933 (0.062)	0.013 (0.007)
Baca, CO	5	4	0.900 (0.161)	0.005 (0.004)
Comanche, KS	14	9	0.912 (0.059)	0.010 (0.006)
Harper, OK	61	22	0.944 (0.012)	0.012 (0.007)
Beaver, OK	27	12	0.912 (0.028)	0.011 (0.006)
Roosevelt, NM	63	9	0.828 (0.027)	0.008 (0.004)

^a Sample size.
^b Number of haplotypes.
^c Haplotype diversity.
^d Nucleotide diversity.

diversity (0.828; Table 3). Nucleotide diversity (π) in New Mexico (0.008) and Baca (0.005) was lower than the average of all populations ($\bar{x} = 0.0108$, SE = 0.004). Genetic diversity attributable to variation among regions, among populations within a region, and within a populations was partitioned as 6.52%, 0.43%, and 93.04%, respectively. Pairwise Φ_{ST} tests indicated low levels of population differentiation with significant differences between Roosevelt and all other populations except Baca, Prowers, and Comanche (Table 4).

The mismatch distributions calculated among haplotypes for the entire sample, the New Mexico population alone, and the entire species excluding New Mexico were all unimodal and consistent with post-glacial range expansion (Fig. 4A–C). In all three analyses the goodness-of-fit tests with the model for expanding population growth showed no significant differences (entire sample: sum of squared deviations = 0.002, $P = 0.67$; New Mexico only: sum of squared deviations = 0.017, $P = 0.31$; excluding New Mexico: sum of squared deviations = 0.004, $P = 0.40$). We found a positive correlation between genetic distance (Φ_{ST}) and geographic distance ($r = 0.692$, $P = 0.004$; Fig. 5A). Subsequently, we analyzed isolation by distance to control for the extreme geographic and genetic distances to the New Mexico populations and found significant isolation by distance ($r = 0.417$, $P = 0.017$) in this subset of data (Fig. 5B).

DISCUSSION

In the late Pleistocene, prairie grouse (*Tympanuchus* spp.) likely experienced a rapid expansion and diversification (Lucchini et al. 2001, Drovetski 2003, Spaulding et al. 2006, Johnson 2008). Furthermore, on the basis of morphology, behavior, and geography, it has been shown that speciation within this genus is recent and lineage sorting among the three species is incomplete (Ellsworth et al. 1994, Spaulding et al. 2006, Johnson 2008). Our data are consistent with previous findings, as we found relatively high levels of genetic diversity

TABLE 4. Pairwise Φ_{ST} values for mtDNA sequencing data from 10 populations of the Lesser Prairie-Chicken in Kansas, Colorado, Oklahoma, and New Mexico. Values of Φ_{ST} in **bold** are significantly different (Bonferroni correction $P \leq 0.001$) in pairwise comparisons across the species' range.

	Kansas					Colorado		Oklahoma		New Mexico
	Gove	Ness	Finney	Kearney	Comanche	Prowers	Baca	Harper	Beaver	Roosevelt
Gove										
Ness	-0.03787									
Finney	0.04250	0.00203								
Kearney	0.05067	0.01057	-0.00690							
Comanche	0.09340	0.02615	0.03832	0.04947						
Prowers	0.02654	-0.00757	-0.03216	0.01012	0.02767					
Baca	0.14881	0.11694	0.02444	0.00025	0.01449	0.08740				
Harper	0.04167	-0.00966	0.01271	0.02514	-0.00285	-0.00234	0.02529			
Beaver	0.10979	0.04780	0.04547	0.02754	-0.01200	0.05395	-0.03916	0.02483		
Roosevelt	0.16793	0.12829	0.12683	0.17040	0.02035	0.12572	0.15051	0.08142	0.12443	

in most populations and no relationship between haplotype phylogeny and geography (Fig. 2, Table 2). Also, 10 of 43 haplotypes of the Lesser Prairie-Chicken exactly matched haplotypes of the Greater Prairie-Chicken (*T. cupido*) deposited in GenBank, while one haplotype matched a sequence identified in both the Sharp-tailed Grouse (*T. phasianellus*) and Greater Prairie-Chicken. One explanation for the lack of phylogeographic structure is a population expansion following the Pleistocene glaciation. Range expansion after the Pleistocene glaciation has been reported for several other avian species including the Great Spotted Woodpecker (*Dendrocopos major*; Zink et al. 2002), Marbled Murrelet (*Brachyramphus marmoratus*; Congdon et al. 2000), King Eider (*Somateria spectabilis*; Pearce et al. 2004), and Mountain Plover (*Charadrius montanus*; Oyler-McCance et al. 2005c). Our results are consistent with these other species, as our haplotype phylogeny was shallow and our mismatch distribution was unimodal.

It has been argued that the Great Plains were subjected to massive ecological perturbation in the Pleistocene, resulting in many extinctions (Mengel 1970). A comparison of species compositions across North America has suggested that the Great Plains' avifauna is low in diversity and relatively undifferentiated morphologically. *Tympanuchus* may have diverged around the Pleistocene Epoch and may have evolved rapidly into the three species recognized today as glaciation constricted or shifted the grassland (Lucchini et al. 2001, Drovetski 2003, Spaulding et al. 2006, Johnson 2008). As glaciers receded, it appears that Lesser Prairie-Chicken populations expanded in concert with the expanding grasslands with the New Mexico population perhaps isolated from the remainder of the species.

Rangewide mtDNA-sequence data suggest that gene flow among Lesser Prairie-Chicken populations persists despite population declines and habitat fragmentation. Haplotype diversity in most of our populations ($h > 0.8$) was higher than in Greater Prairie-Chicken populations ($h < 0.7$) that have

experienced significant population bottlenecks (Bellinger et al. 2003, Johnson et al. 2004). Our analysis revealed significant differences between New Mexico and most of the rest of the range and significant isolation by distance. As found in the Greater Prairie-Chicken by Johnson et al. (2003), however, this isolation by distance may be remnant of past signals. Similarly, given the fragmentation and isolation of each population we sampled (Fig. 1), the lack of differentiation among populations we found may not reflect contemporary gene flow. It is possible that time for drift to influence overall population mtDNA structure has been insufficient, and signals of past isolation by distance still persist. Additional analyses using nuclear markers more sensitive to recent changes in population structure may be necessary for these competing hypotheses to be addressed.

The population in New Mexico was significantly different from all others, lacking of gene flow between Oklahoma, Kansas and Colorado (average $\Phi_{ST} = 0.080 \pm 0.007$). Moreover, in New Mexico haplotype diversity was lower than in all other populations sampled, and three of nine haplotypes found there were unique, further supporting the idea that this population is isolated with the potential risk of inbreeding (Bouzat and Johnson 2004). Lesser Prairie-Chickens from Gove and Ness counties at the northern fringe of range had the greatest haplotype diversity (0.947 and 0.978, respectively) and among the highest nucleotide diversity (0.011 and 0.013, respectively). Hybridization between the Lesser and Greater Prairie-Chickens in northern Kansas has been documented by Bain and Farley (2002), and we captured but did not analyze molecularly five birds that we considered to be hybrids on the basis of morphology. Additionally, two haplotypes unique to the northern fringe of the range match Greater Prairie-Chicken haplotypes in GenBank. Incomplete lineage sorting in *Tympanuchus* could be due to recent range expansion rather than to hybridization (Spaulding et al. 2006, Johnson 2008). However, current

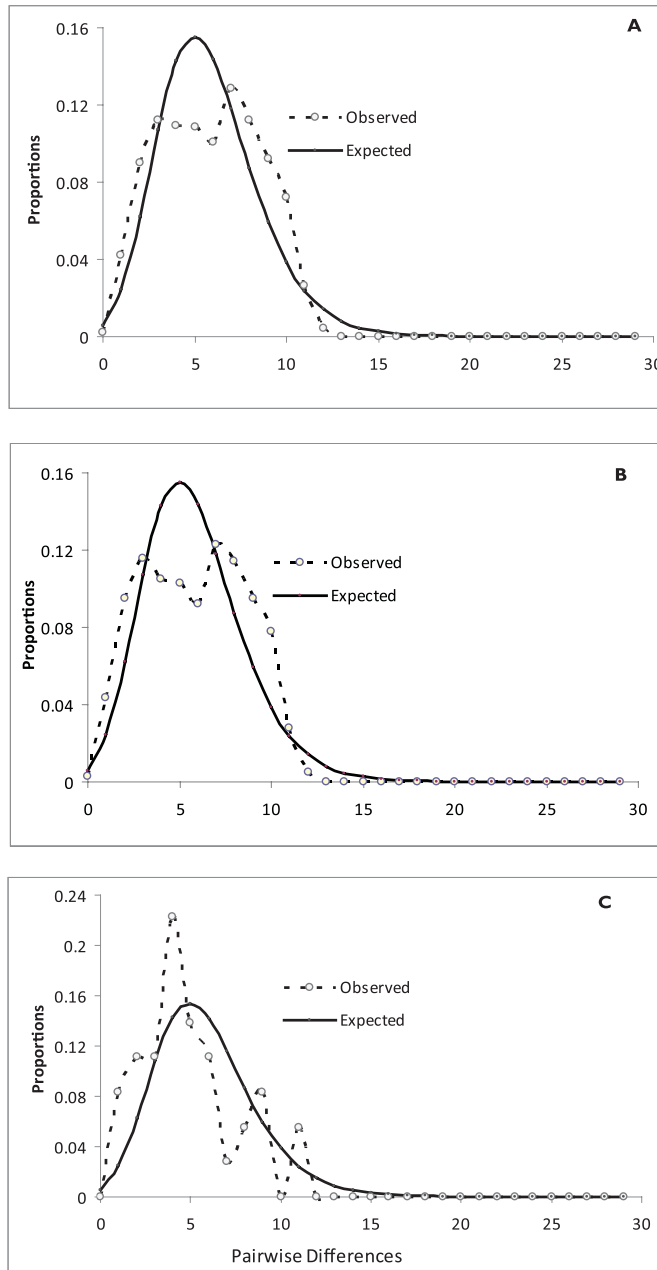


FIGURE 4. Mismatch distribution of the observed variation in haplotypes of the mtDNA control region of the Lesser Prairie-Chicken compared to the theoretical distribution representing population expansion for (A) the entire sample combined, (B) the entire sample excluding the New Mexico population, and (C) the New Mexico population only.

hybridization between the Lesser and Greater Prairie-Chicken in areas of overlap is apparent and may further complicate reconstruction of historical patterns of divergence (Spaulding et al. 2006).

Our study documented the distribution of variation in mtDNA of the Lesser Prairie-Chicken in Kansas and Colorado.

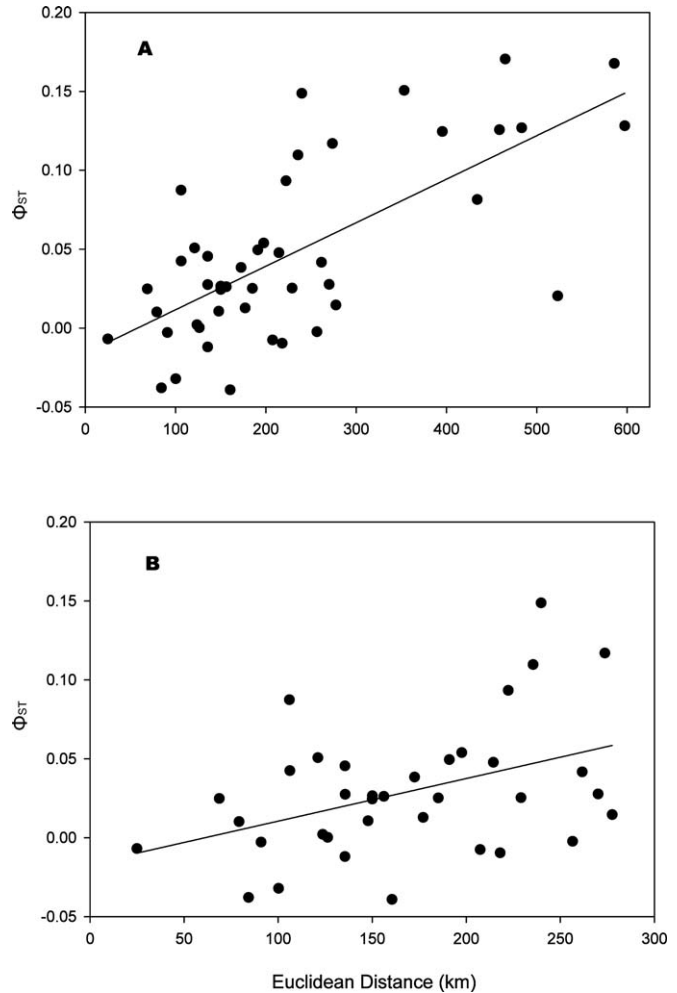


FIGURE 5. Pairwise analysis of isolation by distance for mtDNA (Φ_{ST}) and geographic distance. (A) All populations sampled; (B), and all populations sampled except those in New Mexico.

Our data, combined with those of Van den Bussche et al. (2003), provide a rangewide view of Lesser Prairie-Chicken population genetics, which can be used in a comprehensive management plan for the species. Our study was consistent with previous work indicating a range expansion after the last glaciation. In general, we found levels of haplotype diversity and gene flow that do not suggest barriers to dispersal resulting from habitat fragmentation and population decline. Further work is needed to discern whether these patterns are genetic signals of the past or patterns of contemporary genetic structuring. The New Mexico population, however, is isolated and its genetic diversity is lower than at all other populations. Conservation actions that create or conserve dispersal corridors as well as translocations should be considered. Their effectiveness for increasing the likelihood of genetic viability of the New Mexico populations should be evaluated.

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