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SHORT COMMUNICATION

# Genetic structure among closely spaced leks in a peripheral population of lesser prairie-chickens

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## Abstract

**We evaluated the genetic structure of birds from four closely spaced leks in a peripheral population of lesser prairie-chickens (*Tympanuchus pallidicinctus*). Analyses of molecular variance revealed significant genetic structuring among birds from different leks for six microsatellite loci ($F_{ST}$ = 0.036; $P$ = 0.002), but we found no genetic differentiation at the mtDNA control region. Significant deviations from Hardy–Weinberg revealed an excess of homozygote genotypes within each of the leks studied ($F_{IS}$ = 0.190–0.307), indicative of increased inbreeding. Estimates of relatedness using microsatellite data suggest that the genetic structuring among lesser prairie-chicken leks occurs in part because of a lek mating system in which males at some leks are related. Structuring may also be caused by stochastic effects associated with a historical decline in population size leading to small, semi-isolated leks and high site fidelity by reproductive males. Results from this study suggest that microspatial genetic structuring may occur in lek-mating bird species with low levels of dispersal.**

*Keywords*: genetic structure, lek mating system, peripheral population, relatedness, *Tympanuchus pallidicinctus*

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## Introduction

During the last few years, several studies have assessed the genetic consequences of recent population declines, increased isolation, and habitat fragmentation in wild species (e.g. Bouzat *et al*. 1998a,Bouzat *et al*. 1998b; Gutierrez-Espeleta *et al*. 2000; Uphyrkina *et al*. 2002). Demographic declines have been shown to increase genetic stochasticity, reducing levels of genetic diversity within populations. In some cases these changes have been associated with declines in fitness, leading to potential increases in extinction risk (Saccheri *et al*. 1998; Westemeier *et al*. 1998). The consequences of small population size are especially relevant in peripheral populations, i.e. populations at the edge of a species' distribution range. Peripheral populations are typically small and subject to colonization and founder events, increasing the potential for genetic drift and inbreeding. These populations are valuable for conservation, because they may preserve rare alleles and gene combinations important to local adaptation (Lesica & Allendorf 1995).

The retention of genetic diversity in small populations is related directly to the effective population size, rather than the actual number of individuals present in a population (Lande & Barrowclough 1987). Mating systems can affect effective population size, particularly when variation in mating success leads to a skewed reproductive contribution by some individuals and when reproductive individuals are related to each other. When this occurs, the resulting decreases in effective population size may amplify stochastic genetic effects.

Numerous studies have evaluated the relative importance of stochastic effects and inbreeding in determining population genetic structure over large geographical ranges. However, studies evaluating microspatial genetic structure within populations are less common and restricted mainly to plants. Bird species with lek mating systems and

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low levels of dispersal may be particularly prone to show spatial genetic structuring, as only a few males tend to contribute to reproduction. In these species, low levels of dispersal, site fidelity and potential relatedness among lekking males could decrease levels of genetic variation within leks and increase genetic differentiation among leks.

The lesser prairie-chicken (*Tympanuchus pallidicinctus*) is a grouse species endemic to rangelands in the shortgrass prairie ecosystem of the southern Great Plains of the United States. Since the 1800s its geographical distribution and population sizes have declined dramatically as a result of human activities, particularly agriculture. Current abundance estimates suggest a decline of 97% of the total number of birds estimated to be present in the 1800s, representing the smallest population size of any North American grouse species (Giesen 1998). The species is currently a candidate for listing as threatened under the Endangered Species Act (US Fish & Wildlife Service 2002).

In this species, the effective population size is reduced by a lek mating system. Males aggregate on small display territories visited by females for mating, and only a few older males perform most of the copulations (Giesen 1998). In several lekking species, males displaying on the same lek have been shown to be related (Höglund *et al*. 1999; Petrie *et al*. 1999; Shorey *et al*. 2000); thus, kin-selection provides a possible explanation for why subordinate males join leks when they sire no offspring themselves but enhance the reproductive success of breeding males (Sherman 1999). Relatedness among displaying males, if it occurred in lesser prairie-chickens, could reduce genetic variation on leks and, by extension, in the population as a whole. If leks were strongly differentiated, it is possible that more genetic variation could be maintained at the population level. However, the extreme mating skew that occurs in this species would probably result in an overall reduction in genetic diversity.

Although several studies on the population ecology and behaviour of lesser prairie-chickens exist (e.g. Riley *et al.* 2000; Riley & Davis 1993; Giesen 1994a,b; Woodward *et al.* 2001), only one study has been published on the genetic structure of lesser prairie-chicken populations. Using genetic analyses of microsatellite and mtDNA markers, Van Den Bussche *et al*. (2003) demonstrated genetic structure among leks and populations, particularly at the regional level, suggesting that historical processes have led to genetic differentiation between lesser prairie-chicken populations in Oklahoma and New Mexico.

In this study, we used microsatellites and mitochondrial DNA (mtDNA) analyses to evaluate the genetic structure of four closely spaced leks in a population of lesser prairie-chickens in New Mexico. We focused on the potential effects of recent demographic declines, isolation and relatedness among reproductive individuals on the genetic structure of lesser prairie-chicken leks. In particular, we evaluated the potential effects of genetic drift and inbreeding in defining the microspatial genetic structure in a peripheral population of lesser prairie-chickens. Our general hypothesis is that stochastic processes (i.e. genetic drift) and inbreeding operating in small semi-isolated leks will result in reduced genetic diversity within leks and significant levels of genetic differentiation among leks. We demonstrate demographic declines between 1971 and 2002 and provide behavioural data on site fidelity, which suggest that small effective size and lek fidelity by males may play a role in the microspatial genetic structuring of this population.

## Materials and methods

### *Study population and sample collection*

The study was conducted on the Caprock Wildlife Habitat Management Area, approximately 60 km east of Roswell, Chavez County, New Mexico (Fig. 1). The management area is administered by the Bureau of Land Management, Roswell, New Mexico Field Office (BLM). We trapped prairie-chickens at four traditional lek sites during the peak lek attendance period, late March until early May, 1998–2000. Birds were trapped in circular, welded-wire,

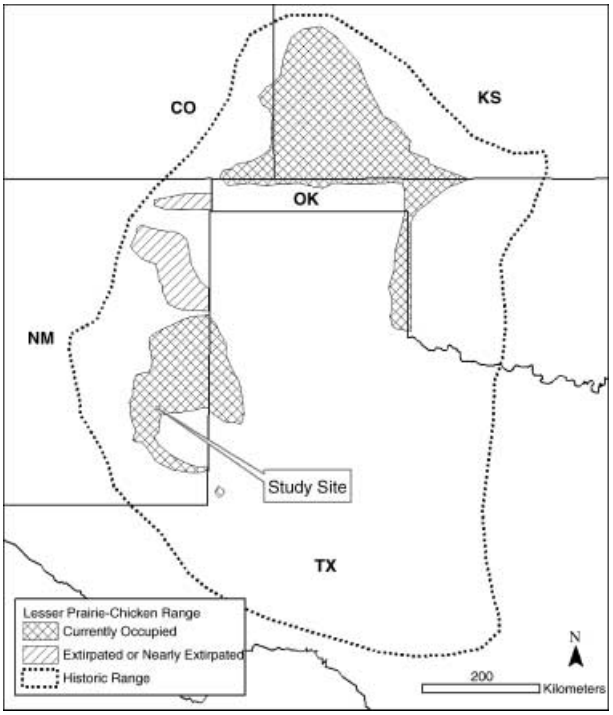


**Fig. 1** Study site relative to current and presumed historic range of lesser prairie-chicken. Ranges adapted from Giesen (1998), Bailey & Williams (1999) and the National Heritage Information System.

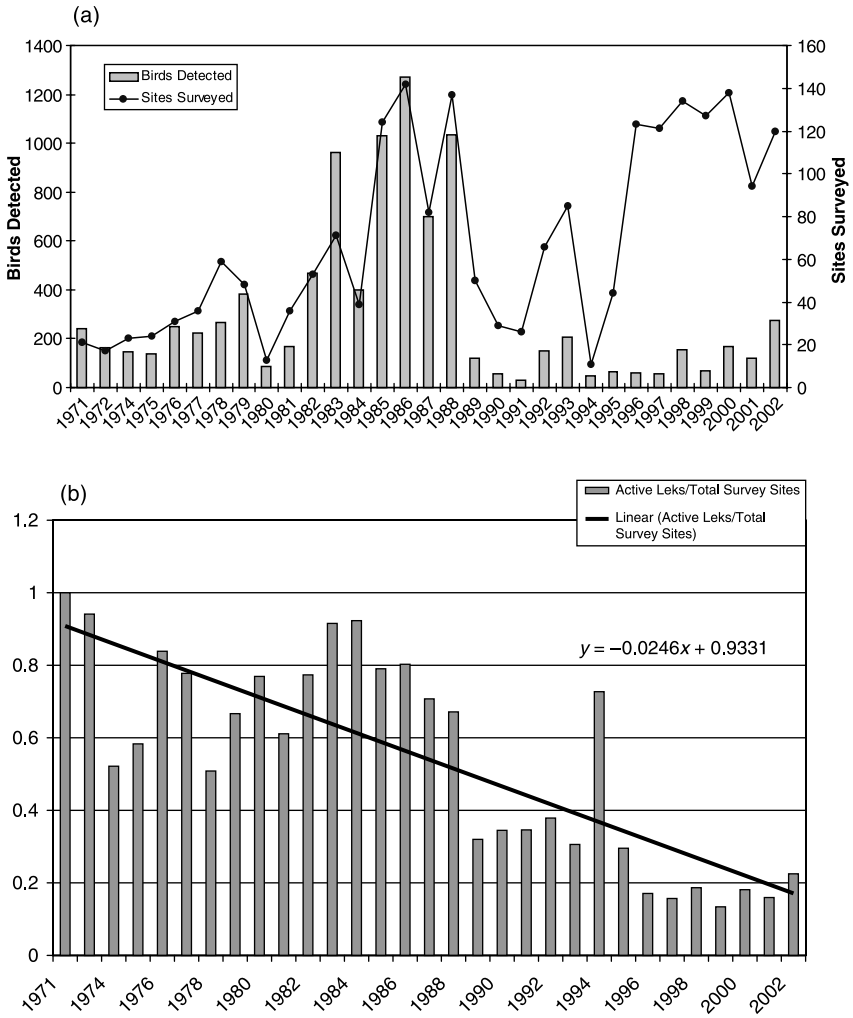


**Fig. 2** (a) Annual average number of birds detected at each lek on the Caprock Wildlife Habitat Management Area, summed over all leks, between 1971 and 2002 showing number of lek sites surveyed. In the 1990s more lek sites were visited, but fewer birds were detected. (b) Annual number of active leks detected per total number of sites surveyed, showing decreasing linear trend.

walk-in traps, placed in an array across each lek site and connected with chicken wire leads (Schroeder & Braun 1991). We collected 10–50 μL of blood via brachial venipuncture and stored it in Queen's lysis buffer (Seutin *et al*. 1991) for later analysis at Bowling Green State University. Birds were banded with a New Mexico Department of Game and Fish numbered aluminium band and a unique combination of plastic colour bands. Females were fitted with radio transmitters. We used a combination of loop necklace and whip antenna, battery-operated transmitters from AVM Instrument Co. (Colfax, CA, USA) and Telemetry Solutions (Concord, CA, USA). The animal welfare protocol was approved by the University of New Mexico Main Campus Animal Care and Use Committee (no. A4023-01). We identified birds at lek sites based on their colour band combinations or, in the case of females, radio frequencies.

We assessed genetic structure among four lesser prairie-chicken leks, based on samples from 49 adult individuals (21 males and 28 females). The four leks were located on average 3 km (range 2.2–3.6) apart. This population is located at the edge of the current lesser prairie-chicken distribution (Fig. 1). Data from annual BLM lek surveys were queried from the Natural Heritage New Mexico database to show population trends between 1971 and 2002 (Fig. 2). We used re-sighting/recapture data from banded birds and telemetry data to estimate site fidelity of males and females.

### *Microsatellite genotyping*

We performed standard phenol–chloroform DNA extractions (see Bouzat *et al*. 1998a for DNA extraction procedures). We used six microsatellite loci that yielded polymorphic amplification products in the lesser prairie-chicken. Three of these (ADL23, ADL146 and ADL230) were designed originally for the domestic chicken (*Gallus gallus*; Cheng *et al*. 1994) and provided successful amplifications in greater and lesser prairie-chickens (Bouzat 1998a,b; Van den Bussche *et al*. 2003). The other three (LLSD4, LLSD9 and LLST1) were designed originally for the red grouse

(*Lagopus lagopus*; Piertney & Dallas 1997) and were shown to be variable in the greater prairie-chicken (Bellinger *et al*. 2003). Polymerase chain reaction (PCR) amplifications were performed in 25 μL volumes using reaction conditions and thermal profiles described in Bouzat *et al*. (1998a) and Bellinger *et al*. (2003). Amplification products were run on 6% acrylamide gels or Spreadex minigels (Elchrom Scientific, Switzerland) and visualized by silver staining or fluorescent detection in a Storm (Amersham Biosciences, Piscataway, NJ, USA) imaging system.

### *DNA sequencing of mtDNA control region*

To assess potential matriline structuring among leks we sequenced a partial section of the mtDNA control region from 18 individuals (six females selected randomly from each of three leks to keep sample sizes even; no females were captured from lek 24 N). We amplified 215 base pairs (bp) of the hypervariable mtDNA control region by PCR, using primers TccRF (5′-CACATACATTTATGGTACCGG-3′) and TccRR (5′-CAATAAATCCATCTGGTACG-3′), designed originally by Dr Allan Arndt (Brandon University, Canada). Amplifications were performed in 25 μL reactions containing approximately 100–200 ng of DNA, 0.4 μM of each primer, 100 μM dNTPs, 2.5 mM $MgCl_2$ and 1 unit of *Taq* polymerase. PCR thermal profiles included 35 amplification cycles of 30 s at 94 °C, 30 s at 50 °C and 45 s at 72 °C. Amplification products were then cloned using a TOPO-TA (Invitrogen, Carlsbad, CA, USA) cloning kit following the manufacturer's instructions. Some PCR products were also sequenced directly. At least two independent clones/PCR products from each sample were sequenced with the Big-Dye Terminator sequencing kit using an ABI 310 automated DNA sequencer (Applied Biosystems, Foster City, CA, USA).

### *Data analyses*

Microsatellite data analyses were performed using GENEPOP, version 1.2 (Raymond & Rousset 1995) and ARLEQUIN, version 2.0 (Schneider *et al*. 2000) computer programs. Allele frequencies, observed heterozygosities and heterozygosities expected under Hardy–Weinberg were estimated using GENEPOP. Deviations from Hardy–Weinberg were assessed using Wright's $F_{IS}$ indices (Wright 1951), estimated according to Weir & Cockerham (1984). Levels of significance for deviations from Hardy–Weinberg were estimated using a Markov chain method implemented in GENEPOP with 1000 randomizations, as described by Guo & Thompson (1992). We corrected for multiple comparisons across loci using a sequential Bonferroni adjustment (Rice 1989). Levels of genetic variation within and among leks were estimated using an analysis of molecular variance (AMOVA; Excoffier *et al*. 1992). Due to sample size limitations, this analysis was restricted to three of the four leks sampled (44 samples, including 16 males and 28 females). Significance levels of variance components were computed in ARLEQUIN, using a nonparametric permutation procedure with 1000 random permutations (Excoffier *et al*. 1992). Levels of genetic differentiation among leks were estimated using the Wright's $F_{ST}$ index (Wright 1951). This index was calculated for each locus independently, as well as for all loci. Significance levels for lek differentiation were obtained through an unbiased estimate of the *P*-value of the Fisher exact test, as described by Raymond & Rousset (1995).

Lek genetic structuring was also evaluated through an assignment test to determine the potential origin of every individual sample. This method provides a likelihood estimate for the assignment of each sample to a specific lek. Likelihood estimates were computed using ARLEQUIN, following the method described originally by Paetkau *et al*. (1997) and Waser & Strobeck (1998). The proportion of correct assignments provides an idea of how genetically distinct individual leks are.

Relatedness among males and females within leks was estimated using RELATEDNESS 5.0.8 (Queller & Goodnight 1989). This program allows estimation of relatedness coefficients among groups of individuals, which can be interpreted in terms of identity by descent. Standard errors were calculated using the jackknife re-sampling technique implemented in RELATEDNESS. We used microsatellite data from 21 males from four leks and 27 females from three leks for the reference population ($n = 48$).

The mtDNA control sequences of the lesser prairie-chicken were aligned using the Clustal-X multiple sequence alignment program (Thompson *et al*. 1997). MEGA 2.1 (Kumar *et al*. 1993) was used to estimate haplotype frequency distributions and levels of genetic variation. Estimates of mean genetic diversity within and among leks and for the entire population were calculated using the Kimura-2 parameter distance (Kimura 1980), which accounts for higher rates of transitions vs. transversions found commonly in animal mitochondrial DNA (Brown *et al*. 1982). These estimates and their standard errors were not significantly different from those obtained using the proportion of nucleotide differences (Kumar *et al*. 1993). Levels of haplotype variation within and among leks were also evaluated through an AMOVA using ARLEQUIN (Excoffier *et al*. 1992).

## Results and discussion

Average observed heterozygosity and number of alleles per locus for the studied population were 0.54 (SE = 0.05) and 6.17 (SE = 1.40), respectively (Table 1). These values are comparable to those obtained by Van Den Bussche *et al*. (2003) across central populations in Oklahoma and New Mexico (observed heterozygosity per lek = 0.22–0.75; allelic diversity = 1.8–4.8). The AMOVA indicated that most of the

**Table 1** Levels of genetic diversity (A: allelic diversity; $H_O$: observed heterozygosity; and $H_E$: expected heterozygosity) estimated for six microsatellite loci at three lesser prairie-chicken leks from the Caprock Wildlife Habitat Management Area in New Mexico

| | Lek 2 N ($n$ = 11) | | | Lek 45 N ($n$ = 24) | | | Lek 47 N ($n$ = 9) | | |
|---|---|---|---|---|---|---|---|---|---|
| Locus | A | $H_O$ | $H_E$ | A | $H_O$ | $H_E$ | A | $H_O$ | $H_E$ |
| ADL23 | 2 | 0.273 | 0.325 | 2 | 0.391 | 0.476 | 2 | 0.555 | 0.569 |
| ADL146 | 5 | 0.273 | 0.710 | 3 | 0.375 | 0.530 | 3 | 0.444 | 0.693 |
| ADL230 | 7 | 0.889 | 0.837 | 4 | 0.636 | 0.754 | 7 | 0.600 | 0.844 |
| LLSD9 | 5 | 0.286 | 0.659 | 5 | 0.409 | 0.670 | 4 | 0.375 | 0.842 |
| LLSD4 | 11 | 0.600 | 0.889 | 7 | 0.565 | 0.868 | 9 | 0.333 | 0.908 |
| LLST1 | 5 | 0.900 | 0.695 | 5 | 0.913 | 0.764 | 5 | 0.875 | 0.733 |
| Mean | 5.83 | 0.537 | 0.686 | 4.33 | 0.548 | 0.677 | 5.00 | 0.530 | 0.765 |
| SE | 0.90 | 0.092 | 0.060 | 0.36 | 0.042 | 0.031 | 0.87 | 0.066 | 0.041 |

**Table 2** Wright's $F$ indices ($F_{IS}$ and $F_{ST}$) and exact $P$-values estimated for six microsatellite loci at three lesser prairie-chicken leks. $P$-values less than 0.05 are shown in bold

| | Lek 2 N | | Lek 45 N | | Lek 47 N | | | |
|---|---|---|---|---|---|---|---|---|
| Locus | $F_{IS}$ | $P$ | $F_{IS}$ | $P$ | $F_{IS}$ | $P$ | $F_{ST}$ | $P$ |
| ADL23 | +0.182 | 0.411 | −0.111 | 1.000 | −0.111 | 1.000 | +0.042 | 0.126 |
| ADL146 | +0.259 | **0.001*** | +0.273 | 0.381 | +0.577 | **0.011*** | +0.050 | **0.000*** |
| ADL230 | +0.137 | 0.470 | +0.250 | **0.048** | −0.067 | 0.924 | +0.056 | **0.021** |
| LLSD9 | +0.366 | 0.055 | +0.558 | **0.002*** | +0.579 | 0.068 | −0.015 | 0.344 |
| LLSD4 | +0.331 | **0.000*** | +0.616 | **0.001*** | +0.337 | **0.020** | +0.045 | **0.000*** |
| LLST1 | −0.230 | 0.238 | −0.210 | 1.000 | −0.317 | 0.085 | −0.015 | 0.893 |
| Mean | +0.217 | **0.000** | +0.190 | **0.001** | +0.307 | **0.007** | +0.036 | **0.002** |

*Significant after Bonferroni correction.

observed genetic variance was explained by differences within (96.4%), rather than among, leks (3.6%). This is not unexpected, because leks are closely spaced and therefore likely to be part of a single population. However, the among-lek variance component led to significant levels of genetic differentiation, as indicated by a significant overall $F_{ST}$ = 0.036 ($P$ = 0.002; Table 2). $F_{ST}$ estimates for each individual locus showed that the overall genetic differentiation among leks resulted mainly from differences in genotype frequencies at ADL146, ADL230 and LLSD4 (Table 2). Levels of genetic differentiation among leks were smaller than those reported by Van Den Bussche *et al*. 2003), in which 6.86% of the observed variance was explained by the among-lek variance component. This result is not unexpected, as that study surveyed a greater number of leks over a larger region.

Lek genetic structuring in our study was also indicated by the likelihood estimates for assigning individual genotypes to a particular lek. Likelihood estimates allowed us to assign 41 of the 49 individuals genotyped correctly (84%), a relatively high percentage given that leks are closely situated (~3 km). Genetic structuring among closely spaced leks probably resulted from stochastic genetic effects associated with small population size (leading to a small average number of birds per lek) and increased isolation at the edge of the distribution of the lesser prairie-chicken.

In addition to the genetic differentiation detected among leks, estimates of genetic diversity within leks suggested increased inbreeding. The analyses of Wright's $F_{IS}$ indices revealed positive values for 12 of the 18 lek-locus combinations (Table 2). Significant deviations from Hardy–Weinberg were detected for seven lek-locus combinations (five significant values when using Bonferroni correction). The overall $F_{IS}$ at each lek was positive and highly significant ($P$ = 0.000–0.007), indicating an excess of homozygote genotypes within each of the leks studied (Table 2).

Our results are consistent with the idea that genetic drift operating in structured populations has led to significant genetic differentiation among small semi-isolated leks (positive $F_{ST}$), whereas inbreeding has increased homozogosity within leks (positive $F_{IS}$). These effects are probably caused in part by the lesser prairie-chicken's lek mating system, in which only a few males sire the majority of offspring, thus increasing genetic stochasticity and the potential for inbreeding. We do not believe that the presence of null alleles is causing the observed excess of homozygote genotypes. The loci in which we detected statistically significant $F_{IS}$ revealed 5–12 alleles with a relatively continuous distribution of allele sizes. In addition, we never had a sample that did not show an amplified product, which would be expected for a homozygote or heterozygote for null alleles.

The lesser prairie-chicken population on the Caprock Wildlife Habitat Management Area has declined during the 32 years since BLM began their annual lek surveys. Annual survey data from 1971 to 2002 show an increase in total numbers from the 1970s to the 1980s, followed by a sharp decline during the 1990s, with no apparent recovery to pre-1980s levels (Fig. 2). The decrease in the average number of birds detected at each lek over the last decade is apparent in spite of increasing sampling effort (Fig. 2a). The decline in abundance in this peripheral population of lesser prairie-chickens contrasts with population trends estimated by Woodward *et al*. (2001), which indicate that central populations of lesser-prairie chickens in New Mexico are stable.

Our estimates of site fidelity suggest that males are faithful to leks. Data from banded males show that from a total of 111 resightings/recaptures in the same (48%) or different (52%) years, 95% (105 observations) were re-sightings at the same lek, suggesting high levels of site fidelity, even between years. Although we have no information on natal dispersal, $F_{ST}$ results could be interpreted as a sign of genetic drift operating among semi-isolated leks.

The analyses of the mtDNA control region revealed no significant levels of lek differentiation. The 18 individuals sequenced resulted in seven mtDNA haplotypes (GenBank Accession nos: AY293569–AY293575). Mean genetic diversity within leks (0.016; SE = 0.006) was not significantly different from that estimated for the entire population (0.015; SE = 0.006). Accordingly, the AMOVA indicated that 100% of the observed mtDNA genetic variance was explained by the within-lek variance component. These results suggest that female dispersal among lesser prairie-chicken leks may prevent local lek genetic differentiation at the mtDNA control region or, alternatively, that there has not been enough time for differentiation to occur. Our field studies showed that from a total of 14 females re-sighted or recaptured 89% of resightings were at the same lek, but unlike for males, 88% occurred in the same year, suggesting that female returns were primarily premating visits to assess potential mates. Low interyear female return rates may be a consequence of lower female site fidelity or higher mortality than in males.

The levels of genetic differentiation detected at the microsatellite loci suggest that genetic structuring among leks may result from high male site fidelity and relatedness among males at some of the leks. Our estimates of the coefficients of relatedness (R) among males indicated that males were related on two of the four leks analysed (lek 24 N: R = 0.363 ± 0.145 (95% CI); lek 45 N: R = 0.170 ± 0.094; lek 2 N: R = –0.046 ± 0.087; lek 47 N: R = –0.112 ± 0.247). In contrast, the coefficients of relatedness among females were not significantly different from zero, suggesting that reproductive females present at leks are unrelated. These results are consistent with our field observations, suggesting higher lek fidelity by reproductive males than by females.

The observed patterns of genetic diversity at microsatellite loci and the mtDNA control region suggest that the genetic structuring in this peripheral lesser prairie-chicken population may be associated with stochastic effects operating in small semi-isolated leks at the edge of the species' range, jointly with a mating system in which related reproductive males (but not females) return to the same leks. These factors may cause genetic structuring, even at the scale of a few kilometres. Our study suggests that microspatial genetic structuring could occur in other lek-mating bird species with low levels of dispersal. Thus, the potential for loss of genetic variation and potential fitness effects as a result of inbreeding should be considered in planning for the conservation and management of these species.

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## References

Bailey JA, Williams SO (1999) Status of the lesser prairie-chicken in New Mexico. *Prairie Naturalist*, **32**, 157–168.

Bellinger R, Johnson JA, Toepfer J, Dunn P (2003) Loss of genetic variation in greater prairie chickens following a population bottleneck in Wisconsin, USA. *Conservation Biology*, **17**, 717–724.

Bouzat JL, Cheng HH, Lewin HA, Westermeier RL, Brawn JD, Paige KN (1998a) Genetic evaluation of a demographic bottleneck in the greater prairie chicken. *Conservation Biology*, **12**, 836–843.

Bouzat JL, Lewin HA, Paige KN (1998b) The ghost of genetic diversity past: historical DNA analysis of the greater prairie chicken. *American Naturalist*, **152**, 1–6.

Brown WM, Prager EM, Wang A, Wilson AC (1982) Mitochondrial DNA sequences of primates: tempo and mode of evolution. *Journal of Molecular Evolution*, **18**, 225–239.

Cheng HH, Levin I, Vallejo RL *et al*. (1994) Development of a genetic map of the chicken with high-utility markers. *Poultry Science*, **74**, 1855–1874.

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distance among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131**, 479–491.

Giesen KM (1994a) Breeding range and population status of lesser prairie-chickens in southeast Colorado. *Prairie Naturalist*, **26**, 175–182.

Giesen KM (1994b) Movements and nesting habitat of lesser prairie-chicken hens in Colorado. *Southwestern Naturalist*, **39**, 96–98.

Giesen KM (1998) Lesser prairie-chicken (*Tympanuchus pallidicinctus*). In: *The Birds of North America*, no. 364 (eds Poole A, Gill F), pp. 1–20. The Birds of North America, Inc., Philadelphia, PA.

Guo SW, Thompson EA (1992) Performing the exact test of Hardy–Weinberg proportion for multiple alleles. *Biometrics*, **48**, 361–372.

Gutierrez-Espeleta GA, Kalinowski ST, Boyce WM, Hedrick PW (2000) Genetic variation and population structure in desert bighorn sheep: implications for conservation. *Conservation Genetics*, **1**, 3–15.

Höglund J, Alatalo RV, Lundberg A, Rintamäki PT, Lindell J (1999) Microsatellite markers reveal the potential for kin selection on black grouse leks. *Proceedings of the Royal Society of London B*, **266**, 813–816.

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, **16**, 111–120.

Kumar S, Tamura K, Nei M (1993) MEGA: *Molecular Evolutionary Genetic Analysis*, Version 1.01. Pennsylvania State University, University Park, PA.

Lande R, Barrowclough GF (1987) Effective population size, genetic variation, and their use in population management. In: *Viable Populations for Conservation* (Soule ME, ed.), pp. 87–123. Cambridge University Press, Cambridge.

Lesica P, Allendorf FW (1995) When are peripheral populations valuable for conservation. *Conservation Biology*, **9**, 753–760.

Paetkau D, Waits LP, Clarkson PL, Craighead L, Strobeck C (1997) An empirical evaluation of genetic distance statistics using microsatellite data from bear (Ursidae) populations. *Genetics*, **147**, 1943–1957.

Petrie M, Krupa A, Burke T (1999) Peacocks lek with relatives even in the absence of social and environmental cues. *Nature*, **401**, 155–157.

Piertney SB, Dallas JF (1997) Isolation and characterization of hypervariable microsatellites in red grouse *Lagopus lagopus scoticus*. *Molecular Ecology*, **6**, 93–95.

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. *Evolution*, **43**, 258–275.

Raymond M, Rousset F (1995) An exact test for population differentiation. *Evolution*, **49**, 1280–1283.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223–225.

Riley TZ, Davis CA (1993) Vegetative characteristics of lesser prairie-chicken brood foraging sites. *Prairie Naturalist*, **25**, 243–248.

Riley TZ, Davis CA, Smith RA (1992) Vegetative characteristics of successful and unsuccessful nests of lesser prairie-chickens. *Journal of Wildlife Management*, **56**, 383–387.

Saccheri I, Kuussaari M, Kankare M, Vikman P, Fortelius W, Hanski I (1998) Inbreeding and extinction in a butterfly metapopulation. *Nature*, **392**, 491–494.

Schneider S, Roessli D, Excoffien L (2000) ARLEQUIN ver 2.000: A software for population genetics data analysis. *Genetics and Bometry Laboratory*, University of Geneva, Switzerland.

Schroeder MA, Braun CE (1991) Walk-in traps for capturing greater prairie-chickens on leks. *Journal of Field Ornithology*, **62**, 378–385.

Seutin G, White BN, Boag PT (1991) Preservation of avian blood and tissue samples for DNA analyses. *Canadian Journal of Zoology*, **69**, 82–90.

Sherman P (1999) Birds of a feather lek together. *Nature*, **401**, 119–120.

Shorey L, Piertney S, Stone J, Höglund J (2000) Fine-scale genetic structuring on *Manacus manacus* leks. *Nature*, **408**, 352–353.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X window interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, **25**, 4876–4882.

Uphyrkina O, Miquelle D, Quigley H, Driscoll C, O'Brien SJ (2002) Conservation genetics of the far eastern leopard (*Panthera pardus orientalis*). *Journal of Heredity*, **93**, 303–311.

US Fish and Wildlife Service (2002) Candidate notice of review. *Federal Register*, **67**, 40667.

Van Den Bussche RA, Hoofer SR, Wiedenfeld DA, Wolfe DH, Sherrod SK (2003) Genetic variation within and among fragmented populations of lesser prairie-chickens (*Tympanucus pallidicinctus*). *Molecular Ecology*, **12**, 675–683.

Waser PM, Strobeck C (1998) Genetic signatures of interpopulation dispersal. *TREE*, **13**, 43–44.

Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.

Westemeier RL, Brawn JD, Simpson SA *et al*. (1998) Tracking the long-term decline and recovery of an isolated population. *Science*, **282**, 1695–1698.

Woodward AJW, Fuhlendorf SD, Leslie DM, Shackford J (2001) Influence of landscape composition and change on lesser prairie-chicken (*Tympanuchus pallidicinctus*) populations. *American Midland Naturalist*, **145**, 261–274.

Wright S (1951) The genetical structure of populations. *Annals of Eugenics*, **15**, 323–354.

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