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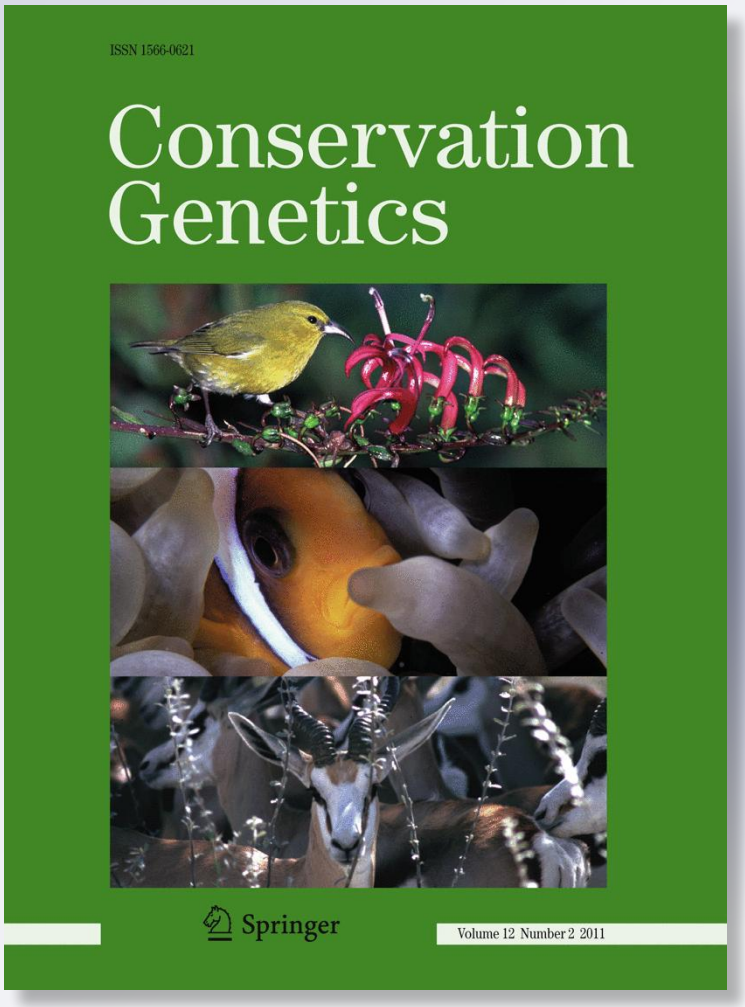

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RESEARCH ARTICLE

Population structure and genetic diversity of greater sage-grouse (*Centrocercus urophasianus*) in fragmented landscapes at the northern edge of their range

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Abstract Range-edge dynamics and anthropogenic fragmentation are expected to impact patterns of genetic diversity, and understanding the influence of both factors is important for effective conservation of threatened wildlife species. To examine these factors, we sampled greater sage-grouse (*Centrocercus urophasianus*) from a declining, fragmented region at the northern periphery of the species' range and from a stable, contiguous core region. We genotyped 2,519 individuals at 13 microsatellite loci from 104 leks in Alberta, Saskatchewan, Montana, and Wyoming. Birds from northern Montana, Alberta, and Saskatchewan were identified as a single population that exhibited significant isolation by distance, with the Milk River demarcating two subpopulations. Both subpopulations exhibited high genetic diversity with no evidence that peripheral regions were genetically depauperate or highly structured. However, river valleys and a large agricultural region were significant barriers to dispersal. Leks were also composed primarily of non-kin, rejecting the idea that leks form because of male kin association. Northern Montana sage-grouse are maintaining genetic connectivity in fragmented and northern peripheral habitats via dispersal through and around various forms of fragmentation.

Keywords Sage-grouse · Genetic structure · Declining population · Genetic diversity · Periphery

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Introduction

The effects of habitat fragmentation and peripheral habitat on genetic diversity are important topics in conservation genetics. Fragmentation can impact gene flow by decreasing dispersal and population size, and increasing genetic drift in isolated pockets (Frankel and Soulé 1981). Declining populations often experience greater loss of genetic diversity, inbreeding, and fixation of deleterious alleles, all of which may increase probability of extinction and reduce adaptive potential of populations (Frankel and Soulé 1981). Species with strong dispersal ability should be more resilient to fragmentation (Galbusera et al. 2004; Veit et al. 2005; Martínez-Cruz et al. 2007), but more sedentary species, particularly galliform birds, often display significant genetic structure and differentiation from fragmentation at varying spatial scales (greater prairie-chicken [*Tympanuchus cupido pinnatus*], Johnson et al. 2003; Johnson et al. 2004; black grouse [*Tetrao tetrix*], Caizergues et al. 2003a; capercaillie [*Tetrao urogallus*], Segelbacher et al. 2003; rock ptarmigan [*Lagopus mutus*], Caizergues et al. 2003b; lesser prairie-chicken [*Tympanuchus pallidicinctus*], Van den Bussche et al. 2003; Bouzat and Johnson 2004).

Peripheral populations are often touted as sources of unique genetic variation, which may allow adaptation to future climate change, habitat alteration, range expansion, or speciation events, but they can also be viewed as genetically depauperate, doomed to extinction, and not worth conservation effort (Eckert et al. 2008). Populations at range peripheries are considered more susceptible to declines because they occupy marginal habitat and are isolated from larger central populations (Lesica and Allendorf 1995; Sargarin and Gaines 2002). Peripheral populations are usually smaller in census and effective population sizes, are more genetically isolated, exhibit founder effects or genetic drift, and are prone to extinction from stochastic or catastrophic events (Lammi et al. 1999; Vucetich and Waite 2003). Some studies have found peripheral populations to be less genetically diverse than central populations (Lammi et al. 1999; Vucetich and Waite 2003; Bouzat and Johnson 2004), while others have not (Kirkpatrick and Ravigne 2002; Eckert et al. 2008). Understanding the genetic consequences of habitat fragmentation and range periphery is crucial for management and conservation of key management species and the ecosystems on which they depend, particularly those experiencing continued anthropogenic fragmentation and range contraction.

In this study we assess how northern range periphery and fragmentation impact genetic diversity and structure in greater sage-grouse (hereafter sage-grouse; *Centrocercus urophasianus*). Sage-grouse are a good model system because large sample sizes are obtainable, they are well studied, and basic biological and habitat parameters are known. Microsatellite markers and baseline genetic data are available for the species (Oyler-McCance et al. 2005). They are also a species of concern in North America due to rapid population declines and habitat destruction (Connelly et al. 2004). Our study encompasses the northernmost portion of the species' distribution, an area that has experienced substantial anthropogenic fragmentation and the most significant declines range-wide (Connelly et al. 2004).

Historically, sage-grouse inhabited three Canadian provinces (Alberta, Saskatchewan, and British Columbia) and 16 U.S. states, but presently occur only in southeastern Alberta, southwestern Saskatchewan, and 11 U.S. states (Schroeder et al. 2004). In Canada, sage-grouse numbers have declined by 66–92% since the 1970s (Aldridge and Brigham 2003) with an estimated 2010 population size of approximately 250 birds (Alberta Fish and Wildlife, Saskatchewan Environment, and Parks Canada, unpubl. data). Populations in the United States have declined at a slower rate, varying from 45 to 80% across the species' range, with the central Montana and central/southern Wyoming regions remaining relatively stable and considered the "core" of the species range (Connelly et al. 2004). Rangewide, the amount of habitat has decreased by over 50% (Schroeder et al. 2004) from conversion of native sage-steppe to agriculture, municipal infrastructure, and energy development (Connelly et al. 2004).

Sage-grouse are polygamous galliforms where males congregate on communal display grounds (leks) in the spring and females select a mate, breed, and incubate and raise the young on their own (Gibson 1996). Leks are highly stable locations that can be used for up to 100 years (Dalke et al. 1963). Although it is generally expected that grouse are philopatric and leks consist of related males (Kokko and Lindström 1996), recent molecular evidence suggests that this may not be the case for sage-grouse (Gibson et al. 2005). If mating success within leks is highly skewed (Wiley 1973), it may be expected that there will be a reduction in effective population size and an increase in genetic structure and inbreeding (Wright 1938; Nunney 1993). These life history constraints combined with recent habitat fragmentation may make sage-grouse susceptible to erosion of genetic diversity by drift.

Determining genetic population structure is essential for managing declining peripheral, populations in a fragmented landscape. Connelly et al. (2004) classified sage-grouse into 41 populations across North America based on spatial isolation from other populations by at least 10 km. The Northern Montana population (NMP; Canada and Montana north of the Missouri River) was split into three subpopulations based on potential habitat barriers (Fig. 1). The Milk River separates subpopulation "A" to the south and an agricultural region in southwester Saskatchewan separates subpopulations "B" and "C" in the north (Fig. 2;

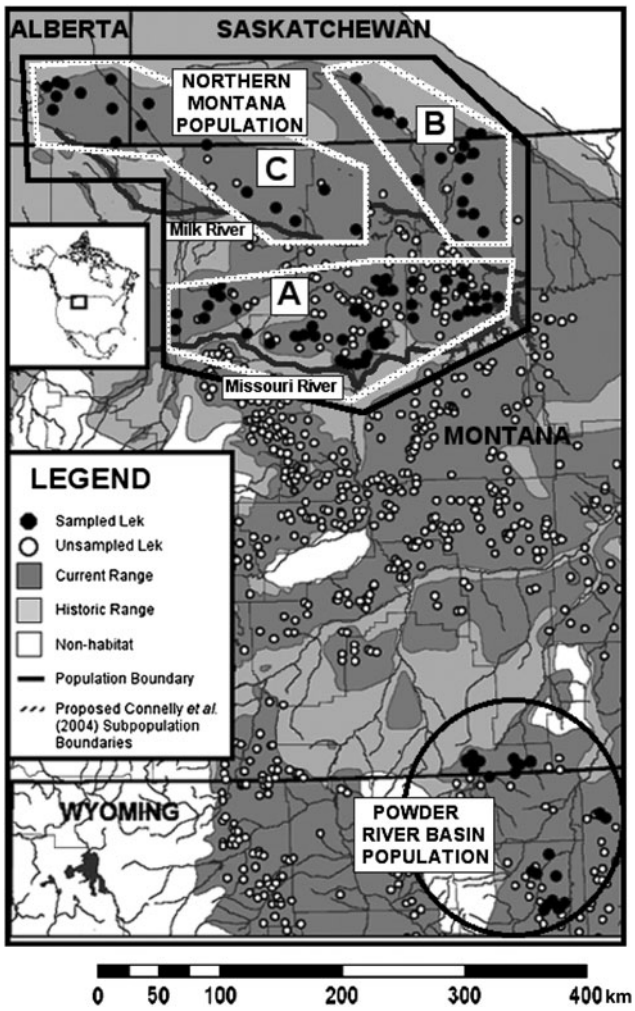

Fig. 1 Study area map with the northern Montana and Powder River Basin populations *highlighted*. *Dashed lines* represent the three NMP subpopulations (*A*, *B*, and *C*) suggested by Connelly et al. (2004). Milk and Missouri Rivers are indicated by wide *grey lines* in the middle and bottom of the northern Montana population, respectively. Map modified from Schroeder et al. (2004)

Connelly et al. 2004). Oyler-McCance et al. (2005) showed isolation-by-distance (IBD) with restricted gene flow across the entire sage-grouse range and identified ten distinct populations using R_{ST} and STRUCTURE (Pritchard et al. 2000). One genetic population included Alberta and most of Montana, but this was a coarse-scale range-wide study, which sampled relatively small numbers of birds from multiple locations across the range.

We used polymorphic microsatellites to test three hypotheses regarding genetic structure and diversity in a fragmented sage-grouse population along the northern edge of the species' range:

(1) birds north of the Missouri river form one highly structured genetic population that is distinct from populations to the south,
(2) leks are not composed of highly related males, and

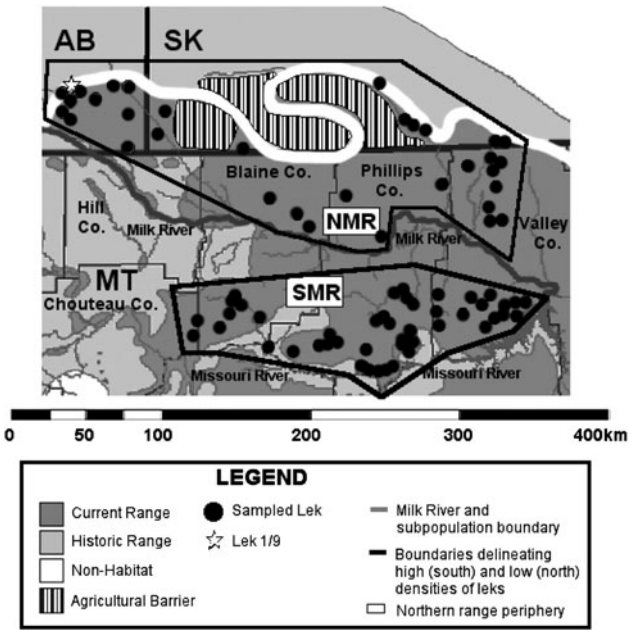

Fig. 2 Map depicting the two subpopulations identified within the northern Montana population by STRUCTURE and partial Mantel analyses: north of the Milk River (NMRS) and south of the Milk River (SMRS). The *white star* in the far north west corner of the range represents the only genetically unique lek identified by STRUCTURE. *Dark lines* represent boundaries delineating high (south of the Milk River) and low (north of the Milk River) densities of sage-grouse and leks. The *white line* represents the northern range periphery. Map modified from Schroeder et al. (2004). To see enlarged maps with all of the sampled leks labeled, go to: http://www.aviangenetics.com/northern_montana_maps/

(3) Northern peripheral and fragmented populations exhibit lower genetic diversity than less peripheral or fragmented regions of the range.

We predicted population structure within the NMP due to substantial declines in lek counts, extensive natural and anthropogenic habitat fragmentation, and isolation at the northern periphery of the species' range. We predicted that leks would not be composed of related males based on Gibson et al.'s (2005) finding that sage-grouse males displayed low levels of relatedness within leks. We anticipated lower genetic diversity in the northern periphery compared to high-density regions near the Missouri River because it is both highly fragmented and geographically distant from core habitat.

Materials and methods

Study location and sample collection

This study was conducted on sage-grouse from the NMP (14.2% of the total sage-grouse range). Samples from the northern Powder River Basin (PRB; Fig. 1) were included

as an outgroup to delineate structure of the NMP. Birds were captured using walk-in funnel traps (Schroeder and Braun 1991), night-lighting (Giesen et al. 1982), rocket nets (Giesen et al. 1982), and drop-nets (Bush 2008). Blood (n = 290), plucked feather (n = 974), mouth swab (n = 104), and shed feather (n = 2,441) samples were collected from adult sage-grouse as part of research projects in the NMP (Alberta [1998–2006] and Montana: Phillips [2001–2005] and Valley [2006] counties) and northern PRB (Montana: Bighorn county [2003–2006] and Wyoming: Sheridan [2003–2006], Campbell [2003–2004], and Johnson [2004–2006] counties). The NMP was sampled using molted feathers collected from leks in Alberta and Saskatchewan (2003–2006), Valley (2005), Blaine (2005 and 2006), Phillips (2006), and Choteau (2006) counties, Montana. Not all active leks were sampled in both populations (Fig. 1). We only sampled leks that were being surveyed and/or studied in the NMP and the PRB. To increase the sample size for birds in Canada, we opportunistically sampled off-lek. Off-lek birds consisted of females captured in the company of radio-collared females, carcasses of unmarked vehicular or predator mortalities, and molted feathers found at roost sites. All birds sampled off lek were assigned an unknown lek status and were not used in any lek-specific analyses. Overall, we collected 3,824 samples (3,616 from 104 leks [83 NMP, 21 PRB] and 208 off-lek). The PRB samples were only used to assess whether the NMP formed a single population and if the Missouri river was a barrier to gene flow. All NMP samples were used for all population and subpopulation level analysis, while only leks over ten (lek-level) and five (sex-specific) sampled birds were used for finer scale analyses.

Microsatellite genotyping

DNA was extracted using Qiagen DNeasy® Tissue and QIAamp® DNA Micro kits using modifications from Bush et al. (2005). All samples were DNA-sexed following the procedure in Bush et al. (2005). Seventy-five galliform microsatellite loci were screened on ten randomly selected sage-grouse DNA samples. Any loci that produced two or more alleles for those ten individuals were retained (21 loci) and were further screened on 96 individuals from every sample type (blood, muscle tissue, plucked feather, molted feather, and saliva). Three loci proved sex-linked (all females appeared homozygous) and/or contained high frequency null alleles (>75% of individuals appeared homozygous) and five loci were very weak on low quality DNA samples (i.e., did not amplify consistently for molted feathers) so these eight loci were excluded from subsequent analyses. We identified null alleles by examining 20 sage-grouse females and their known offspring for mismatches. The 13 microsatellite loci used in this analyses were developed from sage-grouse (SGCA9-2 [redesigned primer set; S. Taylor, pers. comm.] and SGCA5; Taylor et al. 2003), capercaillie (TUT3, TUT4, TUD1, and TUD3; Segelbacher et al. 2000), black grouse (BG6 and BG15; Piertney and Höglund 2001; TTD6 and TTT1; Caizergues et al. 2001; TTT3; Caizergues et al. 2003a), red grouse (*Lagopus lagopus*; LLSD8; Piertney and Dallas 1997), and domestic chicken (*Gallus gallus*; ADL230; Cheng et al. 1995). Microsatellite PCRs (15 µl total volume) were carried out as described in Bush et al. (2005). Forward primers were fluorescently labeled with 6-FAM, TET, and HEX (Applied Biosystems). We followed the PCR cycling conditions outlined for each microsatellite in the original publications using Perkin Elmer Cetus GeneAmp PCR System 9600® and Eppendorf Mastercycler® ep machines. All non-invasive samples were run in triplicate (Bush et al. 2005). The PCR products were visualized using an ABI 377® automated sequencer with GENESCAN ANALYSIS3.1® software (Applied Biosystems). Alleles were scored using GENOTYPER®2.0 software (Applied Biosystems).

Duplicate samples

Shed feathers are normally considered inferior samples, but on leks, most result from fighting and are equivalent in quality to plucked feathers. We quantified the DNA quality of each feather by amplifying the five strongest microsatellites (TUT3, TUT4, SGCA5, SGCA9-2, and TTD6) once and assessing peak height and quality. Then triplicate PCR replicates were performed with 3–5 µl DNA. For shed feathers with lower quality DNA, a maximum of 7–11 microsatellites were successfully amplified in triplicate for each sample. For all other samples, all 13 loci were amplified. In low quality feather samples, low rates of drop out and no false alleles were detected. For all samples that failed to produce the same genotype in three of three replicates for any locus, the genotype for that locus was excluded and only consistent genotypes (three of three replicates) were kept for that sample (i.e., if a sample produced the same genotype at one locus in two of three runs, the genotype for that locus was excluded from the composite genotype, which was composed of all 13 loci). Duplicate samples were identified using GENALEX version 5.1 (Peakall and Smouse 2001). Two samples were considered duplicates if they were identical or differed by no more than one allele at up to two loci in a manner consistent with allelic drop out. Missing data was ignored to allow for matches between fully genotyped samples and samples with one or more missing loci. Probability of identity (PI) was calculated in GENALEX.

Population structure

We investigated spatial genetic structure using the Bayesian program STRUCTURE (Pritchard et al. 2000), which puts individuals into clusters (K) based on multilocus genotypic data, independent of sample location. Highly related individuals (parent-offspring and full-siblings) were identified with COLONY, version 1.2 (Wang 2004a) and all but one relative was removed prior to STRUCTURE analysis to minimize lower-level structure caused by first-order relatives. We examined three levels of population structure to delineate the boundaries for sage-grouse populations in the region. First, all birds from the NMP and the PRB were included to identify the number of populations. Next, the NMP birds were used to identify the number of subpopulations within the population. Finally, we determined lower level structure within the NMP (genetically distinctive leks and lek clusters [groups of related neighboring leks]) by breaking the population into geographic regions containing <20 leks (i.e., Alberta and western Saskatchewan; Fig. 1). We determined K for the number of (1) populations, (2) subpopulations, and (3) lek clusters/leks by running 20 independent simulations for each K (1–20) with 100,000 burn-in iterations and 1,000,000 data repetitions assuming an admixture model, correlated allele frequencies (within the NMP; 0.01), and no prior population information. We used the method of Evanno et al. (2005), which calculates ΔK, a measure of the second order rate of change in the likelihood of K, to estimate the true K. We used this method because both Evanno et al. (2005) and the software documentation note that it is computationally difficult to obtain accurate estimate of K using $\Pr(X|K)$ values and its biological interpretation may not be straightforward.

We examined genetic population structure within the NMP using hierarchical analysis of molecular variance (AMOVA) in ARLEQUIN, version 3.1 (Excoffier et al. 2006) with F_{ST} as the genetic distance measure.

Genetic diversity and differentiation

We calculated expected (H_E) and observed (H_O) heterozygosity for each locus and tested for deviations from Hardy–Weinberg and gametic equilibrium using GENEPOP, version 3.4 (Raymond and Rousset 1995). Number of alleles per locus (A) was calculated in GENALEX and allelic richness (number of alleles corrected for the smallest sample size; AR) in FSTAT, version 2.9.3 (Goudet 2001). Average relatedness (R) within and between-leks was computed in RELATEDNESS 5.0 (Queller and Goodnight 1989). Pairwise-F_{ST} was calculated in GENEPOP and significance tests were performed in FSTAT using 1,000 permutations. The preceding diversity indices were calculated for the NMP, both subpopulations, and all leks. Levels of significance were adjusted using the false discovery rate method (Benjamini and Yekutieli 2001) and Dunn-Sidák method of Bonferroni correction (Sokal and Rohlf 1995) when multiple statistical tests were conducted simultaneously. Tests for differences among groups for AR, H_O, R, and F_{ST} were performed in FSTAT using 1,000 permutations and two-sided tests.

We characterized lek-to-lek genetic differentiation by calculating pairwise-F_{ST} for the population (49 leks), each sex within the population (males = 57 leks, females = 23 leks), each subpopulation (north of the Milk River subpopulation [NMRS] = 22, south of the Milk River subpopulation [SMRS] = 27), and each sex within each subpopulation (NMRS males = 24, NMRS females = 11, SMRS males = 33, SMRS females = 12; see results for subpopulation descriptions). For the analyses at the population and subpopulation levels, all birds and leks were retained. For analyses at the lek level and for each sex, we used leks with a minimum sample size of ten and five, respectively. We regressed F_{ST} against geographic distance to test for IBD and tested for significance using a Mantel test (Mantel 1967) in R-PACKAGE, version 4.0 (Casgrain and Legendre 2001).

We estimated contemporary dispersal between populations (NMP and PRB) and subpopulations (NMRS and SMRS) using assignment tests in STRUCTURE, which places individuals into their most likely population or subpopulation of origin based on the method from Bergl and Vigilant (2007). We used a coalescent-based model to obtain maximum likelihood estimates of asymmetric gene flow ($4Nm$) over time between populations, subpopulations, and groups (MIGRATE 3.0; Beerli and Felsenstein 2001). We applied the following settings: ten short chains with 50,000 trees sampled, 500 trees recorded, three long chains with 500,000 trees sampled, and 5,000 trees recorded. Burn-in was set at 10,000 trees for each chain type. We selected the Brownian motion approximation and assumed equal mutation rates between microsatellite loci. Analyses included ten runs that were replicated five times within a single run. A different random number seed was used each time.

Lek structure

We computed mean coefficients of relatedness (R) for males and females within leks using RELATEDNESS and compared sample means to a null expectation of zero using a t-test to determine whether males and females attending the same lek were more related than expected by chance (Gibson et al. 2005; Bush et al. 2010). Population allele frequencies did not differ significantly between years, sexes, or leks, excluding lek 1/9 (Bush 2009) therefore, we used the NMP frequencies for all analyses. Relatedness

among males and females within leks was estimated and standard errors were calculated using the jackknife re-sampling procedure in RELATEDNESS. Within-lek R was calculated for each sex in each lek along with jackknifed standard errors. To calibrate our estimates of relatedness, we calculated estimates of relatedness within families, specifically known mother-offspring, full-siblings, and half-siblings, in RELATEDNESS and compared the means to a null expectation of 0.5 (mother-offspring and full-siblings) and 0.25 (half-siblings) using a one sample t-test.

Range periphery and fragmentation

To determine whether part (or all) of the NMP fit the assumptions of a peripheral population, we regressed density (males/km^2; range of 0.05–0.40; based on Fig. 13.1 in Connelly et al. 2004), distance to the nearest active neighbor lek, and lek counts (number of males counted on a given lek in a given morning each spring) against geographic distance to the northern range edge. To investigate whether genetic diversity was significantly lower in (a) low density and (b) northern peripheral regions, we calculated AR, H_O, R, and F_{ST} and tested for differences among groups (low density [0.05 males/km^2] vs. high density [>0.15 males/km^2] and northern periphery vs. core) in FSTAT using 1,000 permutations and two-sided tests. Low and high-density regions were categorized using Connelly et al. (2004). Leks situated on the northern range periphery were identified by measuring the geographic distance of each lek to the closest point on the current northern range edge (white line in Fig. 2). All leks within 50 km of the northern range edge were considered peripheral and the rest were classified as core. This is an arbitrary distance, but was chosen because leks were either <50 km or >100 km from the range edge and it provided a natural break for classification purposes. We did not use the species' historic range edge because it was based on several unsubstantiated observations and erroneous locations for historic specimens resulting in an inflated range (Bush 2009). To determine whether proximity to northern range periphery impacted genetic diversity, we regressed all four measures against geographic distance to the range edge and tested for significance using a Mantel test in R-PACKAGE. This test was also performed independently for both sexes.

We tested whether major habitat features acted as dispersal barriers to sage-grouse using partial Mantel tests in R-PACKAGE. Tests using the Missouri and Milk River valleys as barriers served to support the STRUCTURE results (Fig. 2). For a large agricultural region in southwestern Saskatchewan, we performed the test using both lek-to-lek straight-line-distance and distance required to circumvent the disturbance. We also tested other potential barriers (e.g., political boundaries, smaller patches of agriculture and anthropogenic disturbance, and areas of non-habitat [not greatly disturbed, but lacking suitable habitat]) both within the NMP and within each subpopulation. Partial Mantel tests were performed using lek-to-lek F_{ST}, lek-to-lek geographic distance, and a barrier matrix (leks on the same side of the barrier versus leks on the opposite side of the barrier) to assess whether potential barriers impeded gene flow. Tests were performed with sexes combined and separate to detect differences in sex-specific dispersal.

We also regressed diversity indices (AR, H_O, R, and F_{IS}) on distance to the nearest active lek to examine the effects of isolation. Fragmentation levels and type differed greatly between the northern and southern portions of the NMP. Primary causes of fragmentation north of the Milk River included oil and gas development (Alberta; Lungle and Pruss 2008) and agriculture (Saskatchewan [Lungle and Pruss 2008]. Habitat was much less fragmented south of the Milk River (J. Carlson, personal communication), but we could not quantify or test for differences between regions because high-resolution land cover maps were not available for the entire study area.

Results

Identification of unique individuals

A total of 3,810 of 3,824 (99.6%) samples contained enough DNA to amplify seven or more loci in triplicate. Of the 3,824 samples, 2,519 (65.4%) were unique. Because most shed feathers were replicates of another sample (range of replicates = 1–43), most individual samples with one or more loci that failed to amplify could be fully characterized because a duplicate sample filled in the missing gap(s). PI and PI for siblings were set to 0.001 and achieved at four and seven loci respectively. Of the 2,519 samples, 1075 were from the NMRS, 1062 from the SMRS, and 382 from the PRB; 969 (286 NMRS, 380 SMRS, 303 PRB) were female and 1550 (789 NMRS, 682 SMRS, 79 PRB) male.

Population structure

At the population level, the most likely K using the ΔK method was two, with the PRB distinct from the NMP (Table 1). The $\Pr(X|K)$ method selected $K = 3$ as the most likely, with the NMP split into two (Table 1), but there appears to be considerable dispersal between the two regions within the NMP (Table 6 Appendix 1), so we feel that there are two populations (NMP and PRB). No further data are presented from the PRB as it was only included to define the NMP boundary. Within the NMP, the most likely number of subpopulations was two using the

Table 1 Estimates of K and Pr(X|K) for each K in STRUCTURE at the population and subpopulation (within-NMP) levels

K	NMP + PRB		NMP Only	
	ΔK	Pr(X|K)	ΔK	Pr(X|K)
1	–	0	–	0
2	174.1*	0.0005	142.8*	2.6×10^{-195}
3	96.1	0.99*	1.1	2.4×10^{-106}
4	4.7	2.3×10^{-5}	2.4	2.6×10^{-73}
5	9.5	5.1×10^{-7}	7.2	1.4×10^{-34}
6	24.5	7.5×10^{-10}	6.7	0.006
7	3.4	1.4×10^{-32}	4.9	0.99*
8	2.2	8.3×10^{-39}	5.1	5.3×10^{-7}
9	3.6	2.2×10^{-72}	1.8	7.0×10^{-5}
10	3.8	4.4×10^{-56}	3.4	8.9×10^{-11}
11	3.8	5.6×10^{-98}	4.2	1.6×10^{-39}
12	1.6	3.4×10^{-108}	2.1	5.6×10^{-49}
13	15.3	9.2×10^{-123}	4.4	1.5×10^{-54}
14	1.0	6.1×10^{-145}	1.9	6.7×10^{-160}
15	9.9	1.2×10^{-167}	1.2	2.8×10^{-121}
16	3.3	8.8×10^{-192}	0.9	7.8×10^{-139}
17	0.1	6.4×10^{-203}	0.2	6.9×10^{-207}
18	0.6	3.3×10^{-248}	0.7	4.4×10^{-268}
19	0.6	2.1×10^{-273}	0.8	4.5×10^{-296}
20	–	5.7×10^{-365}	–	3.4×10^{-304}

* Signify the most likely K for each method

ΔK method and seven using the Pr(X|K) method, but all seven groups contained birds from all parts of the NMP, therefore we recognized only two subpopulations (Table 1). When leks were plotted for the percentage of birds assigning to subpopulation one, subpopulation two, or being admixed (the product of dispersal between the two subpopulations) and we considered partial Mantel results that identified genetic barriers, the most likely subpopulation boundary was the Milk River. The two subpopulations identified were north (NMRS) and south (SMRS) of the Milk River. We identified no lek clusters in STRUCTURE with the exception of lek 1/9 in Alberta, which appeared distinct when paired with any subset of leks within the NMRS and a $K = 2$ was always the most likely.

Almost all genetic variation (96.8%, $P < 0.001$) detected within the NMP was within leks and due to inter-individual differences (Table 2a). We found very little genetic variation among subpopulations (0.5%, $P < 0.001$) or among leks within subpopulations (2.7%, $P < 0.001$; Table 2a). Independent analysis of subpopulations confirmed that the majority of variation was found within individual leks (NMRS, 96.9%, $P < 0.001$; Table 2b; SMRS, 97.4%, $P < 0.001$; Table 2c).

Genetic diversity and differentiation

We examined departures from Hardy–Weinberg and linkage equilibrium within the NMP. There was disequilibrium after loci were corrected for multiple comparisons using the false discovery rate (FDR) method at both the population (NMP, TUT4 and SGCA9-2; PRB; TUD3) and subpopulation (NMR, TUT4; SMR, SGCA9-2) levels (Appendix 2 in supplementary material). However, at the lek level, no loci deviated from equilibrium at any lek. We tested to the lek level because combining different leks (presumably familial groups) may result in disequilibrium within the population or subpopulation. Sampling across years and within a non-random mating population with

Table 2 AMOVA comparing genetic variation in microsatellite data for the (a) northern Montana population, (b) north of the Milk River subpopulation, and (c) south of the Milk River subpopulation

Source of variation	d.f.	Sum of squares	Variance components	Fixation indices	P value	Percentage of variation
a						
Among subpopulations (n = 2114)	1	13.1	0.003	0.03	<0.001	0.5
Among leks within subpopulations	47	95.4	0.02	0.03	<0.001	2.7
Within leks	3641	2505.2	0.7	0.005	0.001	96.8
Total	3689	2613.7	0.7			
b						
Among NMRS leks (n = 1075)	37	66.9	0.02	0.03	<0.001	3.1
Within NMRS leks	2007	1399.5	0.7	0.03	<0.001	96.9
Total	2114	1466.4	0.7			
c						
Among SMRS leks (n = 1039)	46	103.4	0.03	0.03	<0.001	2.6
Within SMRS leks	2031	2141.2	1.05	0.03	<0.001	97.4
Total	2077	2244.6	1.08			

migration and selection may also lead to deviations from equilibrium (Guo and Thompson 1992) and many leks represented single year samplings. When linkage disequilibrium was examined at the population level, 22 of 78 comparisons were in disequilibrium for the NMP and seven or 78 for the PRB (Appendix 2 in supplementary material). At the subpopulation level, four (SMRS) and 41 (NMRS) comparisons were in disequilibrium, but no two pairs of loci were in disequilibrium across subpopulations. All genetic tests were re-run excluding TUT4 and SGCA9-2 and there were no significant differences in any of the results from those including the two loci; therefore all 13 loci were included in the final analysis.

All loci were polymorphic (6–31 alleles per locus) with high A, AR, H_O, and H_E for the NMP and both subpopulations (Table 3). There was no statistical difference in any genetic diversity or relatedness measure between subpopulations (AR [$P = 0.83$], F_{ST} [$P = 1.00$], R [$P = 1.00$], H_O [$P = 1.00$]). Pairwise-F_{ST} values were low with the exception of those involving lek 1/9 (Appendix 3 in supplementary material).

We observed significant isolation-by-distance relationships between leks for the NMP (Mantel r = 0.21, $P = 0.001$), northern Montana females (Mantel r = 0.27, $P = 0.001$), northern Montana males (Mantel r = 0.18, $P = 0.001$), the NMRS (Mantel r = 0.17, $P = 0.005$, Fig. 3a), the SMRS (Mantel r = 0.37, $P = 0.006$, Fig. 3b), NMRS males (Mantel r = 0.17, $P = 0.001$, Fig. 3c), SMRS males (Mantel r = 0.21, $P = 0.01$, Fig. 3d), and NMRS females (Mantel r = 0.30, $P = 0.05$, Fig. 3e), but not SMRS females (Mantel r = 0.05, $P = 0.28$, Fig. 3f).

We defined first-generation dispersers as individuals assigning >80% to the other population or subpopulation. The assignment test, which investigates contemporary dispersal, revealed eight birds (five males, three females) in the PRB dispersed from the NMP and 37 birds (16 males, 21 females) in the NMP dispersed from the PRB. Dispersal between northern Montana subpopulations was greater from south to north (15; 14 males, 1 females) than north to south (7; 6 males, 1 female). The estimates of dispersal determined using MIGRATE 3.0 were generally higher than contemporary estimates; 18.8 ± 1.5 birds moved from the PRB to the NMP and 23.1 ± 1.4 moved the opposite direction; 44.4 ± 1.7 birds moved from the SMRS to the NMRS and 42.4 ± 1.6 birds moved in the opposite direction.

Lek structure

Mean estimates of R did not differ statistically from the expected value of 0.5 for mother-offspring (mean ± SD = 0.49 ± 0.07, $P = 0.22$) and full-siblings (0.53 ± 0.09, $P = 0.14$) and 0.25 for half-siblings (0.27 ± 0.04, $P = 0.75$). Both average male (mean ± SE = 0.01 ± 0.09, $t = 1.2$, df = 56, $P = 0.24$) and female (mean ± SE = 0.001 ± 0.06, $t = -0.5$, df = 24, $P = 0.65$) R did not differ from zero for all leks combined. When individual leks were examined independently for each sex, only R from males on three of 54 leks; leks 1/9 (Alberta; $R = 0.57 \pm 0.13$, $P = < 0.001$), 35 (Alberta; $R = -0.03 \pm 0.02$, $P = 0.003$) and BL27-19-25 (Montana; $R = -0.23 \pm 0.08$, $P = 0.04$) was significantly different from zero. Female R differed significantly from zero for six of 23 leks; leks 10/11 (Alberta; $R = 0.24 \pm 0.01$, $P = 0.001$), 30 (Alberta; $R = 0.17 \pm 0.06$, $P < 0.001$), Mundell Creek (MC; Saskatchewan; $R = -0.09 \pm 0.06$, $P = 0.01$), Dixon Y (DY; Saskatchewan; $R = -0.10 \pm 0.07$, $P = 0.04$), PH-19 (Montana; $R = -0.08 \pm 0.05$, $P = 0.03$), and PH-33 (Montana; $R = -0.04 \pm 0.08$, $P < 0.001$).

Range periphery and fragmentation

Density ($R^2 = 0.61$, $t = 8.62$, $P < 0.001$) and lek counts ($R^2 = 0.51$, $t = 6.96$, $P < 0.001$) increased significantly with increasing distance from the edge, while distance to nearest neighbor lek ($R^2 = 0.13$, $t = -2.70$, $P = 0.009$) decreased, therefore northern leks fit non-genetic assumptions of peripherality. High and low density leks did not differ in AR ($P = 0.83$), H_O ($P = 1.00$), F_{IS} ($P = 1.00$), or R ($P = 1.00$), nor did peripheral leks ($P = 0.92$, 1.00, 1.00, and 0.89, respectively). For the NMP, which is situated at the species' northern range edge, none of the four measures were related to geographic distance from the northern range edge for all birds combined (Fig. 4), females, and males (Table 4).

Both the Missouri (partial Mantel r = 0.19, $P = 0.001$) and Milk (partial Mantel r = 0.22, $P = 0.001$) River valleys (and associated non-habitat) and the agricultural region in southwestern Saskatchewan (partial Mantel r for straight-line-distance between leks = 0.19, $P = 0.001$) were significant barriers to dispersal. When lek-to-lek distance required to circumvent the agricultural disturbance

Table 3 Estimated genetic variation with standard errors for the northern Montana population and its subpopulations: NMRS and SMRS

(Sub)population	n	A	AR	H_O	H_E	R	F_{ST}
Total population	2137	14.8 (2.0)	12.4 (1.8)	0.66 (0.04)	0.71 (0.04)	0.016 (0.001)	0.009 (0.002)
NMRS	1075	12.0 (1.7)	11.9 (1.7)	0.66 (0.03)	0.71 (0.04)	0.015 (0.002)	0.008 (0.002)
SMRS	1062	12.0 (1.9)	11.8 (1.9)	0.66 (0.04)	0.70 (0.05)	0.008 (0.001)	0.004 (0.0001)

F_{ST} represents the average level of genetic differentiation among leks within NMP and each subpopulation (NMRS and SMRS)

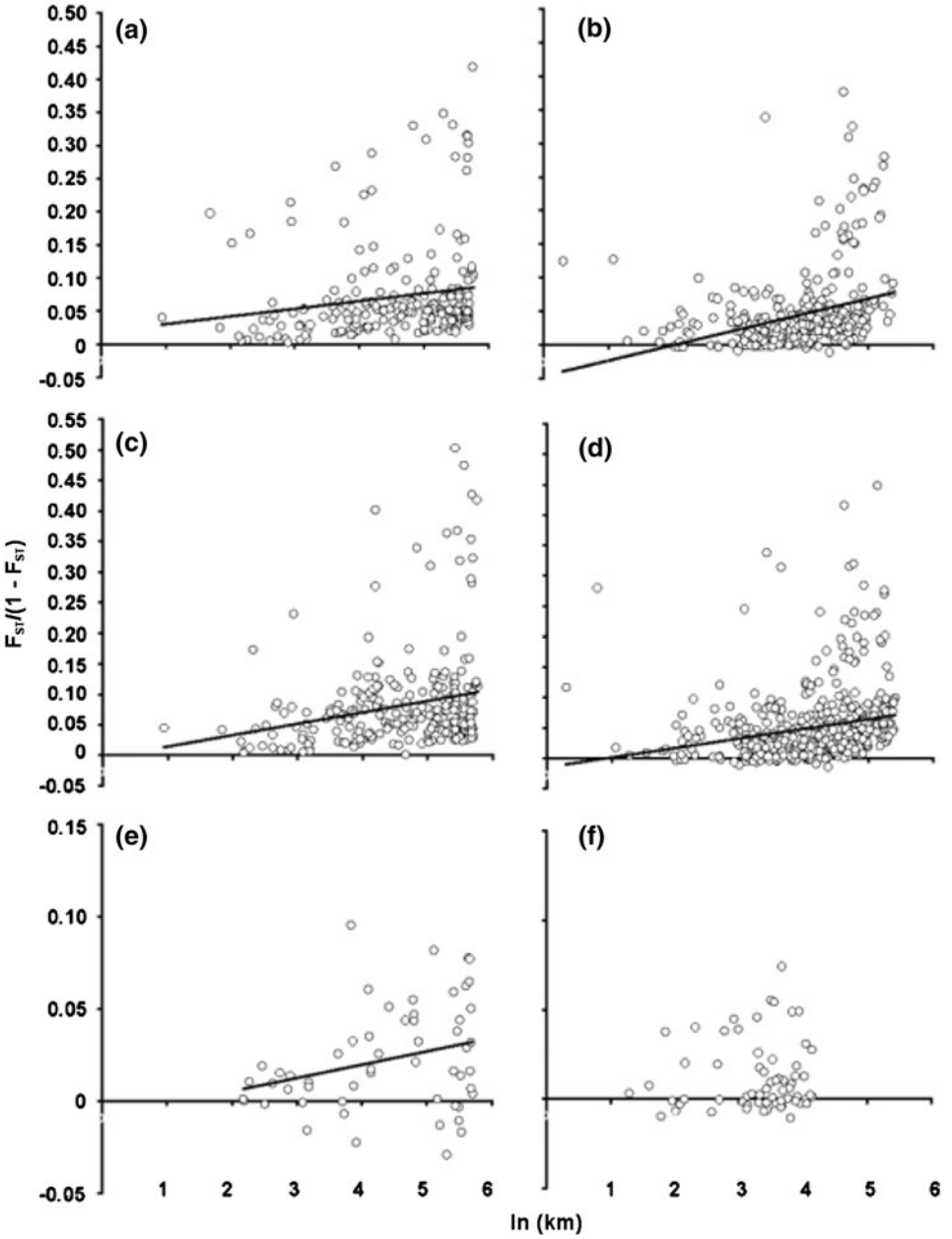

Fig. 3 Plots illustrating spatial genetic structure as an isolation-by-distance correlation between genetic distance ($F_{ST}/(1 - F_{ST})$) and geographical distance (ln[km]) for (**a**) the north of the Milk River subpopulation, (**b**) south of the Milk River subpopulation, males in the (**c**) north and (**d**) south of the Milk River subpopulation, females in the (**e**) north and (**f**) south of the Milk River subpopulation

in Saskatchewan was used, the results were close to significant (partial Mantel r = 0.14, $P = 0.06$). When the sexes were examined independently, the Milk River and surrounding disturbance was a barrier to both males (partial Mantel r = 0.18, $P = 0.001$) and females (partial Mantel r = 0.20, $P = 0.03$). The Saskatchewan cropland was also a barrier for males (partial Mantel r for straight-line-distance between leks = 0.15, $P = 0.001$; circumvent disturbance partial Mantel r = 0.13, $P = 0.07$) and females (partial Mantel r for straight-line-distance between leks = 0.27, $P = 0.001$; circumvent disturbance partial Mantel r = 0.78, $P = 0.001$). No other potential barriers were significant.

A regression of distance to the nearest active lek did not explain variation in any of the diversity indices for either both sexes combined (*P*-values; $AR = 0.83$, $H_O = 0.45$, $R = 0.07$, $F_{is} = 0.66$) or males alone (*P*-values; $AR = 0.95$, $H_O = 0.31$, $R = 0.89$, $F_{is} = 0.42$), but allelic richness vs. distance to the nearest active lek was significant for females (*P*-values; $AR = 0.0002$, $H_O = 0.06$, $R = 0.85$, $F_{is} = 0.40$).

Discussion

We found that all sage-grouse in the NMP formed a single population despite fragmentation and proximity to the

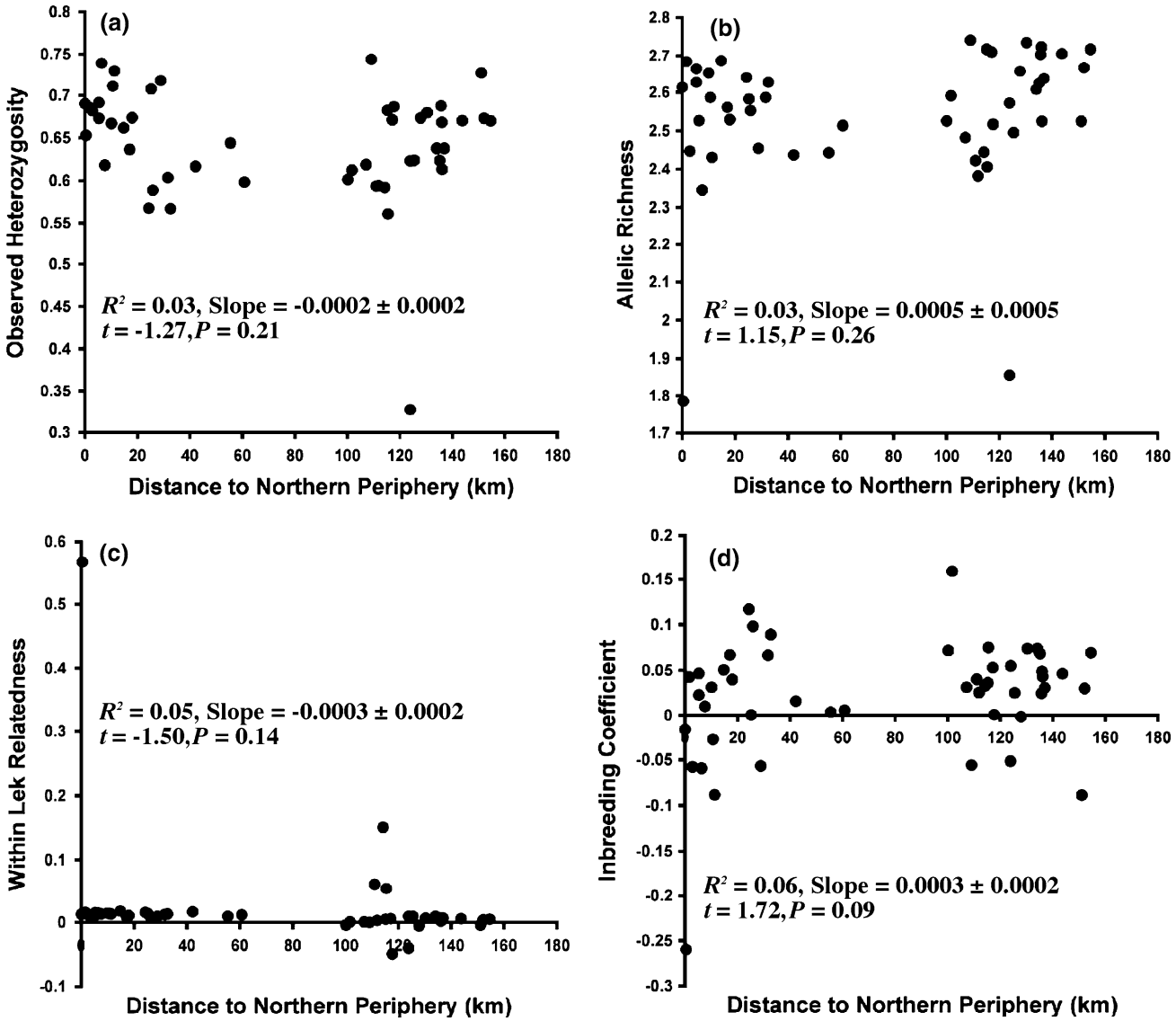

Fig. 4 Regressions of (**a**) observed heterozygosity, (**b**) allelic richness, (**c**) within lek relatedness, and (**d**) inbreeding coefficient (F_{IS}) against geographic distance to the current northern periphery of the species' range for all leks containing more than ten sampled birds in the northern Montana sage-grouse population

range periphery. There was substructure within the NMP north and south of the Milk River, but genetic diversity was high and equivalent in both subpopulations. Male sage-grouse did not form kin groups. Diversity values did not appear to change with distance to the northern range edge, but river valleys (with associated anthropogenic disturbance) and large areas of cropland represented significant barriers to dispersal.

Population structure

We identified two genetically distinct sage-grouse populations, the NMP and the PRB, and two subpopulations within the NMP (NMRS and SMRS). Connelly et al. (2004) predicted both populations and the SMRS using habitat breaks (rivers and areas containing unsuitable habitat; Fig. 1), which suggests gene flow in sage-grouse is impeded by these geographic features. However, the NMRS was not genetically subdivided by agriculture in southwestern Saskatchewan despite it being a significant barrier to gene flow. Birds may circumvent the agricultural disturbance by traveling east–west via corridors of suitable habitat further south (Fig. 2). Potential subpopulation barriers included the Milk River itself, vast agricultural conversion along the Milk River valley, and a change in dominant sagebrush species on either side of the river. The Milk River likely does not pose a barrier because it is narrow, lacks rugged or steep banks in Montana, and sage-grouse commonly fly over small areas of non-suitable habitat (crops, roads, etc.). Agricultural conversion along

Table 4 Regression statistics for allelic richness, observed heterozygosity, F_{IS}, and relatedness against geographic distance to the current northern periphery of the species' range for both sexes with leks containing more than ten sampled birds in the northern Montana sage-grouse population

Sex	Genetic measure	R^2	Slope	t	P
Female	Allelic richness	0.10	0.0007 ± 0.0005	1.57	0.13
	Observed heterozygosity	0.04	0.0009 ± 0.0001	−0.93	0.36
	F_{IS}	0.14	0.0003 ± 0.0002	1.87	0.08
	Relatedness	0.007	0.0007 ± 0.0002	−0.37	0.71
Male	Allelic richness	0.02	0.0005 ± 0.0004	1.18	0.10
	Observed heterozygosity	0.05	−0.0001 ± 0.0001	−1.69	0.10
	F_{IS}	0.05	0.0004 ± 0.0002	1.72	0.09
	Relatedness	0.02	−0.0003 ± 0.0003	−1.09	0.28

the Milk River over the past 30–100 years is likely the largest barrier, and a significant contributor to the population decline, because most sagebrush within the valley has been destroyed and historic leks are inactive (Fig. 1). The change in sagebrush species constitutes another potential barrier because sagebrush is the primary habitat and food source for sage-grouse. Silver sagebrush (*Artemisia cana*) is the only woody sagebrush species present north of the Milk River (Aldridge and Brigham 2003), whereas both silver and big (*A. tridentata*) sagebrush are present south of the river where big sagebrush is the primary food (Sauls 2006). However, using STRUCTURE birds on both sides of the river assign to opposite subpopulations, lek 1/9 birds disperse across the river, and some birds that breed in silver sagebrush winter in big sagebrush south of the Milk River (J. Tack, personal communication) suggesting that sagebrush species is not a barrier.

All leks were genetically undifferentiated from one another, except for one highly differentiated lek (lek 1/9) near the northern range edge in Alberta. Lek 1/9 was genetically and behaviourally unusual. This lek was extirpated in 1977 and refounded in 2001(Alberta Fish and Wildlife; unpublished data) by a single banded male whose offspring produced the males sampled on the lek (Bush 2009). The lek is also unusual because it changes location within approximately 4 km^2 throughout the year and within single days, even though the lek site remains relatively undisturbed (Alberta Fish and Wildlife; unpublished data). Males may shift their display locations in response to female movement, as suggested by Gibson (1996) for migratory sage-grouse. Lek 1/9 was also unusual because within-lek relatedness was higher than for any other lek in the NMP. This result coupled with the unique genetic signature suggests that primarily related birds mate on this lek.

Genetic diversity and differentiation

Most genetic studies on grouse have focused on highly fragmented and isolated populations experiencing extreme population declines (Segelbacher and Storch 2002; Caizergues et al. 2003a; Johnson et al. 2003; Van Den Bussche et al. 2003; Bouzat and Johnson 2004). These studies have found low genetic diversity with extensive population structure and differentiation. Sage-grouse in Canada have undergone dramatic declines, but the NMP exhibited high diversity, little population structure, and low levels of differentiation. The NMP and both subpopulations had H_E in the range of core sage-grouse populations and higher H_E and H_O than fragmented and isolated greater and Gunnison sage-grouse populations (Table 5). Similar to the range-wide analysis on sage-grouse, the NMP exhibited IBD, but lek-to-lek F_{ST} values were considerably lower than for Gunnison sage-grouse (NMP average = 0.05 [range 0–0.40]; Gunnison average = 0.26; Oyler-McCance et al. 2005b). Our average F_{ST} of 0.05 was consistent with regional values for capercaillie (0.05 [range 0–0.15]; Segelbacher and Storch 2002) and greater prairie-chicken (0.008–0.1; Johnson et al. 2003). Sage-grouse inhabit both naturally and anthropogenically fragmented landscapes, but our results suggest that fragmentation either does not greatly impede the ability of northern Montana sage-grouse to disperse or fragmentation has occurred too recently to have had a measurable genetic effect.

Despite various forms of fragmentation, most leks in the NMP were connected by contiguous habitat to at least one other lek, which may facilitate gene flow and prevent isolation of any given region. However, the NMP, NMRS, and SMRS exhibited significant IBD (Fig. 3) suggesting distance limits gene flow. Similarly, both males and females in the NMP and the NMRS and males in the SMRS displayed IBD. Females within the SMRS were the only class not exhibiting IBD, but because we sampled approximately one-third fewer leks in the SMRS containing females, we may have failed to detect an existing pattern. Evidence for both sexes dispersing is contrary to the common assumption that galliform females disperse and males are philopatric (Schroeder and Braun 1993), however similar average dispersal distances for both sexes has been observed in sage-grouse in Colorado (8.8 km for females versus 7.4 km in males; Dunn and Braun 1985). Traditional radio-transmitter studies have documented individual sage-grouse dispersal of only 5–20 km (e.g., Beck et al. 2006), but have not captured maximum dispersal distances and evidence of male dispersal due to infrequency of long distance dispersal events, lack of monitoring juvenile birds prior to and after dispersal, and logistical difficulty of tracking individuals over long distances. These studies have also not accurately

Table 5 Comparison of genetic diversity values for the same microsatellite loci across different sage-grouse studies

Species/study/population subset	Microsatellites											Average for microsatellites used in study
	SGCA5	SGCA9-2	LLSD8	ADL230	TUT3	TUT4	TTD6	TTT1	TTT3	BG6	BG15	
H_E												
Sage-grouse												
(a) NMP	**0.75**	**0.89**	**0.74**	**0.70**	**0.64**	**0.84**	**0.76**	**0.66**	**0.55**	**0.85**	**0.66**	**0.71**
(a) NMRS	**0.73**	**0.88**	**0.74**	**0.70**	**0.64**	**0.84**	**0.73**	**0.67**	**0.55**	**0.82**	**0.71**	**0.71**
(a) SMRS	**0.77**	**0.89**	**0.73**	**0.71**	**0.64**	**0.82**	**0.79**	**0.65**	**0.56**	**0.86**	**0.60**	**0.70**
(b) Core/contiguous	0.66–0.88	0.61–0.93	0.64–0.78	0.70–0.83	–	–	–	–	–	–	–	0.62–0.75
(b) Fragmented Periphery	0.07–0.87	0.42–0.91	0.09–0.79	0.58–0.80	–	–	–	–	–	–	–	0.45–0.71
(c) Gunnison	–	–	–	–	–	–	–	–	–	–	–	0.37–0.57
(d) California	–	–	0.59	–	–	–	–	–	–	–	–	0.64
H_O												
Sage-grouse												
(a) NMP	**0.74**	**0.62**	**0.73**	**0.72**	**0.69**	**0.68**	**0.76**	**0.62**	**0.52**	**0.79**	**0.66**	**0.66**
(a) NMRS	**0.70**	**0.60**	**0.74**	**0.70**	**0.71**	**0.68**	**0.72**	**0.62**	**0.51**	**0.80**	**0.72**	**0.66**
(a) SMRS	**0.78**	**0.64**	**0.72**	**0.74**	**0.67**	**0.68**	**0.80**	**0.62**	**0.53**	**0.79**	**0.60**	**0.66**
(c) Gunnison	–	–	–	–	–	–	–	–	–	–	–	0.36–0.51
(d) California	–	–	0.62	–	–	–	–	–	–	–	–	0.64
(e) California	–	–	0.53–0.60	–	0.64–0.67	–	–	–	–	–	–	0.49–0.53

Values from this study are in *bold*. Studies with multiple populations are shown as *ranges* and *dashes* represent loci not used in specific studies (a) This study, (b) Oyler-McCance et al. (2005a), (c) Oyler-McCance et al. (2005b), (d) Semple et al. (2001), (e) Gibson et al. (2005)

documented the dispersal ability of both sexes because most sage-grouse telemetry studies have been conducted on females. While dispersal is important for bolstering lek size, gene flow is ultimately the most important factor because its presence reveals successful reproduction by dispersers.

Isolation-by-distance suggests that gene flow is constrained by geographic distance across the NMP. However, the low levels of differentiation across the NMP suggests that some dispersers contribute to the gene pool and the higher levels of historic dispersal indicate that this has always been the case. However, the success of dispersers is unknown because we do not know the proportion of birds that successfully reproduce or if dispersers move between leks until they are successful. These questions need to be addressed to understand how dispersal and gene flow are correlated in sage-grouse and the relation of these processes to the species' decline. Gene flow is expected to be more sensitive to fragmentation because fragmentation likely reduces dispersal resulting in fewer injections of new genetic material. Determining dispersal ability and levels of gene flow are important directives for devising management strategies for sage-grouse because if disturbance exceeds movement capabilities, regions can become permanently isolated.

Lek structure

Sage-grouse leks were congregations of primarily unrelated males and females exhibiting little kin association. This is contrary to expectations if male kin selection is responsible for the formation and maintenance of leks (Kokko and Lindström 1996; Sherman 1999). Only one lek in the NMP contained males that were significantly more related to each other than random (a mean expectation of zero), the unusual lek 1/9. Our finding of low within-lek male relatedness is consistent with patterns observed in sage-grouse in California (Gibson et al. 2005) and Alberta (Bush et al. 2010) suggesting that the species does not exhibit kin association on leks. However, ruling this possibility out still leaves many alternative explanations for lek formation ranging from decreased predation risk (Boyko et al. 2004), increased mating opportunity (Höglund and Alatalo 1995), and queuing for future breeding status (Wiley 1973).

Range periphery and fragmentation

Sage-grouse at the northern edge of the range fit the non-genetic assumptions of peripherality. However, the northern edge was no more structured genetically than areas farther from the periphery, was not genetically depauperate

compared to the core, and diversity indices did not vary with distance from the northern periphery. Although our results are consistent with findings for peripheral populations of capercaillie (Seglbacher and Storch 2002), they do not fit the expectations that peripheral regions are more isolated, more differentiated, and have lower diversity than areas closer to the range centre. This could partly be due to the close proximity of the entire population, including the core, to the north and east range peripheries (Fig. 1). Isolation-by-distance, the presence of dispersers between populations, and dispersal between the two subpopulations indicates that birds from across the NMP successfully disperse. We did not detect any association between northern periphery and diversity and only rivers with their associated anthropogenic disturbance and a century-old patch of agriculture were identified as permeable barriers to dispersal. Sage-grouse may violate the genetic assumptions of peripherality at the northern edge of their range for historical reasons. Sage-grouse likely underwent a range expansion and subsequent contraction in the recent past (Fig. 1; Oyler-McCance et al. 2005a) resulting in a system that has not reached equilibrium between mutation, migration, and drift. However, if range contraction and expansion occur, they typically occur at the periphery, making all peripheral regions inherently unstable and panmictic (Hewitt 1999; Channell and Lomolino 2000; Antunes et al. 2006; Johansson et al. 2006; Eckert et al. 2008).

If either northern periphery or fragmentation currently impact sage-grouse, the NMRS should exhibit more differentiation than we are currently detecting between leks, lower diversity, and evidence for genetic isolation of leks or regions. Sage-grouse appear to be counteracting disturbance via dispersal and the extreme population decline may simply be caused by a severe reduction in habitat resulting in fewer birds. Sage-grouse may not be sensitive to the separate or combined effects of peripherality and fragmentation for several reasons. First, sage-grouse are physically capable of long distance dispersal, which homogenizes genetic diversity regardless of location in relation to the range periphery. Only one effective migrant per generation is required to prevent population differentiation (Wright 1964) given differentiation values of less than $F_{ST} = 0.2$ (Wang 2004b), therefore, rare long-distance dispersers and moderate numbers of shorter distance dispersers may maintain high diversity in peripheral and fragmented regions. The presence of significant IBD and gene flow suggests that patches of habitat large enough to support leks form a stepping stone network across the landscape that allows dispersal even in the presence of substantial fragmentation. Second, disturbance is mainly occurring in the NMRS, but silver sagebrush habitat is naturally patchy (Aldridge and Brigham 2003). Peacock and Ray (2001) and Aars et al. (2006) found mammal populations inhabiting patchy habitat retain higher levels of genetic variability compared to high-density continuous populations due to efficient and frequent long-distance dispersal. Both sage-grouse subpopulations exhibit IBD, equivalent diversity, and some long-distance dispersal (Table 3). Therefore patchy habitats may result in an increased propensity for some individuals to move farther in search of new or better quality habitat. This, coupled with dispersal between subpopulations, may have contributed to high diversity and connectivity across the NMP, including areas at the northern periphery of the species' range. Finally, despite northern sage-grouse's adaptations to natural disturbance, anthropogenic disturbance may have occurred too recently to affect genetic diversity due to the species' high dispersal capabilities. The single area of disturbance that we could evaluate (patch of agriculture in Saskatchewan) exhibited a significant genetic effect, but it is also the oldest conversion of land affecting the population (>100 years; Lungle and Pruss 2008). Most other disturbances occurred in the last 30 years (Lungle and Pruss 2008), so there may have not been time for detectable genetic change.

Conservation implications

Our findings reject the idea that sage-grouse inhabiting fringe and fragmented habitats in Alberta, Saskatchewan, and Montana are genetically impoverished or isolated. Subpopulations showed comparable levels of genetic diversity and dispersal, suggesting that gene flow maintains genetic diversity. Nevertheless, both subpopulations are differentiated from each other despite gene flow across the Milk River. Sage-grouse in the NMRS have undergone massive demographic declines in the last half century from increasing fragmentation and destruction of sagebrush along the Milk River. With increasing habitat alteration, fewer dispersers from the SMRS likely disperse across the river, leading to fewer birds supplementing the less productive and declining NMRS. While there are still enough birds dispersing to maintain genetic diversity, increased fragmentation will likely only exacerbate demographic declines. Management efforts need to focus on maintaining current sage-grouse habitat to allow for dispersal and gene flow. In areas that continue to suffer declines, population augmentation from high-density areas in the SMRS may be necessary to maintain a viable breeding population and genetic diversity. Juvenile dispersal corridors need to be identified to better understand when and where birds are dispersing and what forms of fragmentation they can cross.

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Appendix

See Table 6.

Table 6 Proportion of individual birds assigning to the most likely STRUCTURE defined K or populations for (a) ΔK at the population level, (b) Pr(X|K) at the population level, (c) ΔK at the subpopulation level, and (d) Pr(X|K) at the subpopulation level

	STRUCTURE defined K						
	1	2	3	4	5	6	7
(a) ΔK							
NMP	0.69	0.36	–	–	–	–	–
PRB	0.90	0.10	–	–	–	–	–
(b) Pr(X|K)							
NMP	0.43	0.41	0.17	–	–	–	–
PRB	0.08	0.09	0.83	–	–	–	–
(c) ΔK							
NMRS	0.63	0.37	–	–	–	–	–
SMRS	0.35	0.65	–	–	–	–	–
(d) Pr(X|K)							
NMRS	0.18	0.09	0.18	0.15	0.14	0.09	0.16
SMRS	0.10	0.21	0.10	0.14	0.14	0.21	0.11

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