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Genetic variation within and among fragmented populations of lesser prairie-chickens (*Tympanuchus pallidicinctus*)

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Abstract

As a result of recurrent droughts and anthropogenic factors, the range of the lesser prairie-chicken (*Tympanuchus pallidicinctus*) has contracted by 92% and the population has been reduced by approximately 97% in the past century, resulting in the smallest population size and most restricted geographical distribution of any North American grouse. We examined genetic variation through DNA sequence analysis of 478 base pairs of the mitochondrial genome and by assaying allelic variation at five microsatellite loci from lesser prairie-chickens collected on 20 leks in western Oklahoma and east-central New Mexico. Traditional population genetic analyses indicate that lesser prairie-chickens maintain high levels of genetic variation at both nuclear and mitochondrial loci. Although some genetic structuring among lesser prairie-chicken leks was detected within Oklahoma and New Mexico for both nuclear and mitochondrial loci, high levels of differentiation were detected between Oklahoma and New Mexico populations. Nested-clade analysis of mitochondrial haplotypes revealed that both historic and contemporary processes have influenced patterns of haplotype distributions and that historic processes have most likely led to the level of differentiation found between the Oklahoma and New Mexico populations.

Keywords: conservation genetics, habitat fragmentation, lesser prairie-chickens, microsatellites, mtDNA, *Tympanuchus pallidicinctus*

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Introduction

As a result of anthropogenic factors, such as habitat destruction, fragmentation and degradation, numerous species of plants and animals either currently face extinction or have declined drastically during the twentieth century. Habitat fragmentation and loss result in small populations at risk of losing genetic variation because of stochastic demographic, environmental and genetic processes that may contribute to the endangering of fragmented populations (Frankel & Soulé 1981; Shaffer 1981, 1987; Brussard & Gilpin 1989; Srikwan & Woodruff 2000). The lesser prairie-chicken (Aves: Phasianidae: *Tympanuchus pallidicinctus*) is a plains grouse that typically inhabits rangeland dominated by shinnery oak (*Quercus havardii*) or sand sagebrush (*Artemisia filifolia*) in portions of Colorado, Kansas, Oklahoma, New Mexico and Texas (Taylor & Guthery 1980). As a result of recurrent droughts and anthropogenic factors, lesser prairie-chickens currently occupy less than 92% of their historic range and the total number of birds has declined by greater than 97% since the 1800s (Taylor & Guthery 1980), resulting in the smallest population size and most restricted geographical distribution of any native North American grouse (Giesen 1998).

Several studies have been, and continue to be, conducted on various aspects of the ecology and breeding behaviour of lesser prairie-chickens (Taylor & Guthery 1980; Riley *et al*. 1992; Riley & Davis 1993; Giesen 1998; Jamison 2000; Woodward *et al*. 2001). However, little is known about levels of intra- and interpopulation genetic variation, metapopulation dynamics, or how this taxon responds to habitat fragmentation. Because these issues are intimately

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connected and may affect fitness components, the purpose of this study was to ascertain genetic variation within and among lesser prairie-chicken leks in western Oklahoma and east-central New Mexico. These areas were sampled because (i) they represent areas disjunct from one another and from the more northern populations of lesser prairie-chickens, which appear to have greater numbers of individuals (Taylor & Guthery 1980); and (ii) recent data suggest that while a few populations of lesser prairie-chickens in Oklahoma and New Mexico are stable, most are declining (Woodward *et al*. 2001).

To ascertain levels of genetic variation, we sequenced a portion of the mitochondrial control region and assayed allelic variation at six nuclear microsatellite loci. These data were analysed using traditional population genetic statistics that provide measures of genetic variation within populations (observed and expected heterozygosity, allelic diversity, and haplotype and nucleotide diversity) and the partitioning of genetic variation among populations (F_{ST} and ϕ_{ST}). While these statistics provide critical information regarding levels of genetic variation, they fall short of providing objective insight into the evolutionary processes responsible for the observed patterns of genetic variation (Templeton & Georgiadis 1996). Thus, to provide insight into contemporary and historic forces responsible for the partitioning of genetic variation in lesser prairie-chickens, the mitochondrial DNA sequence data were analysed using the intraspecific phylogeographic approach of Templeton *et al*. (1992). The basic premise of this approach is to overlay geography upon the estimated evolutionary tree of mitochondrial DNA (mtDNA) haplotypes. Although intraspecific phylogeographic approaches have been used in previous studies (Avise 2000), the advantage of Templeton's approach for conservation biology is that it provides rigorous statistical testing of the association between geographical data and evolutionary history through the use of a statistical design determined by the haplotype tree and objective criteria for making inferences about historical and contemporary processes, which can be useful for management decisions (Georgiadis *et al*. 1994; Crandall & Templeton 1998). Finally, this approach is statistically sensitive enough to indicate when sampling design is inadequate to make inferences. This last point is a significant advancement over phylogeographic approaches that do not incorporate rigorous statistical testing and is of utmost importance when studying rare and endangered species because it should prevent researchers from making management or conservation recommendations that are not supported by the data.

Materials and methods

Approximately 1 mL of blood was collected from each of 175 lesser prairie-chickens associated with 20 leks in Beaver (n = 29), Ellis (n = 70), and Harper (n = 10) counties, Oklahoma, and Roosevelt (n = 66) county, New Mexico (Fig. 1). These leks varied from 1 to 62 km separation in Oklahoma, and from 1 to 19 km separation in New Mexico. The Oklahoma and New Mexico sites were separated by about 425 km.

Total genomic DNA was extracted from each individual following standard protocol (Longmire *et al*. 1997). Four hundred and seventy-eight base pairs (bp) of the mitochondrial control region were sequenced for each individual using primers L16755 (Nedbal *et al*. 1997) and OSU7713 (5′-CCTGACCGAGGAACCAGAGGCGC-3′). Amplifications

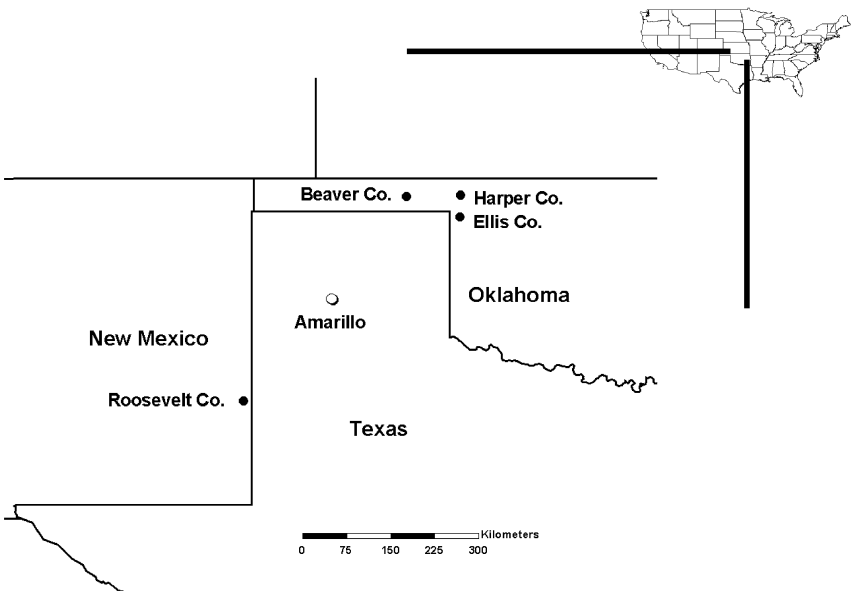

Fig. 1 Map of study region located within the southern Great Plains of the United States showing the location of the 20 lesser prairie-chicken leks examined in this study.

were performed in 50 μL volumes with approximately 500 ng of genomic DNA, 30 pmol of each primer, 0.07 mM dNTPs, 2.0 mM $MgCl_2$, 0.8 mM bovine serum albumin, and 1 unit of *Taq* polymerase. Cycling parameters were 94 °C denaturation for 10 min, followed by 35 cycles of 94 °C denaturation for 50 s, 56 °C annealing for 60 s, and 72 °C extension for 2 min, followed by a single 30 min final extension at 72 °C. Polymerase chain reaction (PCR) products were purified using Wizard PCR Preps (Promega Corporation) and both forward and reverse strands were completely sequenced on an ABI 377 automated DNA sequencer with the Big-Dye Chain Terminating Sequencing kit following the manufacturer's protocols.

The two overlapping fragments of the control region for each individual were compiled using ASSEMBLYLIGN™ (Oxford Molecular Group 1998) and all individuals were aligned using CLUSTAL X (Thompson *et al*. 1997). This multiple alignment was imported into MACCLADE 4 (Madison & Madison 2000) where the alignment was visually inspected. Using the Redundant Taxa option in MACCLADE, all control region sequences were reduced to display the number of unique haplotypes. Estimates of haplotype (h) and nucleotide (π; Nei 1987) diversity within leks were calculated using ARLEQUIN. The extent to which mtDNA sequence variation was partitioned among leks and between New Mexico and Oklahoma was analysed in a hierarchical fashion by the ϕ statistic using the nested analysis of molecular variance (AMOVA) option in ARLEQUIN. Significance of variance estimates for ϕ statistics was obtained using a randomized procedure with 1000 permutations (Excoffier *et al*. 1992).

The program TCS (version 1.13; Clement *et al*. 2001) was used to generate an unrooted haplotype genealogy following the algorithm of Templeton *et al*. (1992) with ambiguities resolved following the recommendations of Crandall & Templeton (1993) and Crandall *et al*. (1994). The resulting haplotype genealogy was converted into a nested design by grouping haplotypes into one-step clades, one-step clades into two-step clades, and so on until all subclades were nested into a single clade using the procedure described by Templeton *et al*. (1987). The resulting nested clade design and geographical distances between all pairs of leks were analysed using GEODIS (Posada *et al*. 2000). Clade distance (D_c — a measure of the geographical range of a particular clade) and nested clade distance (D_n — a measure of how a particular clade is geographically distributed relative to its closest evolutionary sister-clade) were used to calculate average interior distance minus the average tip distances [$(I-T)_c$ and $(I-T)_n$]. These four statistics, with a key provided by the authors (http://bioag.byu.edu/zoology/crandall_lab/geodis.htm), were used to infer biological explanations of the results with the null hypothesis of no association between haplotype genealogy and geographical distribution. Rejection of the null hypothesis provides insight into the role of historic and contemporary processes producing the observed patterns of haplotype distributions (Templeton *et al*. 1995).

To evaluate further the extent of genetic erosion, intra- and interpopulation variation within the nuclear genome was assessed by genotyping each individual at six microsatellite loci (ADL23, ADL42, ADL44, ADL146, ADL162, ADL230) originally isolated from domestic chicken (*Gallus gallus*) and shown to be variable in greater prairie-chickens (*Tympanuchus cupido*; Bouzat *et al*. 1998). PCR amplification of these six loci was conducted in 15 μL volumes containing 50 ng of genomic DNA, 5 pmol of each primer, 9 μL True Allele Premix (Perkin Elmer Applied Biosystems) and 3.8 μL of double-distilled water. The thermal profile consisted of a 12-min denaturation and enzyme activation cycle at 95 °C; 10 cycles of 94 °C for 15 s, 55 °C for 60 s, 72 °C for 30 s; followed by 25 cycles of 89 °C for 15 s, 55 °C for 60 s, and 72 °C for 30 s. A final 72 °C incubation for 30 min was used to ensure that all reactions had gone to completion. Variation at individual microsatellie loci was visualized using a Perkin Elmer Applied Biosystems 377 Automated DNA Sequencer and GENESCAN and GENOTYPER SOFTWARE. Amplicons for each locus from a single individual were mixed (0.5 μL of each PCR product), and 1 μL of this mixture was combined with 3 μL of loading mix (2.5 μL of formamide, 0.5 μL of ROX size standard, 0.25 μL of loading buffer containing blue dextran). PCR-loading mixtures were denatured at 95 °C for 5 min and loaded (1.5 μL) into a single lane of a 5% polyacrylamide gel.

Allele frequencies, mean number of alleles per locus, deviations from Hardy–Weinberg expectations, and population differentiation were assessed using ARLEQUIN 2.0 (Schneider *et al*. 2000). We tested each locus at each lek for deviations from Hardy–Weinberg expectations with the Bonferroni adjustment for multiple comparisons (Rice 1989) as the criterion for statistical significance. The relative genetic variation within each lek was assessed using allele-frequency data, from which the mean number of alleles and unbiased expected heterozygosity (Nei 1987) were calculated. We tested for differences in observed (H_O) and expected (H_E) heterozygosity and mean number of alleles per locus across leks using a mixed model analysis of variance (PROC MIXED; SAS 2000). The Satterthwaite approximation was used to calculate effective degrees of freedom and least squared means separation tests were performed to test for differences across leks with a significance level of $P < 0.05$. The extent to which microsatellite variation was partitioned among leks within states and between states was analysed in a hierarchical fashion using the analysis of molecular variance (AMOVA; Excoffier *et al*. 1992) option in ARLEQUIN. Significance of variance estimates for F-statistics were obtained using a randomized procedure with 1000 permutations (Excoffier *et al*. 1992).

Table 1 Mitochondrial DNA D-loop haplotype distribution and frequency of lesser prairie-chickens within leks sampled from Oklahoma and New Mexico

	mtDNA haplotype																														
Locality	A	B	C	J	D	E	F	G	H	M	N	O	P	R	S	T	V	W	X	Y	Z	AA	BB	CC	DD	EE	I	K	L	Q	U
Oklahoma																															
Ellis co., C-1		2					1	1							1	1					1										
Ellis co., C-2		1				1								1																	
Ellis co., C-3							1			4	1	3		1	1											1					
Ellis co., E-1	1	2	4			1	4	1	5				3				1	1	1				1		1						
Ellis co., E-3						3																									
Ellis co., off lek			1				1				1																				
Harper co., H-2	1					2																		1							
Harper co., H-1	1		1	1		1																									
Beaver co., B-2																1															
Beaver co., B-2B	2	2	1	2	2			5	1											1		1									
Beaver co., B-3		2	1		1																										
Beaver co., B-1			3			1					1																				
New Mexico																															
Lek 1	2		3																									1	2		
Lek M1	3	1	2	1																										1	
Lek 2	2	2	1																										1	1	
Lek 3																											2	1			
Lek 4		2		1																							1	1	1	1	
Lek 5	13	4	3																								2	1			
Lek 6		1	3																												
Off lek																											1	1			1

Results

Mitochondrial DNA

Four hundred and seventy base pairs of the mitochondrial control region were sequenced for 168 individuals. Thirty-six nucleotide positions were variable, resulting in all individuals being assigned to one of 31 haplotypes. Mean per cent sequence divergence among individuals was 1.05%, with a range of 0.00% to 2.63%, whereas mean percentage sequence divergence among haplotypes was 1.39%. Haplotypes A, B, C and J were the most common and were distributed in both Oklahoma and New Mexico, whereas 22 and five haplotypes were unique to Oklahoma and New Mexico leks, respectively (Table 1).

Within-lek haplotype diversity (h), which represents the number and frequency of haplotypes, was high for all leks, and all leks were also characterized by moderate levels of nucleotide diversity (π), which is based on the frequency of haplotypes and sequence divergence among haplotypes within sites (Table 2). Proportions of genetic diversity attributable to variation within leks, among leks within states, and between states were 88.93%, 3.03% and 8.05%, respectively.

Microsatellites

All individuals were examined for six microsatellite loci, but because of difficulties in scoring and obtaining repeatable and reliable results, locus ADL44 was removed from all analyses. After correcting for multiple comparisons (Rice 1989), no locus differed significantly from Hardy–Weinberg proportions. The mean number of alleles per lek, mean H_O and H_E did not differ significantly across leks (Table 2). The proportions of genetic diversity attributable to variation within leks, among leks within Oklahoma and New Mexico, and between Oklahoma and New Mexico were 88.45%, 6.86% and 4.69%, respectively.

Phylogeographic analysis

Using the computer package TCS, which incorporates the formulae given in Templeton *et al*. (1992), mtDNA haplotypes separated by up to nine mutational steps have a probability of ≥ 0.95 of being connected in a parsimonious fashion. Figure 2 shows the nested design, using the rules of Templeton & Sing (1993). Within this network, two loops indicate ambiguous connections that are represented by dashed lines. Despite these ambiguities, the logic of Crandall & Templeton (1993) allows resolution

Table 2 Basic descriptive statistics for each lek based on DNA sequence data of a portion of the mtDNA D-loop (mtDNA) and five microsatellite loci (nDNA)

	mtDNA				nDNA			
Locality	n	A_1	$h \pm$ SE	$\pi \pm$ SE	n	A_2	H_O	H_E
Oklahoma								
Ellis co., C-1	7	6	0.952 ± 0.0955	0.0170 ± 0.0090	7	3.4	0.343	0.554
Ellis co., C-2	3	3	1.000 ± 0.2722	0.0169 ± 0.0135	3	2.2	0.222	0.867
Ellis co., C-3	13	8	0.885 ± 0.0699	0.0156 ± 0.0088	14	4.2	0.386	0.546
Ellis co., E-1	26	13	0.920 ± 0.0289	0.0131 ± 0.0072	27	5.8	0.400	0.631
Ellis co., E-3	12	4	0.773 ± 0.0686	0.0080 ± 0.0049	12	4.4	0.400	0.596
Ellis co., off lek	3	3	1.000 ± 0.2722	0.0085 ± 0.0072	3	2.6	0.417	0.817
Harper co., H-2	4	3	0.833 ± 0.2224	0.0144 ± 0.0103	5	3.2	0.400	0.720
Harper co., H-1	4	4	1.000 ± 0.1768	0.0057 ± 0.0045	5	3.0	0.400	0.622
Beaver co., B-2b	2	2	1.000 ± 0.5000	0.0254 ± 0.0264	3	2.6	0.750	0.750
Beaver co., B-2	17	9	0.897 ± 0.0534	0.0116 ± 0.0066	17	4.4	0.318	0.527
Beaver co., B-3	4	3	0.833 ± 0.2224	0.0088 ± 0.0066	4	2.6	0.375	0.750
Beaver co., B-1	5	3	0.700 ± 0.2184	0.0034 ± 0.0028	5	3.6	0.400	0.644
New Mexico								
Lek 1	8	4	0.821 ± 0.1007	0.0054 ± 0.0037	8	3.8	0.475	0.598
Lek M1	8	5	0.857 ± 0.1083	0.0103 ± 0.0064	8	4.2	0.450	0.647
Lek 2	7	5	0.905 ± 0.1.33	0.0113 ± 0.0071	7	3.6	0.400	0.580
Lek 3	3	2	0.667 ± 0.3143	0.0057 ± 0.0050	3	2.4	0.417	0.733
Lek 4	7	6	0.952 ± 0.0955	0.0131 ± 0.0081	7	3.6	0.429	0.580
Lek 5	23	5	0.652 ± 0.0937	0.0048 ± 0.0030	23	4.8	0.409	0.587
Lek 6	4	2	0.500 ± 0.2652	0.0042 ± 0.0036	4	3.4	0.500	0.821
Off lek	3	3	1.000 ± 0.2722	0.0071 ± 0.0061	3	1.8	0.583	0.583

n = sample size; A_1 = number of different haplotypes; $h \pm$ SE, haplotype diversity; $\pi \pm$ SE, nucleotide diversity; A_2 = mean number of alleles per locus; H_O = mean observed heterozygosity; H_E = mean expected heterozygosity.

Table 3 Nested contingency analysis of geographical associations based upon 1000 iterations. Clades are the same as in Fig. 1 and include only those with a probability value less than 0.05, indicating significant geographical structuring

Clade	Permutational χ^2 statistic	Probability
1–5	9.00	0.025
1–18	13.79	0.022
2–9	47.96	0.001
2–10	33.70	0.000
3–2	9.80	0.041
4–1	18.75	0.001

of these loops. Table 3 presents the results of the nested contingency analyses in which sampling localities are treated as categorical variables. Although this analysis did not incorporate geographical distance information, it revealed significant associations between the clades 1–5, 1–18, 2–9, 2–10, 3–2, 4–1, and their geographical locations. No other nested contingency tests were significant at the 5% level. Therefore, a geographical association within Oklahoma and between Oklahoma and New Mexico is detectable when the haplotype tree is used to generate a nested statistical design.

Table 4 Interpretation of the results of Fig. 2 using the key of inference

Clade	Chain of inference	Demographic event inferred
1–5	1–12–11–12–13–14	Long Distance Colonization
1–18	1–2–No	Inconclusive Outcome
1–20	1–2–11–12–No	Contiguous Range Expansion
2–9	1–2–3–4–No	Restricted Gene Flow with Isolation by Distance
2–10	1–2–11–12–No	Contiguous Range Expansion
2–11	1–2–11–12–No	Contiguous Range Expansion
3–2	1–2–11–17–4–No	Restricted Gene Flow with Isolation by Distance
3–4	1–2–3–4–No	Restricted Gene Flow with Isolation by Distance
3–5	1–2–3–5–15–No	Past Fragmentation
4–1	1–2–11–12–No	Contiguous Range Expansion
4–2	1–2–11–12–No	Contiguous Range Expansion
Total	1–2–No	Inconclusive Outcome

The null hypothesis of no association between geographical distribution of haplotypes and mtDNA genealogy was rejected for 11 clades (Table 4; Fig. 2). Use of the inference key led to the conclusion that the null hypothesis was rejected in favour of long distance dispersal for clade 1–5.

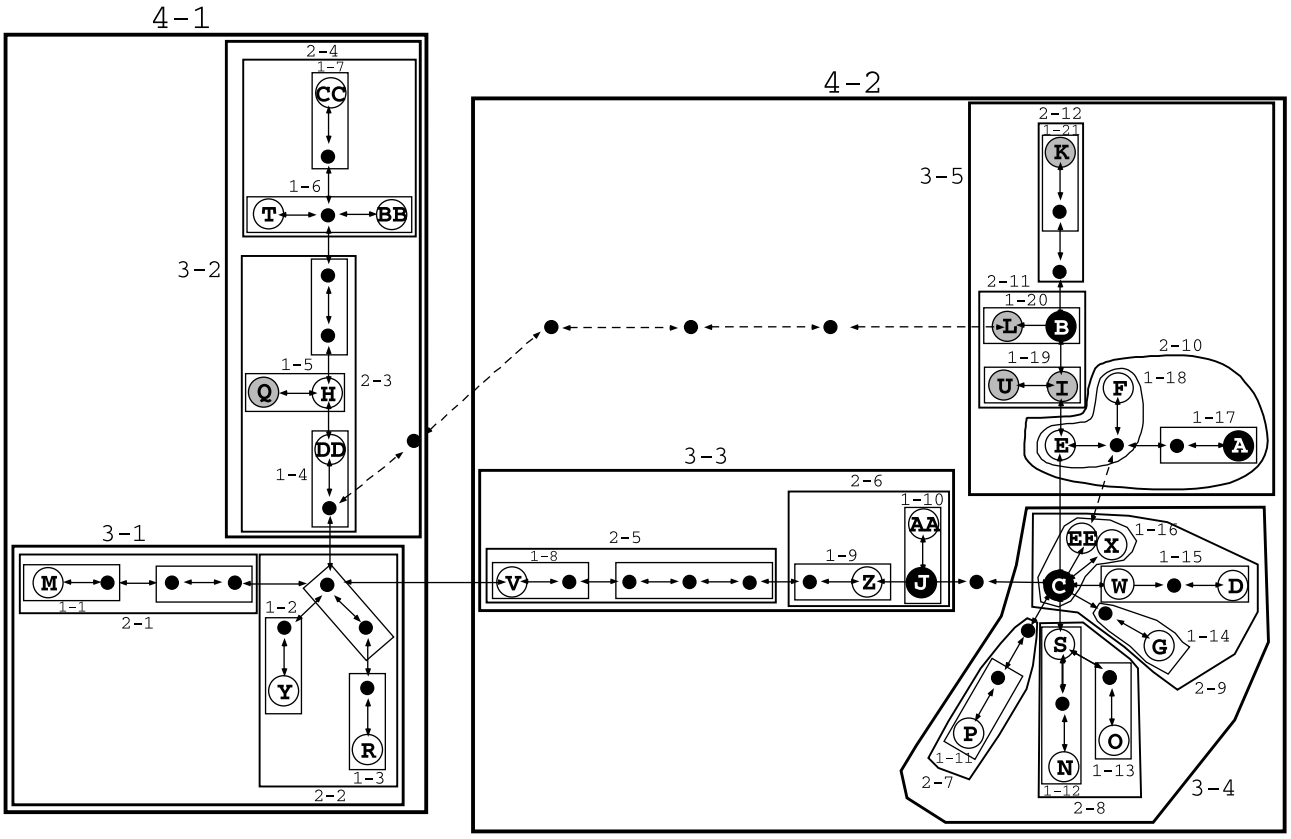

Fig. 2 Unrooted estimated 95% parsimony cladogram of 31 haplotypes detected in lesser prairie-chickens. Each line in the network represents a single mutational event. Haplotypes are represented by letters A–EE whereas solid circles represent intermediate haplotypes that are not present in the sample, but are necessary to link all observed haplotypes. Ambiguities (haplotypes interconnected forming a closed loop that can be broken at several places) are represented by dashed lines in the network. The five haplotypes identified by the grey circles (I, K, L, Q, U) were detected only in lesser prairie-chickens from New Mexico; the four haplotypes identified by black circles with white letters (A, B, C, J) were the most abundant haplotypes, being distributed throughout Oklahoma and New Mexico; the remaining 22 haplotypes indicated by open circles were restricted to lesser prairie-chickens from Oklahoma.

The distribution of haplotypes within clades 1–20, 2–10, 2–11, 4–1 and 4–2 are interpreted as being the result of contiguous range expansion. The null hypothesis was rejected in favour of restricted gene flow with isolation by distance for clades 2–9, 3–2 and 3–4. Although the null hypothesis was rejected for clades 1–18 and the total cladogram, the process that produced their distribution of haplotypes was inconclusive. Finally, the null hypothesis was rejected for clade 3–5 with past fragmentation contributing to its distribution of haplotypes.

Discussion

Woodward *et al*. (2001) examined 13 populations of lesser prairie-chicken from Oklahoma, Texas and New Mexico and found that in general, populations of lesser prairie-chickens were declining in Oklahoma but those in New Mexico remained stable. They attributed this decline in population number to greater landscape changes and greater loss of shrubland cover-types. Moreover, this loss appeared greater in Oklahoma because of smaller ownership tracts in Oklahoma. For example, Woodward *et al*. (2001) found that the landscape change index for Oklahoma landscapes changed at a rate of 11% per decade compared with 1% in New Mexico and 3% in Texas. One potential consequence of such habitat fragmentation is decreased genetic variation in isolated populations as a result of random genetic drift and inbreeding, which is manifested by the loss of heterozygosity, loss of alleles and mitochondrial haplotypes. Although a lack of genetic data or tissues from lesser prairie-chickens prior to habitat fragmentation and subsequent population declines hampers our estimation of genetic erosion, analysis of both mtDNA and microsatellite data indicate that at least in the southern portion of their range (Oklahoma and New Mexico), lesser prairie-chickens maintain high levels of genetic variation (Tables 1, 2). Moreover, levels of heterozygosity, allelic, nucleotide and haplotypic diversity were not different between Oklahoma and New Mexico, even though populations of lesser prairie-chickens in Oklahoma appear

less stable than those in New Mexico (Woodward *et al*. 2001).

Partitioning of genetic variation within leks, among leks within Oklahoma and New Mexico, and between Oklahoma and New Mexico for mitochondrial and nuclear loci produced similar results. Approximately 89% of the variation was partitioned within leks, whereas 3.0% (mtDNA) and 6.7% (microsatellites) of the variation was partitioned among leks within Oklahoma and New Mexico. Finally, the levels of genetic variation partitioned between Oklahoma and New Mexico for mtDNA (8.05%) and microsatellites (4.7%) suggest low historic levels of gene flow between Oklahoma and New Mexico. Although lesser prairie-chickens possess a lek system, in which gene flow is female biased, the higher levels of mitochondrial differentiation relative to the nuclear loci examined are most likely the result of the smaller effective population size of the mitochondrial genome.

Nested clade analysis has been shown to have more statistical power for detecting population subdivision than traditional *F*-statistics, and can distinguish between contemporary and historic processes (Templeton 1998). Twenty-five clades were detected in the mtDNA haplotype network for lesser prairie-chickens (Fig. 2). The null hypothesis of no association between haplotype distribution and geography could not be rejected for 13 of these clades; failure to reject the null hypothesis results from panmixia, small sample sizes, or inadequate geographical sampling. The 12 clades for which the null hypothesis was rejected along with the interpretation of why the null hypothesis was rejected are shown in Table 4.

Beginning with clade 4–1, the null hypothesis for this clade was rejected because of contiguous range expansion with each haplotype in this clade being detected in only one to three leks but with the totality of the nine haplotypes covering the geographical area sampled. Moving up the tree for clade 3–2, the null hypothesis was rejected in favour of restricted gene flow with isolation by distance. As discussed by Templeton (1998), restricted gene flow implies limited movement by individuals during any given generation; therefore, newly arisen haplotypes will not have had sufficient time to spread geographically. All haplotypes in clade 3–2 have restricted geographical distributions. Finally, the last clade in this portion of the haplotype network for which the null hypothesis was rejected is 1–5, and the conclusion drawn for this clade is long distance colonization. With long distance colonization, haplotypes found in the ancestral population serving as the source of the range expansion become geographically widespread and some of the haplotypes found in the expanding population therefore can become very distant from older haplotypes that are confined to the ancestral, pre-expansion area (Templeton 1998). Because the majority of haplotypes detected in the pre-expansion area are found in Ellis County, Oklahoma, the detection of haplotype Q in New Mexico documents long distance dispersal.

The same logic was used to draw inferences regarding the processes that gave rise to the distribution of haplotypes on the right side of the network. The only clade requiring further explanation is 3–5. For clade 3–5, the null hypothesis of no association between haplotype distribution and geography was rejected in favour of past fragmentation. This clade contains two widespread haplotypes (A and B), two haplotypes found throughout most Oklahoma leks sampled (E and F), and four of the five haplotypes restricted to New Mexico. Although samples were not available for our study, lesser prairie-chickens occur in Lipscomb, Hemphill and Wheeler counties in eastern Texas. These three counties are located on the border of Oklahoma and Texas and are adjacent to the Ellis County Oklahoma, sample, probably representing the same population of lesser prairie-chickens. Similarly, lesser prairie-chickens occur in Cochran, Hockley and Yoakum counties, Texas, which are in close proximity to the samples from Roosevelt County, New Mexico, and probably represent the same breeding population. Other than these six counties, lesser prairie-chickens are scarce or absent from the panhandle of Texas between our study areas. The conclusion of past fragmentation indicates that lesser prairie-chickens between Oklahoma and New Mexico have been fragmented or separated as a result of historic factors and that current anthropogenic factors that have fragmented habitat in the panhandle of Texas only continues to maintain this past fragmentation. However, such a conclusion must be cautionary for two reasons. First, past fragmentation typically is characterized by longer than average branch lengths (Templeton 1998). Even though four of the eight haplotypes in clade 3–5 are restricted to New Mexico, with two of the remaining clades being present in essentially all populations sampled (Fig. 2), we failed to detect longer than average branch lengths separating the clades nested within clade 3–5. Second, it is possible that breeding populations of lesser prairie-chickens occur in the northern portion of the panhandle of Texas as well as in northeastern New Mexico. If additional breeding populations of lesser prairie-chickens are found in these regions, it could provide an alternative route for gene flow between these populations.

In summary, although recent data suggest that habitat changes in Oklahoma may be contributing to the decline of lesser prairie-chickens in this portion of their range, both mitochondrial and nuclear loci indicate that they maintain levels of genetic variation similar to those found in lesser prairie-chickens from east-central New Mexico, where numbers seem to be stable, or possibly increasing (Woodward *et al*. 2001). However, future studies on lesser prairie-chickens should (i) ascertain whether breeding populations occur in the Texas panhandle, and, if so, determine levels of genetic

differentiation and gene flow among Oklahoma, New Mexico and Texas populations, and (ii) include samples from populations in Colorado and Kansas to assess genetic variability within and differentiation among the remaining relict populations of lesser prairie-chickens.

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This work is the result of a collaborative project to understand better the effects of population declines and habitat fragmentation on the viability of populations of lesser prairie-chickens. R.A.V.D.B. is an Associate Professor in the Department of Zoology at Oklahoma State University and S.R.H. is a PhD student working under his guidance. These two authors are interested in the processes that give rise to phylogeographic and phylogenetic patterns. D.A.W., D.H.S. and S.K.S. are research staff at the Sutton Avian Research Center, a unit of the Oklahoma Biological Survey, University of Oklahoma. Their interests are in the ecology and conservation biology of lesser prairie-chickens.
