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RESEARCH ARTICLE

Rangewide genetic analysis of Lesser Prairie-Chicken reveals population structure, range expansion, and possible introgression

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Abstract The distribution of the Lesser Prairie-Chicken (*Tympanuchus pallidicinctus*) has been markedly reduced due to loss and fragmentation of habitat. Portions of the historical range, however, have been recolonized and even expanded due to planting of conservation reserve program (CRP) fields that provide favorable vegetation structure for Lesser Prairie-Chickens. The source population(s) feeding the range expansion is unknown, yet has resulted in overlap between Lesser and Greater Prairie-Chickens (*T. cupido*) increasing the potential for hybridization. Our objectives were to characterize connectivity and genetic diversity among populations, identify source population(s) of recent range expansion, and examine hybridization with the Greater Prairie-Chicken. We analyzed 640 samples from across the range using 13 microsatellites. We identified three to four populations corresponding largely to ecoregions. The Shinnery Oak Prairie and Sand Sagebrush Prairie represented genetically distinct populations ($F_{ST} > 0.034$ and $F_{ST} > 0.023$ respectively). The Shortgrass/CRP Mosaic and Mixed Grass ecoregions appeared admixed ($F_{ST} = 0.009$). Genetic diversity was similar among ecoregions and N_e ranged from 142 (95 % CI 99–236) for the Shortgrass/CRP Mosaic to 296 (95 % CI 233–396) in the Mixed Grass Prairie. No recent migration was detected among ecoregions, except asymmetric dispersal from both the Mixed Grass Prairie and to a lesser extent the Sand Sagebrush Prairie north into adjacent Shortgrass/CRP Mosaic ($m = 0.207$, 95 % CI 0.116–0.298, $m = 0.097$, 95 % CI 0.010–0.183, respectively). Indices investigating potential hybridization in the Shortgrass/CRP Mosaic revealed that six of the 13 individuals with hybrid phenotypes were significantly admixed suggesting hybridization. Continued monitoring of diversity within and among ecoregions is warranted as are actions promoting genetic connectivity and range expansion.

Keywords *Tympanuchus pallidicinctus* · Spatial genetic population structure · Gene flow · Genetic diversity · Hybridization

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Introduction

Effective management and conservation of a threatened species requires information about connectivity and spatial structure of populations throughout its geographic range. The size of populations, their spatial configuration in the landscape, and the capacity with which individuals move

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through their environment are all key factors that affect how species are able to respond to changing environments (Gilpin and Hanski 1991; Hanski 1998; Noon et al. 2003). In the face of ongoing habitat loss and fragmentation, managers must consider facilitating inter-population movement where appropriate by protecting or developing corridors essential to maintain connectivity (Morrison and Reynolds 2006). The ongoing effort to protect North American grassland birds is a good example of how conservation requirements and economic progress may disagree in the management of prairie habitat. Many grassland bird species have declined in range and census size by >80 % during the past 150 years, and numerous management strategies exist among species as a result of such declines (Brennan and Kuvlesky 2005).

The Lesser Prairie-Chicken (*Tympanuchus pallidicinctus*), is one of three obligate grassland species in the genus *Tympanuchus* or "prairie grouse" given their adaptation to tall and short-grass habitats in North America. Conservation concern for the Lesser Prairie-Chicken dates back to the Dust Bowl era, when the species was thought extinct after a decade of catastrophic habitat conditions in the 1930s (USFWS 1966). More recent declines in the species' distribution at approximately 16 % of the estimated historical range at the time of European settlement (Giesen and Hagen 2005; Van Pelt et al. 2013) have resulted in its listing as a threatened species under the U. S. Endangered Species Act (USFWS 2014), although that ruling is currently under litigation. In addition to overall range contraction, both agricultural and urban development have divided the geographic range into at least two widely separated regional populations (Giesen and Hagen 2005). The Lesser Prairie-Chicken currently persists in four ecoregions distributed among five states in the central United States including: (1) a mosaic of Shortgrass Prairie intermixed with land enrolled in the conservation reserve program (CRP) in northwestern Kansas; (2) Sand Sagebrush (*Artemisia filifolia*) Prairie in eastern Colorado, western Kansas and the Oklahoma panhandle; (3) Mixed Grass Prairie in south-central Kansas, northwestern Oklahoma, and the northeastern Texas panhandle; and (4) Shinnery Oak (*Quercus havardii*) Prairie of eastern New Mexico and the western Texas panhandle (Fig. 1; McDonald et al. 2014).

The primary threats to the Lesser Prairie-Chicken are habitat loss and fragmentation that have mostly resulted from anthropogenic land use, such as conversion of grassland habitat for agriculture, encroachment of eastern redcedar (*Juniperus virginianus*) due to fire suppression, and energy development, that are incompatible with the species' life-history (Woodward et al. 2001; Pruett et al. 2009; Hagen et al. 2011; Pruett et al. 2011). It is unclear how the species' demography and genetic diversity have been impacted by such changes to the landscape. The effects of habitat fragmentation and small population size on genetic diversity have been well-documented in other grouse species including the Greater Prairie-Chicken (*T. cupido*; Bouzat et al. 1998; Westemeier et al. 1998; Johnson et al. 2004), Greater Sage-Grouse (*Centrocercus urophasianus*; Benedict et al. 2003; Oyler-McCance et al. 2005a, 2014) and Gunnison Sage-Grouse (*C. minimus*; Oyler-McCance et al. 2005b). Several isolated or peripheral populations of Lesser Prairie-Chickens have been documented to have lower genetic diversity than continuous populations (Bouzat and Johnson 2004; Hagen et al. 2010; Corman 2011; Pruett et al. 2011). To date, regional scale studies based on mitochondrial DNA (mtDNA) suggest relatively high levels of genetic connectivity across the species' range, yet such patterns likely represent historical rather than current connectivity (Van den Bussche et al. 2003; Hagen et al. 2010; Pruett et al. 2011).

The species' reproductive behavior likely contributes to its increased susceptibility to genetic diversity loss with declining population size. The Lesser Prairie-Chicken, like other prairie grouse, possesses a lek breeding behavior with high variance in male mating success (Nooker and Sandercock 2008; Stiver et al. 2008). Species with highly skewed mating systems typically have reduced effective population sizes (N_e) compared to census size because a high proportion of males do not reproduce, thereby increasing the loss of overall genetic diversity due to genetic drift when populations are small. Reduced population connectivity and a subsequent decline in N_e in the Greater Prairie-Chicken led to increased genetic drift and inbreeding depression as documented by reduced hatching success (Westemeier et al. 1998; Bouzat et al. 2009) and reduced diversity among immune-related genes (Bollmer et al. 2011; Eimes et al. 2011; Bateson et al. 2015). As such, documenting and monitoring levels of N_e is an important tool for the conservation and management of Lesser Prairie-Chicken.

The Lesser Prairie-Chicken rangewide population trends remain precarious. However, recent responses to changes in available habitat both inspire optimism yet raise additional concerns (Garton et al. 2016). Portions of the historical range in Kansas were recently reoccupied by Lesser Prairie-Chickens, and the distribution in northwestern Kansas now extends beyond the species' known historical range (Fig. 1; Giesen and Hagen 2005; Van Pelt et al. 2013). The current range shifts have coincided with the planting and maintenance of CRP fields that provide the necessary vegetation structure for Lesser Prairie-Chicken throughout the shortgrass prairies (Rodgers and Hoffman 2005; Ripper et al. 2008). Population trends in this region have increased with changes in distribution, yet the source population(s) involved in the range expansion are not known. Moreover, the range expansion has resulted in geographic overlap between Lesser and Greater Prairie-

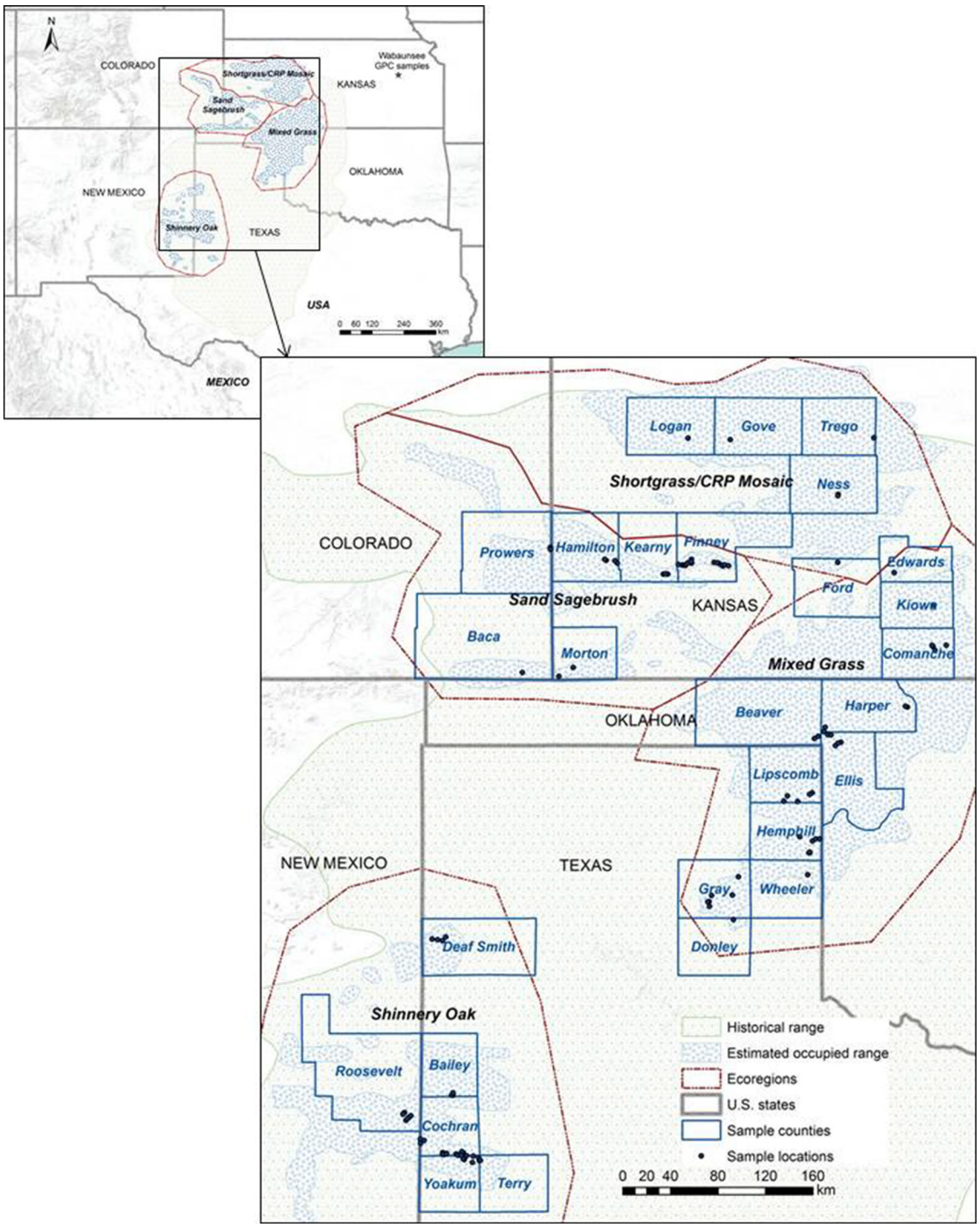

Fig. 1 Map of Lesser Prairie-Chicken range with sampling locations, ecoregions, and historical distribution noted

Chickens, increasing the potential for hybridization, genetic introgression, and conservation status complications (Bain and Farley 2002, USFWS 2012).

Fewer genetic studies have focused on the Lesser Prairie-Chicken relative to the Greater Prairie-Chicken. Therefore, the effects of habitat loss and fragmentation on population structure, population connectivity, and genetic diversity remain largely unknown for the Lesser Prairie-Chicken. Our goal in this study was to characterize the genetic structure and gene flow throughout the species' geographic distribution to identify potential threats to population viability and aid in conservation and management. Specifically, our objectives at the range-wide scale were to (1) characterize patterns of connectivity, (2)

document levels of genetic variability among populations, (3) identify the source population(s) for the region of recent range expansion, and (4) determine the level of introgression with the Greater Prairie-Chicken in areas where the two species overlap in distribution.

Methods

Sample collection

We obtained Lesser Prairie-Chicken samples from 28 counties throughout its current geographic distribution (Fig. 1; Table 1). Most samples (588 or 92 %) were obtained from blood or plucked feathers taken from individuals captured in passive funnel traps during the spring and fall seasons as described previously (Hagen et al. 2010; Pruett et al. 2011). In addition, feather samples were collected non-invasively from the ground on leks in geographic areas previously not sampled. Blood samples were obtained by clipping a toenail or by drawing from the brachial vein of each individual and stored in microfuge tubes coated with EDTA (Brinkman). All blood samples were kept frozen at −20 °C, whereas feather samples were stored in dry paper envelopes until DNA extraction.

To investigate potential hybridization, we also sampled areas in northwestern Kansas (Gove, Ness, and Trego Counties), where the Lesser and Greater Prairie-Chickens are sympatric. A total of 49 Lesser and 28 Greater Prairie-Chickens were sampled in this area, as well as six putative hybrids based on morphology and behavior and seven individuals that were possible hybrids based on morphology, yet not verified by vocalization. We also obtained samples from 20 Greater Prairie-Chickens collected in east Kansas (Wabaunsee County; Johnson et al. 2003), >300 km from the closest Lesser Prairie-Chicken population, to use as a more geographically distant Greater Prairie-Chicken population for comparison purposes where hybridization is unlikely.

Table 1 Lesser Prairie-Chicken samples collected in this study

State	County	*n*
Colorado	Baca	8
	Prowers	12
Kansas	Comanche	17
	Edwards	7
	Finney	120
	Ford	5
	Gove	32
	Hamilton	32
	Kearny	27
	Kiowa	8
	Logan	2
	Morton	2
	Ness	9
	Trego	8
Oklahoma	Beaver	40
	Ellis	49
	Harper	26
New Mexico	Roosevelt	48
Texas	Bailey	5
	Cochran	30
	Deaf Smith	15
	Donley	4
	Gray	27
	Hemphill	30
	Lipscomb	26
	Terry	22
	Wheeler	1
	Yoakum	28

DNA extraction and microsatellite amplification

DNA was extracted from blood using either standard phenol–chloroform methods or an ammonium acetate protocol (modified from the PUREGENE kit; Gentra Systems). DNA was isolated from feather samples using a DNeasy 96 Blood and Tissue Kit (QIAGEN) following the user-developed protocol for the DNA purification from nails, hair, or feathers with the exception of a modified elution step (eluted in 100 µl Buffer AE after 5 min incubation at room temperature). Samples were amplified across 13 microsatellite loci: MSP11, SGMS06.2, SGMS06.3 (Oyler-McCance and John 2010), TUT3, TUD1 (Segelbacher et al. 2000), BG6, BG18 (Piertney and Höglund 2001), TTT1, TTD6 (Caizergues et al. 2001), ADL230 (Cheng et al. 1995), SG24, SG27, SG31 (Fike et al. 2015). The sex of each sample was determined by amplifying a region of the CDH gene using the primers 1237L and 1272H (Kahn et al. 1998). Amplifications were performed in four 10 µL multiplexed PCRs.

The loci were grouped based on their annealing temperature, size and primer label. Multiplexes were comprised of the following loci: Set 1a—MSP11, SGMS06.2, and BG18; Set 1b—TTT1 and TTD6; Set 2—SG24, ADL230, TUD1, and SG31; Set 3—TUT3, SGMS06.3, BG6, SG27, and 1237L/1272H (a locus that identifies gender). All PCRs consisted of 2–40 ng of template DNA, 0.2 mM of each dNTP, 1 U GoTaq Flexi DNA polymerase (Promega), 2.25 mM $MgCl_2$ and 1X GoTaq Flexi Buffer (Promega). Primer concentration varied depending on locus intensity and primer label type. M13-labeled primer concentrations (loci SG27 and SG31) were 0.03 µM of

m13-tailed forward primer, 0.075 μM of reverse primer, and 0.075 μM of m13 dye-labeled primer, and the remaining dye-labeled primer concentrations ranged from 0.075 to 0.20 μM.

Amplification conditions for multiplex sets 1a, 2, and 3 were as follows: 94 °C for 2 min, then 94 °C for 1 min, annealing temperature (set 1a: 53 °C, set 2: 60 °C, set 3: 57 °C) for 1 min, 72 °C for 1 min for 40 cycles, then 60 °C for 45 min and a final extension at 72 °C for 10 min. Multiplex set 1b was amplified using a 'Touchdown' protocol: 94 °C for 3 min, 10 cycles of 30 s denaturation at 94 °C, 30 s of annealing starting at 65 °C and dropping by 1 °C per cycle, and 30 s of extension at 72 °C, followed by a further 20 cycles consisting of 30 s denaturation at 94 °C, 30 s of annealing at 55 °C, 30 s of extension at 72 °C, with a 10 min period of extension at 72 °C following the last annealing step. PCR products from sets 1a and 1b were loaded together whereas sets 2 and 3 were run separately on an AB3500 Genetic Analyzer (Applied Biosystems). PCR products were combined with GeneScan LIZ 600 internal lane size standard. Allele sizes were determined for each locus using GENEMAPPER v4.1 software (Applied Biosystems).

All feather samples collected non-invasively were amplified at least twice at each locus to verify genotypes. Repeated amplification resulted in the generation of multiple multi-locus genotypes for each feather sample. Multilocus genotypes were compared and a consensus genotype was generated. As is common with low quantity/quality DNA, some genotypes did not match with both amplification attempts. For mismatch genotypes, individuals were scored as heterozygotes at a locus if they were heterozygous in at least one of the PCR attempts. Genotypes were scored as missing data if more than two alleles were obtained for two amplification attempts. We further assessed both allelic dropout and identified feather samples belonging to the same individual using the program DROPOUT (McKelvey and Schwartz 2005). After removing duplicate samples and those that did not amplify well, a total of 52 additional individuals were identified from the non-invasive feather samples.

Analysis of genetic diversity

Multiple sampled counties (particularly those on the periphery of the species' range) had small sample sizes (<10 individuals, Table 1) and were subsequently combined with neighboring counties to form sampling locales (Table 2). Further, we consolidated counties into the four ecoregions identified by McDonald et al. (2014) and calculated summary statistics both by sampling locale and by ecoregion (Table 2). Microsatellite loci were tested by sampling locale for departures from Hardy–Weinberg equilibrium (Guo and Thompson 1992) using the program ARLEQUIN 2.001 (Excoffier and Lischer 2010) after correcting for multiple comparisons using the B-Y False Discovery Rate method (Narum 2006), resulting in $\alpha = 0.008$.

The mean number of microsatellite alleles per locus and observed (H_o) and expected (H_e) heterozygosity values for each sampling locale and ecoregion were calculated using GENALEX (Peakall and Smouse 2012). Allelic richness, which adjusts for discrepancies in sample size by incorporating a rarefaction method, was estimated in FSTAT 2.9.3.2 (Goudet 1995). We tested whether the allelic richness and observed heterozygosity differed significantly between all pairs of ecoregions using two tailed Wilcoxon matched-pairs signed-ranks tests. We also tested whether the sampling locale with the lowest allelic richness was significantly lower than other sampling locales using a one-tailed Wilcoxon matched-pairs signed ranks test. Allelic richness values between ecoregions were considered to be significantly different at $\alpha \leq 0.020$ after controlling for multiple comparisons, while differentiation between the lowest sampling locale and all others was significant at $\alpha \leq 0.014$. We also estimated N_e for each sampling locale and ecoregion using the linkage disequilibrium method (Waples and Do 2008), as implemented in NeESTIMATOR V2.01 (Do et al. 2014). To limit bias, alleles with frequencies <2 % were not included in the N_e estimates.

Analysis of genetic structure

We calculated pairwise F_{ST} values among both sampling locales and ecoregions using ARLEQUIN 2.001 (Excoffier and Lischer 2010). Differences were tested using 10,000 permutations among groups with Fisher's exact test. Sampling locales were considered significantly different at $\alpha \leq 0.009$ after controlling for multiple comparisons ($n = 153$), while differentiation between ecoregions (6 comparisons) was significant at $\alpha \leq 0.020$. To explore isolation-by-distance, we computed pairwise values of Weir and Cockerham's (1984) F_{ST} among sampling locales using the program SPAGeDi 1.4 (Hardy and Vekemans 2002); statistical significance was assessed using 10,000 permutations of individuals between pairs of groups. Next, we computed linearized F_{ST}, $F_{ST}/(1 - F_{ST})$, because this quantity is expected to have a linear relationship with geographic distance (Rousset 1997). We tested the correlation between linearized F_{ST} and geographic distance among sampling locales using a Mantel procedure in the computer program ISOLATION BY DISTANCE WEB SERVICE (Jensen et al. 2005). The intercept and slope were estimated using a reduced major axis regression (Hellberg 1994) and statistical significance was assessed based on 1000 permutations.

To further examine genetic structure, we used STRUCTURE 2.00 (Pritchard et al. 2000), where individuals were assigned to clusters without a priori assignment

Table 2 Genetic diversity measures and effective population size for sampling locales (combining neighboring counties with small samples) broken out by ecoregion

Ecoregion	N	Mean # alleles	H_o	H_e	AR	N_e	Jackknife CI for N_e
Sand Sagebrush Prairie							
Prowers	12	5.46	0.75	0.72	5.28	109.2	36–∞
Hamilton	32	7.00	0.77	0.75	5.54	27.3	22–36
Kearny	27	7.00	0.71	0.75	5.69	47.4	34–76
Finney	120	8.31	0.75	0.74	5.64	104.8	84–136
Baca/Morton	10	5.46	0.75	0.70	5.46	∞	54–∞
All Sand Sagebrush Prairie combined	201	8.85	0.75	0.75	7.91	198	159–254
Shortgrass/CRP Mosaic							
Logan/Gove/Trego/Ness/Ford	56	8.92	0.73	0.76	6.21	141.6	99–236
All Shortgrass/CRP Mosaic combined	56	8.85	0.73	0.76	8.81	142	99–236
Mixed Grass Prairie							
Edwards/Kiowa/Comanche	32	7.54	0.75	0.74	5.96	46.9	35–67
Harper	26	8.00	0.76	0.75	6.25	145.5	68–∞
Beaver	40	7.92	0.71	0.75	6.07	80.5	61–115
Ellis	49	7.92	0.73	0.75	6.00	95.8	67–156
Lipscomb	26	7.15	0.71	0.74	5.94	68.2	41–163
Hemphill	30	7.00	0.71	0.72	5.55	20.5	16–28
Wheeler/Gray/Donely	32	7.00	0.65	0.69	5.62	39.5	28–62
All Mixed Grass Prairie combined	235	10.46	0.72	0.76	9.14	296	233–396
Shinnery Oak Prairie							
Deaf Smith	15	4.92	0.69	0.62	4.61	37.8	22–102
Roosevelt	48	7.62	0.71	0.71	5.53	91.6	64–151
Bailey/Cochran	35	7.39	0.69	0.72	5.67	205.3	85–∞
Yoakum	28	6.77	0.73	0.73	5.59	251.1	86–∞
Terry	22	6.69	0.70	0.70	5.61	134	59–∞
All Shinnery Oak Prairie combined	148	8.39	0.71	0.73	7.83	252	179–404

N Sample size, H_o observed heterozygosity, H_e expected heterozygosity, AR allelic richness, and N_e effective population size

to a particular sampling locale or ecoregion while assuming an admixed model of population structure and correlated allele frequencies. The most likely number of unique genetic clusters (K) given the data was initially estimated by conducting ten independent runs each for $K = 1$ to 10 with 500,000 Markov Chain Monte Carlo (MCMC) repetitions and using 250,000 initial iterations as the burn-in period. We explored the optimal value of K using two methods. First, we plotted the average [Pr($X|K$)] values across ten runs for each value of K from STRUCTURE and chose the smallest K where the highest likelihood values plateau (Pritchard et al. 2000). Second, we calculated ΔK where the highest ΔK represents the likely value of genetic clusters (Evanno et al. 2005). We visualized these metrics in STRUCTURE HARVESTER (Earl and VonHoldt 2012). The use of multiple runs to evaluate K in STRUCTURE can produce several distinct solutions due to multimodality and label switching across replicates. To alleviate this issue, we used CLUMPP to produce our final output (Jakobsson and Rosenberg 2007). Results from CLUMPP were visualized using the software DISTRUCT (Rosenberg 2004).

Population structure also was explored using the software program GENELAND version 4.0, which uses both genotype data and the spatial information associated with each sample to estimate the number of unique genetic clusters or populations (Guillot et al. 2005). We used the spatial model with uncorrelated allele frequencies in GENELAND to infer K among all Lesser Prairie-Chicken samples. In our initial runs, K was allowed to vary from 1 to 15 under the following conditions: 10,000 stored iterations of the Markov Chain, maximum rate of the Poisson process equal to the number of samples, minimum population set to 1 and maximum to 15, and the number of nuclei in the Poisson–Voronoi tessellation set at three times the sample size (Guillot et al. 2005). The noise parameter associated with uncertainty in the spatial coordinate was set to 100, which corresponds to $\sim$1 km. Convergence was

verified by comparing results with longer runs of the Markov chain.

We used spatial autocorrelation to quantify fine-scale genetic structure within the four sampled ecoregions. We used Moran's *I* (Moran 1950; Sokal and Oden 1978) to assess spatial autocorrelation because Moran's *I* has been well tested in empirical studies and simulations (Hardy and Vekemans 1999; Epperson 2004). We computed mean pairwise values of Moran's *I* within pre-defined distance intervals; standard errors were estimated by jackknifing over loci. To assess statistical significance, we used 10,000 permutations of individuals among spatial groups for within-lek comparisons and spatial group locations among spatial groups for distance intervals. We used 2.5-km distance intervals to allow about 100 pairs of individuals in each distance interval, enabling more precise estimates of autocorrelation. Because fewer females were sampled, we analyzed females at 5-km intervals to increase the number of pairs per interval. Analyses of spatial autocorrelation were performed using the computer program SPAGeDi 1.3a (Hardy and Vekemans 2002).

Analysis of contemporary gene flow

A Bayesian assignment-based method using multi-locus genotypes as implemented in BayesAss v.3.0.3 (Wilson and Rannala 2003) was used to estimate the direction and rate of contemporary gene flow between designated Lesser Prairie-Chicken ecoregions. The method does not assume that sampled populations are at genetic equilibrium with respect to drift or mutation or that genotypes are in Hardy–Weinberg equilibrium, but it does assume that loci used in the analysis are in linkage equilibrium for each population. Each run was performed with 20×10^6 iterations after a burn-in of 2×10^6 and with a sampling frequency of 100. Delta values for allele frequency, mutation rate, and inbreeding were adjusted to where accepted numbers of changes were between 40 and 60 % of the total number of iterations. To minimize convergence problems, 10 runs with different initial seeds were conducted and the one with the lowest Bayesian deviance value was selected for further analysis (Faubet et al. 2007; Meirmans 2014). The migration rate (m) into a population per generation is estimated as the proportion of individuals in the population of interest that is derived from another population (Wilson and Rannala 2003).

Analysis of hybridization

To look for evidence of hybridization between Lesser and Greater Prairie-Chickens in the area of geographic overlap in the Shortgrass/CRP ecoregion, we calculated a hybridization index HINDEX (Buerkle 2005) in the R package INTROGRESS (Gompert and Alex Buerkle 2010) for each of the putative hybrids and the unknown samples. The hybridization index is the proportion of alleles inherited from each of two parental populations and ranges from 0.0 to 1.0, where 0.0 is represented by a Greater Prairie-Chicken from Eastern Kansas and 1.0 is represented by a Lesser Prairie-Chicken from the Mixed Grass Prairie. An intermediate value indicates introgression of parental populations, or a hybrid individual.

Results

Genetic diversity

For most individuals (88.4 %), all loci amplified effectively, producing complete multilocus genotypes for 13 loci. Of the 234 locus/sampling locale combinations, only four were not in Hardy–Weinberg Equilibrium (HWE) and they occurred in different loci in different sampling locales. Because all of the loci consistently failed to reject HWE across sampling locales, we used all 13 loci for our analyses. The mean number of alleles per sampling locale ranged from 4.92 to 8.31 and among ecoregions ranged from 8.39 to 10.46 (Table 2, Supplementary Table 1). Among sampling locales, H_o values were similar (Table 2), with the highest value in Hamilton (0.77) and the lowest in Wheeler/Gray/Donely (0.65). Among ecoregions, H_o values were also similar, ranging from 0.71 in the Shinnery Oak Prairie to 0.75 in the Sand Sagebrush Prairie. Allelic richness was similar among sampling locales with the highest (6.25) in Harper and lowest (4.61) in Deaf Smith. Allelic richness in Deaf Smith was significantly lower ($P < 0.01$) than all but two other sampling locales. Among ecoregions, allelic richness was generally similar (Table 2) with only Mixed Grass Prairie possessing a significantly greater value than Sand Sagebrush Prairie ($P < 0.02$).

N_e could not be estimated in all samples because the calculation resulted in infinite confidence intervals (Table 2). Among sampling locales, the point estimate of N_e ranged from 20.5 (95 % CI 16–28) in Hemphill to 104.8 (95 % CI 84–136) in Finney, yet some confidence intervals were wider (Table 2). Based on ecoregions, N_e ranged from 142 (95 % CI 99–236) for the Shortgrass/CRP Mosaic to 296 (95 % CI 233–396) in the Mixed Grass Prairie, although the confidence intervals overlapped in all comparisons (Table 2).

Genetic structure

Sampling locale pairwise F_{ST} values varied from 0.002 to 0.134 (Table 3). A total of 144 out of 153 pairwise comparisons (94 %) were significant ($P < 0.009$), most of which were between sampling locales in different ecoregions (111/144; 77 %). Only one (Harper & Kearny) of the

Table 3 Pairwise F_{ST} values between all pairs of sampling locales

	Prowers	Hamilton	Kearny	Finney	B/M	L/G/T/N/F	E/K/C	Harper	Beaver	Ellis	Lipscomb	Hemphill	W/G/D	Deaf Smith	Roosevelt	BC	Yoakum
Prowers																	
Hamilton	**0.017**																
Kearny	0.008	**0.014**															
Finney	**0.023**	**0.014**	**0.019**														
Baca/Morton	0.020	**0.028**	0.032	0.013													
Logan/Gove/Trego/Ness/Ford	**0.034**	**0.030**	**0.021**	**0.027**	**0.036**												
Edwards/Kiowa/Comanche	**0.041**	**0.031**	**0.020**	**0.035**	**0.039**	**0.018**											
Harper	**0.028**	**0.032**	0.018	**0.040**	**0.044**	**0.011**	**0.018**										
Beaver	**0.039**	**0.033**	**0.030**	**0.041**	**0.052**	**0.013**	**0.024**	**0.011**									
Ellis	**0.040**	**0.033**	**0.025**	**0.035**	**0.048**	**0.014**	**0.014**	0.005	**0.008**								
Lipscomb	**0.041**	**0.033**	**0.022**	**0.033**	**0.035**	**0.017**	**0.023**	**0.018**	**0.013**	**0.009**							
Hemphill	**0.053**	**0.056**	**0.037**	**0.058**	**0.072**	**0.025**	**0.045**	**0.022**	**0.029**	**0.024**	**0.033**						
Wheeler/Gray/Donely	**0.067**	**0.056**	**0.046**	**0.058**	**0.073**	**0.032**	**0.040**	**0.033**	**0.032**	**0.023**	**0.024**	**0.048**					
Deaf Smith	**0.118**	**0.115**	**0.098**	**0.107**	**0.110**	**0.078**	**0.115**	**0.096**	**0.095**	**0.104**	**0.101**	**0.134**	**0.121**				
Roosevelt	**0.075**	**0.062**	**0.054**	**0.059**	**0.056**	**0.041**	**0.051**	**0.043**	**0.049**	**0.045**	**0.043**	**0.084**	**0.053**	**0.066**			
Bailey/Cochran	**0.069**	**0.049**	**0.051**	**0.046**	**0.050**	**0.035**	**0.045**	**0.046**	**0.049**	**0.042**	**0.045**	**0.086**	**0.051**	**0.062**	**0.009**		
Yoakum	**0.060**	**0.050**	**0.051**	**0.053**	**0.055**	**0.035**	**0.046**	**0.037**	**0.046**	**0.042**	**0.047**	**0.079**	**0.053**	**0.045**	**0.017**	−0.002	
Terry	**0.055**	**0.046**	**0.038**	**0.050**	**0.063**	**0.032**	**0.043**	**0.031**	**0.046**	**0.038**	**0.046**	**0.078**	**0.048**	**0.076**	**0.015**	0.007	0.004

Values shown in bold are statistically significant. Within Eco-region comparisons are shaded

Table 4 Pairwise F_{ST} values between all pairs of ecoregions

	Sand Sagebrush Prairie	Shortgrass/CRP Mosaic	Mixed Grass Prairie
Sand Sagebrush Prairie			
Shortgrass/CRP Mosaic	**0.023**		
Mixed Grass Prairie	**0.027**	**0.009**	
Shinnery Oak Prairie	**0.047**	**0.034**	**0.039**

Values shown in bold are statistically significant

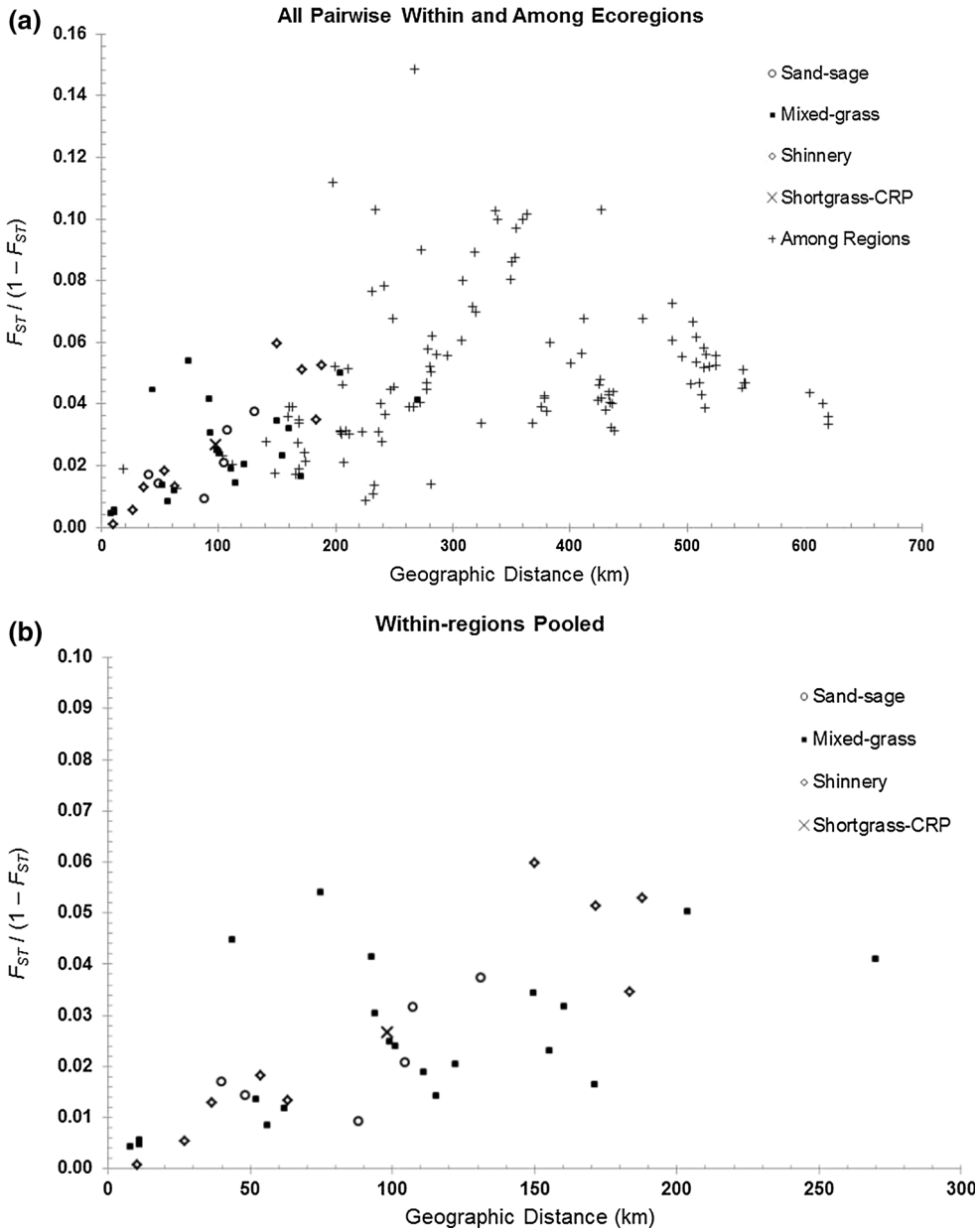

Fig. 2 Relationship between linearized F_{ST} and geographic distance across the entire range (**a**) and within ecoregions (**b**)

9 sampling locale pairwise comparisons that was not significant were in different ecoregions. When sampling locales within an ecoregion were combined, pairwise F_{ST} values among all ecoregions were significant, suggesting little or no gene flow among ecoregions (Table 4). We observed a statistically significant correlation between linearized F_{ST} and geographic distance (Fig. 2a) over the entire dataset ($R = 0.46$, $P < 0.001$) suggesting an isolation-by-distance pattern of population structure. Within ecoregions (Fig. 2b), the only significant correlation

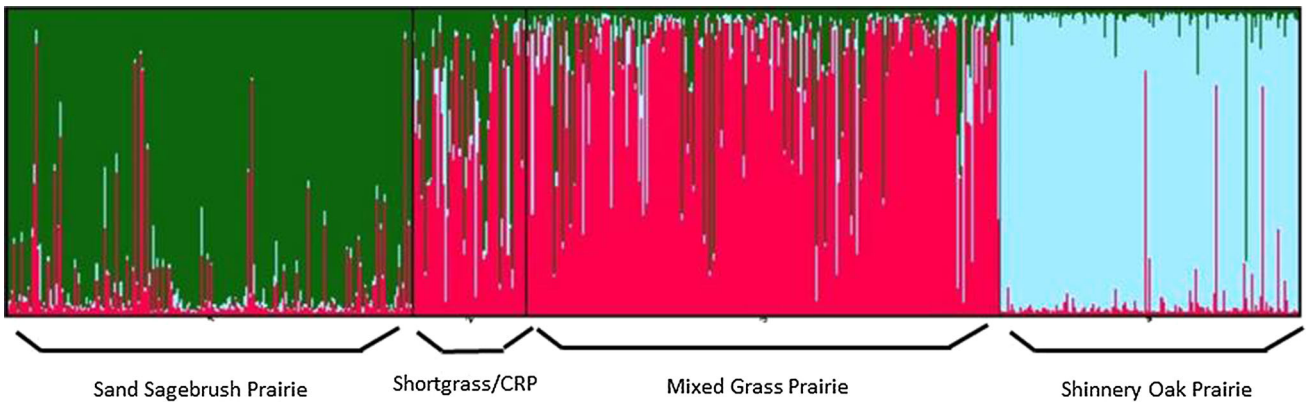

Fig. 3 Estimated population genetic structure based on allele frequency variation from 13 microsatellite loci as calculated in STRUCTURE. Ecoregions and sampling locales are ordered in a north to south direction. Genetic structure among all individuals with the optimal number of distinct genetic clusters (K) of three. Each distinct cluster is represented by a *unique color*. Each *vertical bar* represents an individual Lesser Prairie-Chicken. The *colors* on each *vertical bar* represent the individual's estimated membership in each of the three unique genetic clusters. (Color figure online)

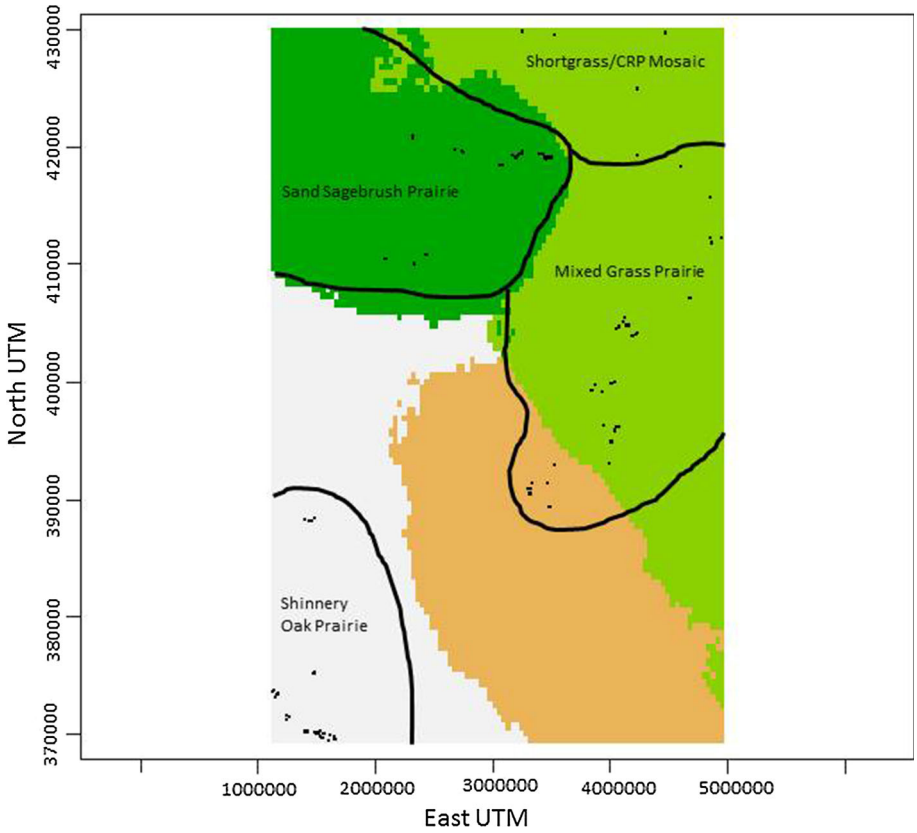

Fig. 4 Map delineating estimated cluster membership of the four distinct genetic clusters of Lesser Prairie-Chickens based on allele frequency variation from 13 microsatellite loci as calculated in GENELAND. The *four colors* represent the four different genetic clusters. Geneland assigned all individuals in the Shinnery Oak Prairie ecoregion to the cluster shown in white and all individuals in the Sand Sagebrush Prairie ecoregion to the cluster shown in *dark green*. All individuals from the Shortgrass/CRP Mosaic clustered with the northern part of the Mixed Grass Prairie ecoregion, while the southern part of the Mixed Grass Prairie group made up their own cluster. Ecoregion boundaries are approximate. (Color figure online)

between linearized F_{ST} and geographic distance occurred in the Shinnery Oak region ($R = 0.93$, $P = 0.037$), yet there was only one data point in the Shortgrass/CRP.

In the STRUCTURE analysis, both criteria to choose the optimal number of genetic clusters supported $K = 3$ (Fig. 3). One cluster was made up largely of Lesser Prairie-Chickens from the Sand Sagebrush Prairie (shown in green). The second cluster (red) was comprised of birds from the Shortgrass/CRP Mosaic and the Mixed Grass Prairie, while the third cluster (blue) formed the Shinnery Oak Prairie ecoregion (Fig. 3). GENELAND results were similar to those obtained with STRUCTURE analysis, with the exception that GENELAND inferred the highest likelihood for $K = 4$ (Fig. 4). Similar to STRUCTURE, the Sand Sagebrush Prairie was identified as a distinct cluster (dark green), and the Shortgrass/CRP belonged to a second

cluster along with the northern Mixed Grass Prairie samples (light green; Fig. 4). Individuals in the southern portion of the Mixed Grass Prairie were assigned to third cluster (orange) and all of the samples from the Shinnery Oak Prairie (white) formed a distinct fourth cluster.

We observed statistically significant positive autocorrelation of Moran's *I* for males in all 4 regions (Fig. 5). Data were sparse for the Sand Sagebrush Prairie, with no pairs of males in distance intervals >10 km, and too few pairs to analyze for females overall; few pairs of females were available between 5 and 20 km in the Shortgrass/CRP Mosaic region. For males, autocorrelation became non-significant after the third or fourth distance class for the Shinnery Oak Prairie, Mixed Grass Prairie, and Shortgrass/CRP Mosaic regions, suggesting an intercept of about 5–7 km. The Sand Sagebrush Prairie displayed positive autocorrelation only for within-lek comparisons, suggesting an intercept of <1 km. Within-lek autocorrelation values were positive and statistically different from 0 in all but the Shinnery Oak Prairie. Females displayed lower autocorrelation values in general than males in the within-lek category, and sporadic positive values within 5 km and >10 km in the Mixed Grass Prairie.

Contemporary gene flow

The results from BayesAss further confirmed those obtained from F_{ST}, STRUCTURE, and GENELAND. We found no evidence for recent migration for the majority of pairwise comparisons between ecoregions, with two exceptions. We documented a low level of asymmetric migration from the Sand Sagebrush Prairie into the Shortgrass/CRP Mosaic ($m = 0.097$, 95 % CI 0.010–0.183) and a much higher rate of asymmetric migration from the Mixed Grass Prairie also into the Shortgrass/CRP Mosaic ($m = 0.207$, 95 % CI 0.116–0.298). The rates of migration from the Shortgrass/CRP Mosaic into both the Sand Sagebrush Prairie and the Mixed Grass Prairie were negligible and not significantly different from zero (Table 5). Minimal variability was detected across runs with deviance values ranging from 53,816 to 53,823 (data not shown). These results suggest, therefore, that Lesser Prairie-Chicken populations based on ecoregions were largely isolated, however, individuals that occupy the core area within the species' distribution in western Oklahoma and Kansas (i.e., Mixed Grass Prairie and to a lesser extent the Sand Sagebrush Prairie) have dispersed north into the species' northernmost ecoregion (i.e., Short Grass/CRP Mosaic) but not in the opposite direction.

Hybridization

A proportion of the individuals suspected of hybrid origin were confirmed hybrids based on their multilocus microsatellite genotypes using INTROGRESS. The hybridization indices for putative hybrids and unknowns ranged from 0.29 to 1.00 with relatively wide confidence intervals (Fig. 6). At least four of the seven unknowns and two of the six putative hybrids possessed hybridization indices <0.5 indicating a high degree of genetic introgression with Greater Prairie-Chicken, and the lower 95 % confidence interval for all but two of the unknowns and putative hybrids included values <0.5.

Discussion

Genetic structure among ecoregions

Our analyses of genetic data identified either three or four distinct Lesser Prairie-Chicken populations that were largely defined by ecoregion boundaries (sensu McDonald et al. 2014), which has important implications for the management and conservation of this species. The Shinnery Oak Prairie ecoregion in New Mexico and west Texas harbored a genetically distinct population recognized in both the STRUCTURE and GENELAND analyses (Figs. 3, 4) and large and significant F_{ST} values ($F_{ST} > 0.034$; Table 4), as reported in previous studies (e.g., Pruett et al. 2011). Additional sampling in this study further documented that the Sand Sagebrush Prairie ecoregion also represented a distinct population, whereas the Shortgrass/CRP Mosaic and Mixed Grass Prairie ecoregions appeared admixed (Figs. 3 and 4) despite significant F_{ST} values between all ecoregion pairwise comparisons (Table 4). These results were further confirmed through the investigation of migration rates between ecoregions. BayesAss indicated no contemporary gene flow between ecoregions with the exception of movement into the Shortgrass/CRP Mosaic largely from the Mixed Grass Prairie and to a lesser extent from the Sand Sagebrush Prairie ecoregions.

Schulwitz et al. (2014) documented a strong relationship between population genetic structure and habitat type, or ecoregions, for the Greater Sage-Grouse in Wyoming, but this is the first study to identify a similar relationship for prairie grouse species. The Lesser Prairie-Chicken ecoregions were defined on the basis of grassland, prairie, or shrubland habitats (Short-grass/CRP, Sand Sagebrush, Mixed Grass, and Shinnery Oak) independent of genetic structure (McDonald et al. 2014). The strong genetic patterns associated with ecoregions may be a result of the environmental variation across its geographic range. Assuming that the Lesser Prairie-Chicken existed in a large contiguous population prior to European settlement (e.g., Van den Bussche et al. 2003) and have only recently started to show differentiation, the observed contemporary boundaries corresponding to ecoregions may suggest that

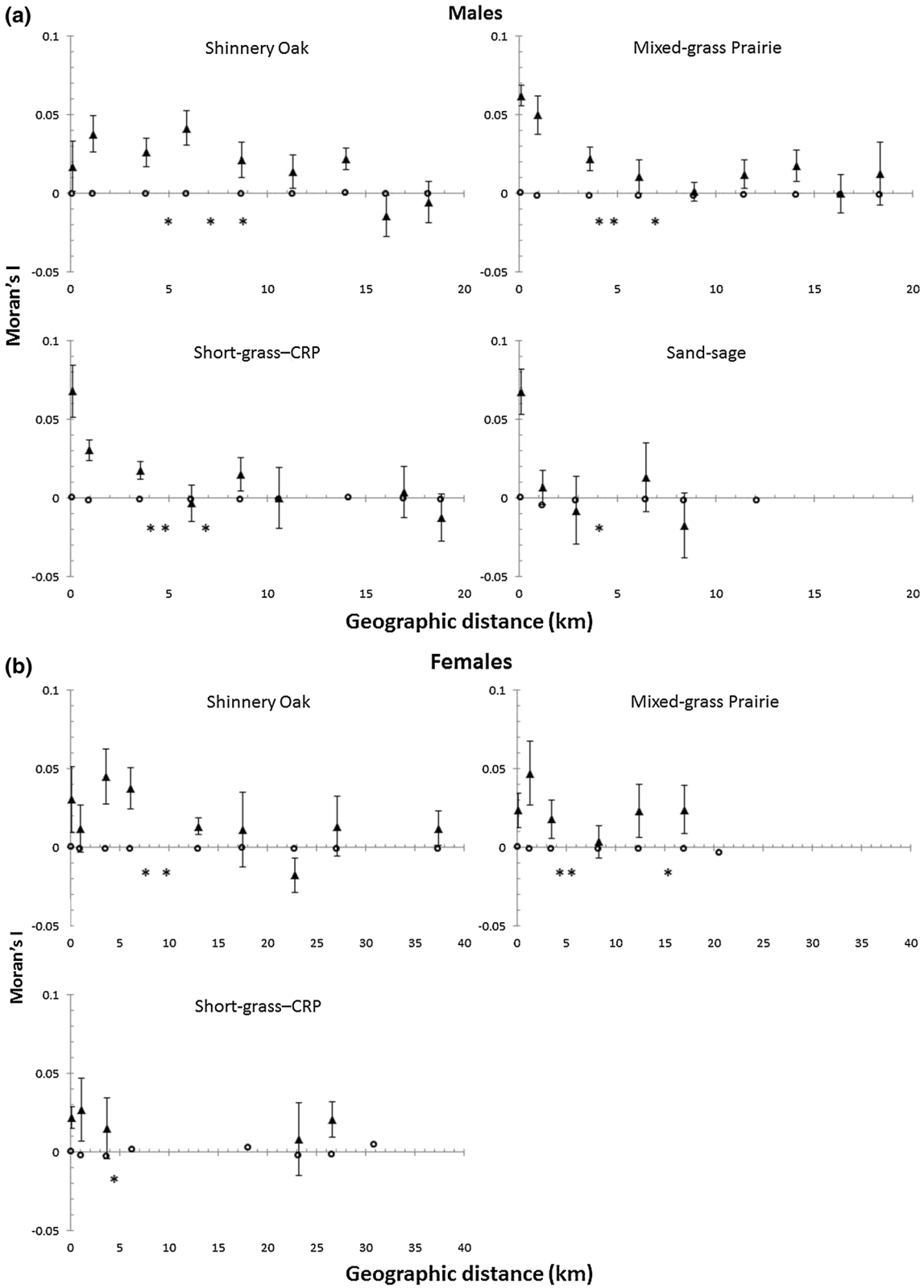

Fig. 5 Mean autocorrelation coefficients (Moran's *I*) and Euclidean distance among pairs of individuals in 2.5- or 5-km distance classes for Lesser Prairie-Chickens. *Open circles* represent null values based on 10,000 permutations of individual locations. *Error bars* indicate ±1 SE, and were computed by jackknifing over loci. **a** Spatial autocorrelation for male Lesser Prairie-Chickens captured on leks in four ecoregions. **b** Spatial autocorrelation for female Lesser Prairie-Chickens

Table 5 Means of the posterior distributions of contemporary migration rate (*m*) into each Lesser Prairie-chicken population with 95 % credible intervals indicated in parentheses

Migration into	Migration from			
	Sand Sagebrush	Shortgrass/CRP	Mixed Grass	Shinnery Oak
Sand Sagebrush Prairie	**0.982** (0.966–0.998)	0.003 (0.000–0.007)	0.013 (0.000–0.028)	0.002 (0.000–0.006)
Shortgrass/CRP Mosaic	0.097 (0.010–0.183)	**0.686** (0.656–0.716)	0.207 (0.116–0.298)	0.010 (0.000–0.029)
Mixed Grass Prairie	0.005 (0.000–0.013)	0.004 (0.000–0.010)	**0.986** (0.973–1.000)	0.006 (0.000–0.014)
Shinnery Oak Prairie	0.004 (0.000–0.010)	0.003 (0.000–0.009)	0.003 (0.000–0.010)	**0.990** (0.979–1.000)

Values along the diagonal (in bold) are the proportion of individuals derived from the source population each generation. Migration rates greater than 0.100 are underlined

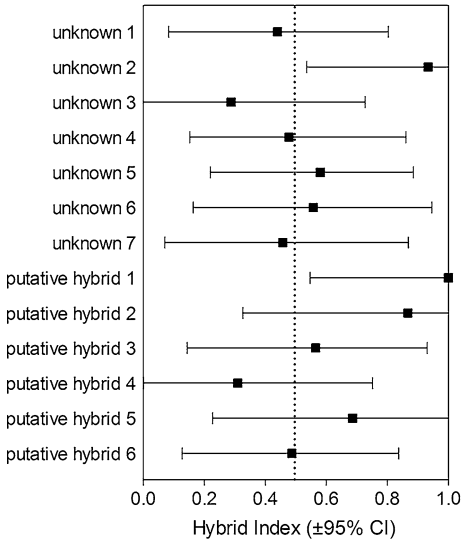

Fig. 6 Hybridization indices for unknowns and putative hybrids with 95 % CI. Indices <0.5 indicate individuals with a high degree of genetic introgression by Greater Prairie-Chicken

niche conservatism is important (Wiens and Graham 2005; Wiens et al. 2010; Pyron et al. 2015). Niche conservatism has been described as the phenomenon in which ecological traits are retained across time and may be an important concept for conservation as it can be used to help predict the consequences of changing climates on adaptation to new environmental conditions as ecological niches begin to shift (Pyron et al. 2015).

Another reason for the spatial patterns documented here may be that Lesser Prairie-Chickens tend to have short natal dispersal distances, generally on the order of a few kilometers for males (Pitman et al. 2006) even though they have the potential to move longer distances. Seasonal movements of Lesser Prairie-Chicken may be much larger than natal dispersal as Lesser and Greater Prairie-Chicken have some of the largest space requirements of any grouse species (Winder et al. 2015). Grouse in general are large-bodied and relatively strong fliers as is evidenced by seasonal migration patterns between breeding and nonbreeding areas (Schroeder and Braun 1993), yet their natal dispersal abilities tend to be limited. For example, Red Grouse (*Lagopus l. scoticus*) in Scotland were documented not to disperse across a river valley that separated broad patches of suitable highland vegetation resulting in genetic structure and a strong signal of isolation by distance (Piertney et al. 1998). It is plausible that the patterns of genetic structure we detected in the Lesser Prairie-Chicken, particularly among the three northern ecoregions, are due to reduced gene flow across inhospitable habitats that are not particularly far apart geographically. Conversely, the strong differentiation observed with the Lesser Prairie-Chicken population in the Shinnery Oak Prairie ecoregion in eastern New Mexico is most likely the result of geographic isolation because of the large distance that exists with the nearest population to the northeast.

Before European settlement of the Great Plains, populations of the Lesser Prairie-Chicken were most likely connected across the Oklahoma and Texas Panhandle or at least from eastern New Mexico to southeast Colorado (e.g., Giesen and Hagen 2005; Elmore et al. 2009). Thus, the Lesser Prairie-Chicken population in the Shinnery Oak Prairie ecoregion has likely been isolated for over a century (Pruett et al. 2011). Research on the Greater Prairie-Chicken in Wisconsin revealed that genetic differentiation between proximate areas ($\sim$20 km) emerged in less than 50 years due to disruption of gene flow from land use changes and reduced N_e (Johnson et al. 2004). It seems plausible that the Shinnery Oak Prairie population was isolated by distance (sensu Wright 1943) before it was isolated physically. Habitat fragmentation and genetic drift have likely exacerbated the observed differentiation.

The results of our spatial autocorrelation analysis support the idea that male relatives are present on leks and that male dispersal is proximate (see Pitman et al. 2006) because we detected genetic structure within 5–7 km and perhaps even less in the Sand Sagebrush Prairie ecoregion (Fig. 5). Dispersal patterns for females were less clear as sample size and sample distribution may have affected this

analysis, although previous research has shown that female activity is also centered on leks (Winder et al. 2015). Elevated relatedness of males on leks has been previously documented with the Lesser Prairie-Chicken in New Mexico (Bouzat and Johnson 2004) and Black Grouse (*Tetrao tetrix*) in Europe (Höglund et al. 1999; Lebigre et al. 2008), although no such pattern has been found in the Greater Sage-Grouse (Gibson et al. 2005; Bush et al. 2010). Further, the general pattern of shorter dispersal movements in males than in females has been shown in field studies of the Lesser Prairie-Chicken (Pitman et al. 2006) and many other grouse species (Dunn and Braun 1985; Giesen and Braun 1993; Warren and Baines 2002; Caizergues et al. 2003) and is likely the case here.

Levels of genetic diversity were relatively similar among ecoregions. The Shinnery Oak Prairie ecoregion had the lowest allelic richness and the lowest heterozygosity (yet not significantly, Table 2). Effective population sizes were similar for all ecoregions and had overlapping confidence intervals. This suggests that no population has experienced a steep enough decline relative to other populations to influence levels of genetic diversity differently between ecoregions. The relatively low N_e values, however, may suggest that significant losses of genetic diversity are on the horizon, especially if long-term population declines continue. Differences in genetic diversity among populations that differ in N_e have been detected in the Greater Prairie-Chicken (Bouzat et al. 1998; Johnson et al. 2003, 2004; Bollmer et al. 2011; Eimes et al. 2011; Bateson et al. 2015) and in Gunnison (Oyler-McCance et al. 1999, 2005b) and Greater Sage-Grouse (Benedict et al. 2003; Oyler-McCance et al. 2005a; Schulwitz et al. 2014). If similar patterns were detected in the Lesser Prairie-Chicken, it could be cause for concern because significant reductions of genetic diversity have also been correlated with reduced fitness in the Greater Prairie-Chicken (Westemeier et al. 1998). The Shinnery Oak Prairie population had the lowest genetic diversity among the surveyed ecoregions, and therefore continued efforts should be made to monitor this population's genetic diversity given its current isolation. One area in particular within the Shinnery Oak Prairie, the Deaf Smith sampling locale, had significantly lower allelic richness than almost all other sampling locales in this study. Efforts could be made to monitor the Deaf Smith population and maintain potential gene flow to prevent further decline in diversity.

Evidence for population expansion in CRP

While the geographic range of the Lesser Prairie-Chicken has contracted markedly from pre-settlement times due to human-induced changes on the landscape, the northern front of the range appears to be expanding into historically occupied habitat and previously unoccupied or sparsely occupied areas (Van Pelt et al. 2013). The enrollment of agricultural fields into the CRP may have allowed the observed Lesser Prairie-Chicken range expansion in western Kansas (Van Pelt et al. 2013) or this may be a response due to drought conditions and climate change effects influencing geographic distributions (Rodgers and Hoffman 2005; Fields et al. 2006). Our genetic data suggest that although contemporary gene flow is limited among ecoregions (Table 5), contemporary gene flow does exist from the Mixed Grass Prairie into the Shortgrass/CRP Mosaic ecoregion and to a lesser extent from the Sand Sagebrush Prairie into the Shortgrass/CRP Mosaic. Thus, birds largely from the Mixed Grass Prairie are moving northward into the Shortgrass/CRP Mosaic and into previously unoccupied or sparsely occupied habitat, which further highlights the benefit of CRP management practices for prairie grouse conservation efforts (Rodgers and Hoffman 2005; Fields et al. 2006). This finding is particularly important as it reveals that habitat management through enrollment in CRP is a strategy that may result in the expansion of Lesser Prairie-Chicken populations and that can potentially increase connectivity among populations. Additionally, the idea that the Lesser Prairie-Chicken can expand its geographic range highlights the species' ability to track changing conditions and provides hope that the species, if managed in a solicitous way, could respond to changing habitats due to climate change.

Evidence for hybridization with the Greater Prairie-Chicken

The area of range expansion in northwest Kansas also favors Greater Prairie-Chickens, and has been described as an area with limited mixing of both species of prairie-chicken (Van Pelt et al. 2013; McDonald et al. 2014). Our results further document that hybridization has occurred to some extent between Lesser and Greater Prairie-Chickens in western Kansas (see also Bain and Farley 2002) as nearly half of the unknown or putative hybrid individuals that were tested had a hybrid index intermediate between Greater and Lesser Prairie-Chickens, with confidence intervals that did not overlap 0 or 1 (Fig. 6). Recent research exploring the phylogenetic relationships among prairie grouse has also shown that hybridization between the two species has occurred, suggesting that the introgression is largely female biased with male hybrids limiting introgression due to extrinsic post-zygotic reproductive barriers associated with sexually-selected traits (Galla and Johnson 2015). No research has been conducted to determine if offspring produced from hybrid Lesser and Greater Prairie-Chicken females possess reduced fitness. The two

species share a common ancestor between 150,000 and 475,000 years before present (Galla and Johnson 2015), so outbreeding depression may be of concern (Frankham et al. 2011). To what degree the extent of hybridization occurs between the two species deserves further study.

Implications for conservation

Our genetic data can inform conservation and management of the Lesser Prairie-Chicken. We found genetic structuring along ecoregion boundaries and minimal gene flow between them, suggesting that landscape composition and configuration may be important drivers of gene flow. A formal landscape genetic analysis to estimate permeability for the Lesser Prairie-Chicken is beyond the scope of this study but could provide additional valuable information regarding the interaction between gene flow and landscape composition (e.g., Row et al. 2015).

The strong relationship between ecoregion boundaries and genetic structure has several implications for management planning and conservation policy. First, as ecoregions represent relatively discrete populations, any translocations to boost population size or restore genetic diversity should be considered carefully (Bouzat et al. 2009; Press et al. 2013). Given the genetic divergence among ecoregions (particularly involving the Shinnery Oak Prairie), translocations among ecoregions may dampen local adaptation or habitat-specific evolutionary potential and is not advisable. For example, mixing individuals from largemouth bass (*Micropterus salmoides*) populations that were genetically and geographically distinct resulted in offspring with reduced resistance to infectious disease (Goldberg et al. 2005). Based on these results and minimal differentiation within ecoregions, translocations within an ecoregion are not likely to affect local adaptation and are therefore preferable if the goal is population augmentation (e.g., Deaf Smith sampling locale). If inter-ecoregional translocations are deemed necessary, then a careful cost-benefit analysis is warranted including a risk assessment of outbreeding vs. inbreeding depression (Lynch 1991; Frankham et al. 2011). We suggest that bolstering population recovery through habitat restoration and the creation and enhancement of corridors may be more effective in linking genetic connectivity.

Second, in addition to monitoring census population size and maintaining adequate numbers as conservation management objectives, it is equally important to establish objectives for the maintenance of genetic diversity (Laikre et al. 2010; Hagen and Elmore 2016). Similar to other lek-breeding grouse species, the Lesser Prairie-Chicken is susceptible to loss of genetic diversity with increasing habitat fragmentation and isolation (Bouzat et al. 1998; Benedict et al. 2003; Johnson et al. 2003, 2004; Oyler-McCance et al. 2005a, b; Johnson and Dunn 2006; Schulwitz et al. 2014). As populations become smaller in size, their lek-breeding behavior (high variance in male reproductive success; Robel 1970; Wiley 1973; Behney et al. 2012) and fairly high annual mortality (~50 %; Hagen et al. 2005, 2007) increase their propensity for local extirpation (Soule and Mills 1998). Monitoring levels of genetic diversity among and within ecoregions, particularly the Shinnery Oak Prairie ecoregion and the Deaf Smith locale, will provide a framework for assessing connectivity among areas (Schwartz et al. 2007).

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