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BIRDS OF A FEATHER DO NOT ALWAYS LEK TOGETHER: GENETIC DIVERSITY AND KINSHIP STRUCTURE OF GREATER SAGE-GROUSE (*CENTROCERCUS UROPHASIANUS*) IN ALBERTA

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ABSTRACT.—Endangered species are sensitive to the genetic effects of fragmentation, small population size, and inbreeding, so effective management requires a thorough understanding of their breeding systems and genetic diversity. The Greater Sage-Grouse (*Centrocercus urophasianus*) is a lekking species that has declined by 66–92% during the past 35 years in Alberta. Our goals were to assess the genetic diversity of Greater Sage-Grouse in Alberta and to determine the degree of sex-specific relatedness within and among leks. Six hundred and four individuals sampled in 1998–2007 were genotyped at 13 microsatellite loci. Levels of genetic diversity were high, with the exception of one recently founded lek, and did not change over time. Overall, we did not observe isolation-by-distance among leks, and most leks were not differentiated from one another, which suggests that gene flow occurs across the study area. Males and females exhibited similar patterns of isolation-by-distance, so dispersal was not sex-specific. Overall relatedness was close to zero for both sexes at the level of the province, lek, and year, which suggests that neither sex forms strong kin associations. However, we found relatedness within leks at the year level to be greater than zero, which indicates interannual variation. We also found no evidence that Greater Sage-Grouse follow the typical avian pattern of male philopatry. Although the species is endangered in Alberta and occurs in fragmented habitat, it has maintained genetic diversity and connectivity. *Received 8 September 2008, accepted 22 September 2009.*

Key words: *Centrocercus urophasianus*, dispersal, genetic diversity, Greater Sage-Grouse, kin selection, population genetics, relatedness.

Les oiseaux semblables ne s'assemblent pas toujours dans des leks : diversité génétique et structure de la parenté chez *Centrocercus urophasianus* en Alberta

RÉSUMÉ.—Les espèces en péril sont sensibles aux effets génétiques de la fragmentation, d'une petite taille de population et de la consanguinité. Une gestion efficace requiert une compréhension approfondie de leurs systèmes d'accouplement et de leur diversité génétique. *Centrocercus urophasianus* est une espèce de type « lek » qui a subi un déclin de 66–92 % au cours des 35 dernières années en Alberta. Nos objectifs étaient d'évaluer la diversité génétique chez cette espèce en Alberta et de déterminer le degré de parenté spécifique au sexe dans les leks et entre ceux-ci. Pour ce faire, 604 individus échantillonnés en 1998–2007 ont été génotypés à 13 loci microsatellites. Les niveaux de diversité génétique étaient élevés, à l'exception d'un lek récemment formé, et n'ont pas varié dans le temps. Dans l'ensemble, nous n'avons pas observé d'isolement par la distance entre les leks et la plupart des leks n'étaient pas différenciés les uns des autres, ce qui suggère qu'un flux de gènes se produit dans toute la zone d'étude. Les mâles et les femelles présentaient des patrons d'isolement par la distance similaires; la dispersion n'était donc pas spécifique au sexe. Globalement, le niveau de parenté s'approchait de zéro pour les deux sexes à l'échelle de la province, du lek et de l'année, ce qui suggère qu'aucun des sexes ne forme d'association marquée entre parents. Toutefois, nous avons trouvé que les liens de parenté dans le lek à l'échelle de l'année étaient supérieurs à zéro, ce qui indique une variation interannuelle. Nous n'avons trouvé aucune preuve que *C. urophasianus* suit le patron avien typique de philopatrie des mâles. Bien que l'espèce soit en péril en Alberta et qu'elle soit présente dans des habitats fragmentés, elle a su maintenir une diversité génétique et une connectivité.

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Understanding the genetic structure and diversity of threatened and endangered populations, especially those that occur in fragmented or disturbed habitats, is necessary for devising effective management strategies to preserve these populations, determine their risk of extirpation, and aid in their recovery (Crozier 1997, Kraaijeveld-Smit et al. 2005). Increased fragmentation as a result of changes in human land use is a major threat that limits gene flow by reducing dispersal, decreasing population size, and increasing genetic drift in remnant habitat patches (Sherwin and Moritz 2000, Frankham 2003, Coulon et al. 2004). Most threatened species, regardless of habitat disturbance, exhibit decreased genetic diversity compared with their nonthreatened taxonomic relatives (Spielman et al. 2004) because they are at higher risk of erosion of genetic diversity, fixation of deleterious alleles, and inbreeding (Crozier 1997, Kraaijeveld-Smit et al. 2005). Because birds are mobile, they are expected to withstand the effects of fragmentation better than more sedentary animals (Veit et al. 2005). However, galliforms have been found to be particularly susceptible to the genetic effects of disturbance (Caizergues et al. 2003a, b; Johnson et al. 2003; Segelbacher et al. 2003; Bouzat and Johnson 2004).

Greater Sage-Grouse (*Centrocercus urophasianus*; hereafter "sage-grouse") are endangered at the provincial (Alberta Sage-Grouse Recovery Action Group 2005) and federal (Lungle and Pruss 2008) levels in Canada, where they are located at the northern periphery of the species' range. Sage-grouse in Alberta have declined by 66–92% over the past 35 years (Aldridge and Brigham 2003), with an estimated population size of <150 birds in spring 2009 (Bush 2009). Suggested causes for the decline include oil and gas development (Braun et al. 2002), intensive grazing practices (Aldridge et al. 2004), wildlife viewing, changes in the predator community, climate change, and widespread destruction of sagebrush habitat in neighboring Montana (Alberta Sage-Grouse Recovery Action Group 2005, Bush 2009). Sage-grouse occur in the mixed-grass ecoregion of southeastern Alberta but are primarily limited to the distribution of Silver Sagebrush (*Artemisia cana*), which keeps its leaves year round and is the main food and source of cover for sage-grouse (Alberta Sage-Grouse Recovery Action Group 2005). The distribution of silver sagebrush is naturally patchy, so birds have adapted to move large distances to find suitable habitat.

Sage-grouse exhibit lekking behavior: males congregate on communal display grounds (leks) and females select a mate, breed, and then incubate eggs and raise young on their own (Wiley 1973). Many hypotheses have been proposed to explain why males participate in leks when the majority of males apparently fail to mate. Explanations include anticipation of future breeding opportunities (Wiley 1973), unpredictable female copying behavior (Kokko 1997), reduced predation risk (Boyko et al. 2004), parasite–host coevolution (Boyce 1990), increased mating opportunity (Höglund and Alatalo 1995), and kin selection (Kokko and Lindström 1996), and the latter hypothesis has been tested on lekking grouse species using genetic data (Höglund et al. 1999, Bouzat and Johnson 2004, Gibson et al. 2005, Lebigre et al. 2007, Segelbacher et al. 2007). Kin selection is thought to drive the participation of low-ranking males in leks because they may indirectly and directly increase their own fitness by joining male relatives (Kokko and Lindström 1996, Sherman 1999). Subordinate males may benefit indirectly if their presence at the lek increases the reproductive success of related males. Direct benefits to subordinate males include increased mating opportunities with increased lek size, increased number of females attending the lek, or attraction of females to the lek that might be interested in males other than dominant individuals (Kokko and Lindström 1996, Sherman 1999, Sæther 2002). Several genetic studies have found evidence of kin association on leks (Höglund et al. 1999, Petrie et al. 1999, Bouzat and Johnson 2004), but others have not (McDonald and Potts 1994, Martín et al. 2002, Höglund and Shorey 2003, Madden et al. 2004, DuVal 2007, Loiselle et al. 2007, Segelbacher et al. 2007, Knopp et al. 2008), including the only study on sage-grouse (Gibson et al. 2005).

We used polymorphic microsatellites to answer two main questions. First, what is the genetic diversity and connectivity of sage-grouse in Alberta? Second, are leks composed of related males? We expected to find low diversity and high differentiation between leks because of low estimated population size and extensive habitat fragmentation across the species' range in Alberta. For within-lek relatedness, we predicted low levels of male kinship within leks because a study of sage-grouse in California found that males were typically unrelated (Gibson et al. 2005), and there is no evidence to suggest that sage-grouse in Alberta would show different patterns of lek organization.

Methods

Study location and sample collection.—Our study was conducted on sage-grouse from the extreme southeastern corner (4,000 km^2; Aldridge and Brigham 2003) of Alberta, near Manyberries (Fig. 1). Birds were captured using walk-in funnel traps (Schroeder and Braun 1991), night lighting (Giesen et al. 1982), and drop nets (Bush 2008). Blood, feather, and mouth swab samples were collected from captured sage-grouse between 1998 and 2006. All captured birds were aged following Eng (1955). "Yearlings" were birds entering their first breeding season, and "adults" were birds entering their second (or subsequent) breeding season (Dalke et al. 1963). Vehicular and predator mortalities were opportunistically sampled and molted feathers were collected on leks from 2003 to 2007. All samples were collected during the lekking season (mid-March to mid-May) after dispersal had taken place and included both adults and yearlings. We did not attempt to separate birds into age categories for analysis because most of our samples were molted feathers that could not be aged. Survival of chicks was low (12%; Aldridge and Brigham 2003), so we had few samples from yearlings. In total, we collected 1,422 samples (327 blood, plucked feather, mouth swab, and road kill and 1,095 molted feathers); 1,391 were from the 11 known active leks in Alberta and 31 samples were collected 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 31 birds sampled off-lek were assigned an "unknown" lek status and were not used in any lek-specific analyses. Nine leks were retained for analyses because only one male was sampled on lek 28, and leks 1 and 9 were combined into "lek 1/9" because the lone bird from lek 9 relocated to lek 1's site.

Microsatellite genotyping.—DNA was extracted using DNeasy Tissue and QIAamp DNA Micro kits (Qiagen, Valencia, California), incorporating modifications from Bush et al. (2005).

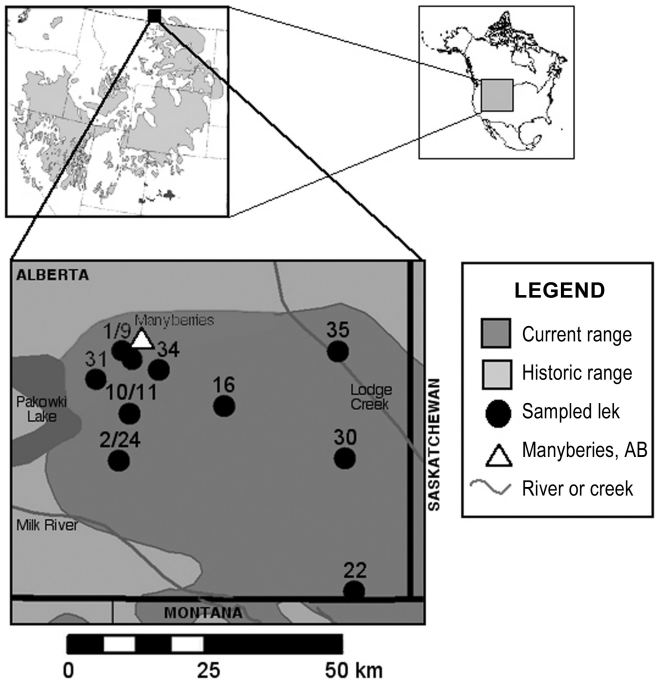

Fig. 1. Map of the study area in Alberta, with sampled Greater Sage-Grouse leks highlighted.

All samples were sexed using DNA methods following Bush et al. (2005). We used 13 microsatellite loci developed from sage-grouse (SGCA9-2 [redesigned primer set; S. Taylor pers. comm.] and SGCA5; Taylor et al. 2003), Capercaillie (*Tetrao urogallus*; TUT3, TUT4, TUD1, and TUD3; Segelbacher et al. 2000), Black Grouse (*T. tetrix*; BG6 and BG15, Piertney and Höglund 2001; TTD6 and TTT1, Caizergues et al. 2001; TTT3, Caizergues et al. 2003b), Red Grouse (*Lagopus lagopus scoticus*; LLSD8, Piertney and Dallas 1997), and domestic chicken (*Gallus gallus*; ADL230, Cheng et al. 1995). We assessed the presence of null alleles by examining 20 female sage-grouse and their known offspring (full nests; offspring were not included in the general analyses). We detected no null alleles; therefore, the 13 loci were used for all analyses. Microsatellite polymerase chain reactions (PCRs; 15 μL total volume with 3, 4, or 5 μL extracted DNA) were carried out as described in Bush et al. (2005). Forward primers were fluorescently labeled with 6-FAM, TET, and HEX (Applied Biosystems, Foster City, California). We followed the PCR cycling conditions outlined for each microsatellite in the publications cited above using Perkin Elmer Cetus GeneAmp PCR System 9600 and Eppendorf Mastercycler ep machines. All noninvasive samples were run in triplicate using the modified multiple-tubes approach (Segelbacher and Steinbrück 2001) as outlined in Bush et al. (2005). The PCR products were visualized using an ABI 377 automated sequencer with GENESCAN ANALYSIS, version 3.1 (Applied Biosystems). Alleles were scored using GENOTYPER, version 2.0 (Applied Biosystems).

Duplicate samples.—Molted feathers are normally considered noninvasive sources of genetic material because their collection does not involve handling birds. On leks, we observed that most molted feathers were pulled out during fights between males, which resulted in DNA equivalent in quality to hand-plucked feathers. Duplicate samples were identified using Microsoft EXCEL MICROSATELLITE TOOLKIT (Park 2001). For all noninvasive samples, the triplicate runs were first compared to one another. If the genotype for a given microsatellite was the same in all three runs, that genotype was retained. If inconsistent genotypes were found (different alleles in different runs) for a locus, no genotype was assigned and the locus was considered missing in all analyses. This approach decreased the likelihood of allelic dropout and limited error. 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.

We determined DNA quality of each feather by amplifying five microsatellites (TUT3, TUT4, SGCA5, SGCA9-2, and TTD6) once and assessing peak height (amplification strength) and peak quality (presence–absence and amplitude of stutter peaks) on GENESCAN ANALYSIS electropherograms. Each feather was then classified as high-quality (high peaks with no stutter), medium-quality (medium-height peaks with little to no stutter), or low-quality (short peaks and those exhibiting stutter), and triplicate PCR replicates were performed with 3, 4, and 5 μL DNA, respectively. Identification of genotyping errors was performed in MICRO-CHECKER (Van Oosterhout et al. 2004). Probability of identity (PI), the probability that two unrelated individuals drawn from a single population have the same multilocus genotype, was calculated in GENALEX, version 5.1 (Peakall and Smouse 2006) using the Paetkau and Strobeck (1994; random mating) and Taberlet and Luikart (1999; siblings) methods.

Genetic diversity, differentiation, and gene flow.—We used the Bayesian program STRUCTURE (Pritchard et al. 2000) to investigate spatial genetic substructure within Alberta. Previous research using STRUCTURE had shown that Alberta birds are part of the northern Montana sage-grouse population (Alberta, Saskatchewan, and Blaine, Choteau, Phillips, and Valley counties in Montana) and belong to a subpopulation that occurs north of the Milk River (Alberta, Saskatchewan, and north Blaine, Phillips, and Valley counties; Bush 2009). We ran 20 independent simulations for each K (1–19) with 100,000 burn-in iterations and 1 million data repetitions, assuming an admixture model 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 chance in the likelihood of K, to estimate the true K, or number of clusters.

We calculated all genetic diversity measures at the provincial (all birds combined), lek, and year levels. We calculated expected (H_E) and observed (H_O) heterozygosity for each locus and tested for deviations from Hardy-Weinberg and linkage equilibrium in GENEPOP, version 3.4 (Raymond and Rousset 1995). Number of alleles per locus (A) was calculated in MICROSATELLITE TOOLKIT. Allelic richness (AR; number of alleles corrected to the smallest sample size) and the inbreeding coefficient F_{IS} were calculated using FSTAT, version 2.9.3 (Goudet 2001); F_{IS} was calculated using Weir and Cockerham's (1984) estimator. Average relatedness (R) within leks and pairwise-R between leks and individuals were calculated in SPAGEDI, version 1.1 (Hardy and Vekemans 2002) using the relationship coefficient of Queller and Goodnight (1989). We used Wald statistics to test whether diversity changed over time using linear mixed models in SPSS, version 15.0 (SPSS, Chicago, Illinois), fitting year as a covariate.

To evaluate lek differentiation and dispersal within Alberta, we calculated average lek-to-lek *R* for leks with >5 birds sampled both annually and overall (1998–2007) for both sexes combined, for males, and for females. We regressed average lek-to-lek *R* onto lek-to-lek geographic distance (5.4–61.3 km; Fig. 1) to test for isolation-by-distance (IBD) and determined significance using a Mantel test (Mantel 1967) in R-PACKAGE, version 4.0 (Casgrain and Legendre 2001). We assessed IBD of males and females separately to identify sex-specific differences in dispersal.

Lek genetic structure.—We computed mean coefficients of relatedness for males and females within leks for each year using SPAGEDI. All birds belonged to a single population (Alberta; see STRUCTURE results); therefore, we used allelic frequencies from the entire population across years for all analyses. *R* among males, females, and overall (males and females combined) within Alberta, leks, years, and lek-years was estimated and standard errors were calculated using the jackknife resampling procedure in SPAGEDI. To determine whether males and females attending the same lek in a given year were more related than expected by chance, we compared sample means to a null expectation of zero using a one-sample *t*-test (Gibson et al. 2005).

RESULTS

Duplicate samples.—Of the 1,095 molted feather samples, 1,093 (99.8%) contained enough DNA to successfully amplify 7–13 loci in triplicate. For low- and medium-quality molted feathers and several plucked feathers with limited DNA quantities, a maximum of 11 microsatellites were successfully amplified for each sample. Amplification rates were consistent across lek (mean number of complete genotypes per sample ± SE = 12.0 ± 0.3), year (12.1 ± 0.4), sex (12.1 ± 0.2), and sample type (12.1 ± 0.6), and individual loci did not fail to amplify for an entire year, lek, sex, or single sample type. Therefore, it is unlikely that our estimations of genetic diversity or relatedness were biased because of missing data. For all samples that failed to produce the same genotype in two of three replicates (as a result of drop-out), the genotype for that locus was excluded and only consistently accurate genotypes (3 of 3 replicates) were included in the duplicate analysis to minimize error. Of the 1,422 samples, 604 were unique and 82% of these samples were genotyped at all 13 loci. Some birds were sampled up to 43 times by molted feathers. Probability of identity (PI) and PI for siblings were set at 0.001 and achieved at four and seven loci, respectively.

Of the birds genetically sampled more than once on a single lek, 98 males (59.0%) and 28 females (80.0%) were identified in only 1 year, whereas 68 males (41.0%) and 7 females (20.0%) were sampled over multiple years at the same lek. Three leg-banded males were genetically sampled on more than one lek, but not in the same year; and once a male relocated to a new lek, it stayed on that lek. One male moved from a lek that disbanded to the next closest lek (8.7 km), one male relocated to a slightly larger lek 8.8 km away, and one male relocated to a lek of approximately the same size 8.8 km away. No female was genetically detected on a lek other than the one where it was first captured or sampled, but 12 females were either physically recaptured or were detected via radiotelemetry on or near different leks during counts. Females attended different leks, both in the same year and across years, separated by 8.7 km ($n = 5$), 8.8 km ($n = 1$), 11.7 km ($n = 2$), 13.7 km ($n = 2$), 17.5 km ($n = 1$), or 24.1 km ($n = 1$).

Genetic diversity, differentiation, and gene flow.—Twelve of 13 loci were in Hardy-Weinberg disequilibrium at the provincial level after corrections for multiple comparisons. At the lek level, all loci were in equilibrium. Nine of 78 comparisons were in linkage disequilibrium in Alberta, but because no loci were in disequilibrium at the lek level, all loci were considered unlinked and retained for analysis.

All microsatellite loci were polymorphic, with 5–23 alleles per locus at the provincial level and 1–19 alleles at the lek level (Table 1). Global (across years) genetic diversity measures and relatedness were consistent with annual estimates within leks and across most leks (Table 1). Allelic richness (*AR*) was highest in the larger leks (average lek counts of ≥8 males; 10/11, 16, 30, 31, and 34) and was lowest in lek 1/9. Observed heterozygosity (H_O) was consistent across all leks. Relatedness (*R*) was high (0.63) and F_{IS} low (–0.33) for lek 1/9. Diversity did not vary across years (H_O, Wald = 1.53, $P = 0.13$; *AR*, Wald = 1.39, $P = 0.16$; F_{IS}, Wald = 1.37, $P = 0.17$).

The most likely number of genetic clusters within Alberta was 1 ($\Delta K = 12.3$ for $K = 1$ vs. the next highest $\Delta K = 4.8$ for $K = 3$). There was a weak negative relationship between lek relatedness and geographic distance for all birds combined, males, and females (Table 2). When individual years were examined, there were significant negative relationships for both sexes combined in 2002, 2003, and 2004; for males in 2003 and 2004; and for females in 1998 and 2002 (Table 2 and Fig. 2).

TABLE 1. Genetic characteristics of active Greater Sage-Grouse leks in Alberta from 1998 to 2007 (*n* = number of individuals analyzed, *AR* = allelic richness or number of alleles corrected to a sample size of 6, H_O = mean observed heterozygosity, *R* = average relatedness of individuals, F_{IS} = inbreeding of individual in relation to that of its lek). Values in parentheses are ranges of annual averages.

Lek	*n*	*AR*	H_O	*R*	F_{IS}
1/9	6	2.6	0.69	0.64	–0.33
2/24	26	4.4 (2.4 to 2.7)	0.70 (0.68 to 0.79)	0.02 (–0.08 to 0.2)	0.01 (–0.2 to 0.07)
10/11	84	4.5 (2.3 to 2.8)	0.69 (0.65 to 0.73)	0.01 (–0.01 to 0.08)	–0.01 (–0.02 to 0.1)
16	171	4.7 (2.3 to 2.7)	0.69 (0.67 to 0.71)	–0.01 (–0.01 to 0.003)	0.03 (0.009 to 0.05)
22	41	4.1 (2.2 to 2.5)	0.65 (0.62 to 0.72)	0.09 (–0.2 to 0.2)	0.01 (–0.1 to 0.04)
30	67	4.5 (2.3 to 2.7)	0.66 (0.62 to 0.72)	0.01 (–0.3 to 0.07)	0.05 (–0.06 to 0.1)
31	77	4.6 (2.3 to 2.7)	0.69 (0.63 to 0.72)	–0.01 (–0.03 to 0.04)	0.02 (–0.02 to 0.1)
34	74	4.6 (2.3 to 2.7)	0.69 (0.68 to 0.71)	0.02 (–0.09 to 0.04)	0.01 (–0.04 to 0.01)
35	37	4.3 (2.3 to 2.7)	0.69 (0.66 to 0.81)	0.06 (0.02 to 0.32)	–0.03 (–0.3 to 0.1)
Global Alberta average	604	4.7	0.68	–0.01	0.03

Table 2. Correlation between average lek-to-lek relatedness and geographic distance among leks by sex, year, and combined in Greater Sage-Grouse in Alberta. See Figure 2 for the associated isolation-by-distance plots for both sexes combined for each year of the study (1998–2007) and across all years. Asterisk denotes significant difference from zero, $\alpha = 0.05$.

	Sexes combined			Males			Females		
Year	Mantel *r*	*n* (birds)	*N* (leks)	Mantel *r*	*n* (birds)	*N* (leks)	Mantel *r*	*n* (birds)	*N* (leks)
1998	0.14	68	6	−0.78	41	5	−0.68*	25	4
1999	−0.11	81	5	−0.32	32	4	−0.79	47	4
2000	−0.40	87	6	0.08	31	5	−0.28	55	5
2001	−0.54	91	5	−0.47	28	5	−0.20	63	5
2002	−0.46*	99	6	0.04	42	6	−0.58*	57	5
2003	−0.45*	109	8	−0.37*	55	8	0.41	53	5
2004	−0.36*	96	9	−0.34*	65	9	0.98	29	3
2005	0.11	186	8	0.07	123	8	0.02	61	6
2006	0.26	166	7	0.15	111	7	−0.25	48	5
2007	0.15	48	6	0.17	47	6	NA	NA	NA
Global Alberta average	−0.09	604	9	−0.34	375	9	0.10	229	8

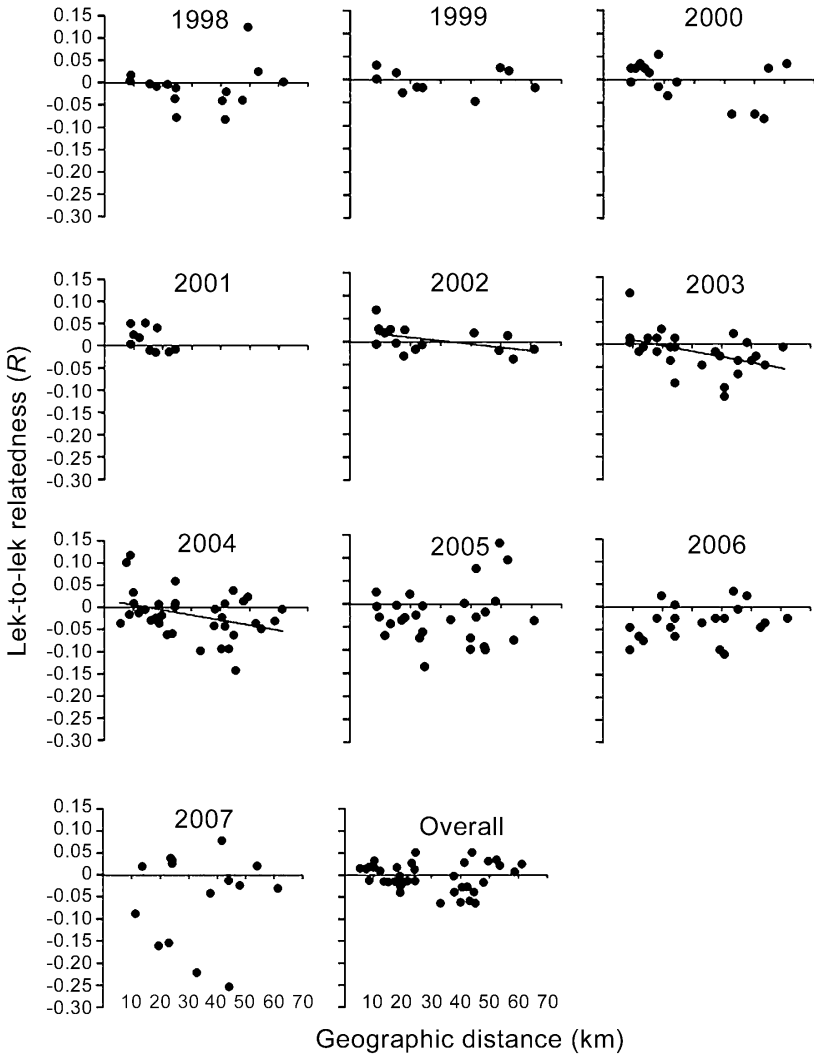

Fig. 2. Average lek-to-lek relatedness plotted versus geographic distance between Greater Sage-Grouse leks in Alberta for each year of the study (1998–2007) and overall.

TABLE 3. Average relatedness of males, females, and both sexes combined in Greater Sage-Grouse on nine leks in Alberta (1998–2007). Global Alberta averages were calculated by combining all birds across years, and standard errors were generated by jackknife resampling in SPAGEDI. Means across leks were calculated by taking the average of the lek averages, and standard errors are based on the range in leks. Asterisk denotes significant difference from zero, $\alpha = 0.05$.

	Sexes combined		Males		Females	
Lek	$R \pm$ SE	n	$R \pm$ SE	n	$R \pm$ SE	n
1/9	0.64 ± 0.090*	6	0.64 ± 0.090*	6	N/A	0
2/24	0.02 ± 0.020	26	0.04 ± 0.030	13	0.0002 ± 0.030	13
10/11	0.01 ± 0.010	84	0.02 ± 0.010*	48	0.006 ± 0.010	36
16	−0.008 ± 0.008	171	−0.01 ± 0.010	97	0.02 ± 0.020	74
22	0.09 ± 0.040*	41	0.08 ± 0.040*	32	0.18 ± 0.080*	9
30	0.01 ± 0.010*	67	0.007 ± 0.010	54	0.07 ± 0.040*	13
31	−0.007 ± 0.010	77	0.02 ± 0.020*	46	−0.006 ± 0.020	31
34	0.02 ± 0.020	74	0.03 ± 0.020*	43	0.03 ± 0.020*	31
35	0.06 ± 0.020*	37	0.09 ± 0.030*	30	0.04 ± 0.070	7
Mean across leks	0.09 ± 0.003*	604	0.1 ± 0.003*	375	0.04 ± 0.004	229
Global Alberta average	−0.002 ± 0.001*	604	0.001 ± 0.005	375	0.01 ± 0.007*	229

Lek genetic structure.—Global provincial average R for males across years was near zero, whereas overall and female R were slightly but significantly different from zero (Table 3). Birds on several individual leks were significantly positively related (3 leks for both sexes combined, 6 leks for males only, and 3 leks for females only; Table 3). Most of these cases involved the 3 most eastern and isolated leks (Fig. 1). Lek 22 was the most geographically isolated lek and exhibited the most positive within-lek R for all three categories (combined, males, and females; Table 3). When means were taken across leks, R was close to zero for females, but greater than zero for males and both sexes combined.

For all years, birds were consistently more related within leks than between leks (Table 4). Males and females displayed similar relatedness within leks (Table 4). Within-lek R varied among years and was highest in 2005 and 2006 for all three categories (Table 4). Averages based on all years were close to zero for all three categories for most leks, with the exception of lek-22 males and females, lek-35 overall and males, and lek-30 females (Fig. 3). Within individual leks, variation in R could be attributed to lek-years when <5 birds were sampled, lek location (22, 30, and 35 were peripheral leks), and lek size (2/24 was small; Fig. 3).

Over the study period, annual within-lek R varied greatly between leks and sexes. R increased for some leks (lek-2/24 females and overall; lek-10/11 and lek-16 males, females, and overall; lek-22 males and overall), decreased for others (lek-2/24 males; lek-30, -31, and -35 females; lek-34 males, females, and overall), or remained relatively constant (lek-30, -31, and -35 males and overall; data not shown).

TABLE 4. Mean relatedness of Alberta Sage-Grouse within and among leks by year and overall (all years combined) for both sexes combined, for males, and for females. Global Alberta averages were calculated by combining all birds across years, and standard errors were generated by jackknife resampling in SPAGEDI. Means across leks were calculated by taking the average of the lek averages, and standard errors are based on the range in years. Asterisk denotes significant difference from zero, $\alpha = 0.05$.

	Sexes combined		Males		Females	
Year	Within lek ($R \pm$ SE)	Between lek ($R \pm$ SE)	Within lek ($R \pm$ SE)	Between lek ($R \pm$ SE)	Within lek ($R \pm$ SE)	Between lek ($R \pm$ SE)
1998	0.03 ± 0.050	−0.02 ± 0.002*	0.07 ± 0.060*	−0.03 ± 0.004*	−0.03 ± 0.050	−0.04 ± 0.005*
1999	−0.001 ± 0.050	−0.02 ± 0.002*	0.004 ± 0.080	−0.04 ± 0.003*	−0.02 ± 0.040	−0.03 ± 0.003*
2000	−0.004 ± 0.060	−0.01 ± 0.002*	−0.01 ± 0.090	−0.04 ± 0.006*	0.06 ± 0.050*	−0.02 ± 0.004*
2001	0.01 ± 0.030	−0.01 ± 0.002*	0.07 ± 0.080	−0.05 ± 0.007*	0.007 ± 0.030	−0.02 ± 0.002*
2002	0.03 ± 0.040	−0.01 ± 0.001*	0.01 ± 0.060	−0.02 ± 0.003*	0.02 ± 0.040	−0.02 ± 0.003*
2003	0.02 ± 0.040	−0.01 ± 0.003*	0.05 ± 0.060	−0.02 ± 0.003*	−0.002 ± 0.050	−0.02 ± 0.004*
2004	0.09 ± 0.060*	−0.01 ± 0.003*	0.08 ± 0.070*	−0.03 ± 0.004*	−0.01 ± 0.030	−0.03 ± 0.007*
2005	0.09 ± 0.050*	−0.005 ± 0.003*	0.09 ± 0.050*	−0.009 ± 0.003*	0.07 ± 0.080	−0.02 ± 0.007*
2006	0.09 ± 0.060*	−0.01 ± 0.001*	0.12 ± 0.060*	−0.02 ± 0.001*	0.12 ± 0.070*	−0.03 ± 0.006*
2007	0.01 ± 0.040	−0.05 ± 0.009*	0.01 ± 0.040	−0.05 ± 0.009*	0.006 ± 0.180	NA
Mean across leks	0.04 ± 0.005*	−0.02 ± 0.003*	0.05 ± 0.007*	−0.03 ± 0.004*	0.03 ± 0.007	−0.03 ± 0.005*
Global Alberta average	0.02 ± 0.002	−0.04 ± 0.002*	0.03 ± 0.002*	−0.006 ± 0.001*	0.04 ± 0.004	−0.04 ± 0.002*

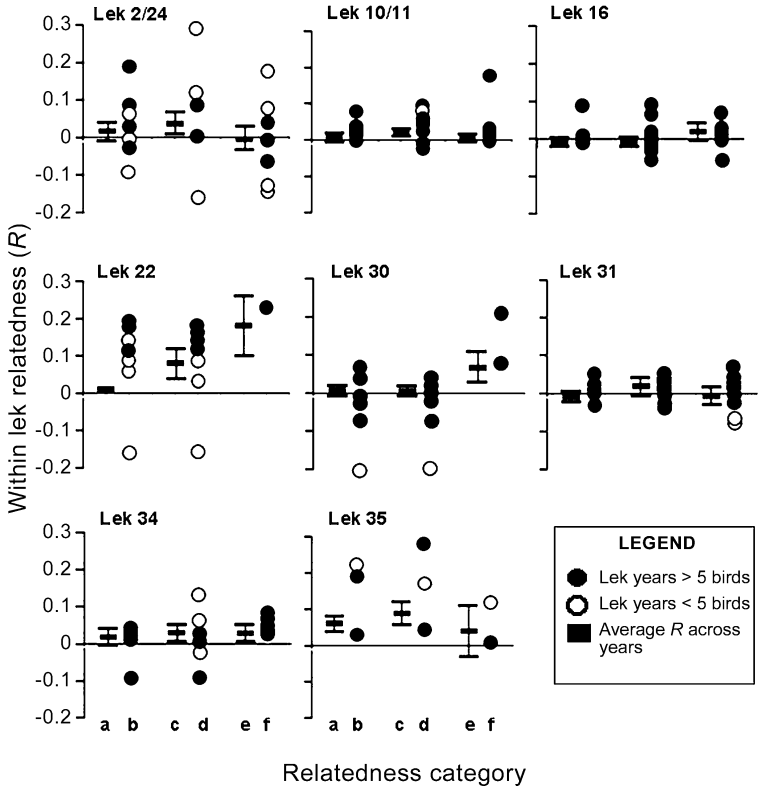

Fig. 3. Average and annual within-lek relatedness for Alberta Sage-Grouse leks, 1998–2007. Relatedness within each lek is presented as (a) average $R \pm$ SE for both sexes combined across all years, (b) average R for both sexes combined for each year with >2 individuals sampled, (c) male average $R \pm$ SE across all years, (d) male average R for each year with >2 individuals sampled, (e) female average $R \pm$ SE across all years, and (f) female average R for each year with >2 individuals sampled.

Discussion

Endangered sage-grouse in Alberta exhibited high genetic diversity and connectivity. Leks were not primarily composed of kin, as indicated by levels of within-lek relatedness. Leks in Alberta were not highly differentiated from one another despite population declines and habitat fragmentation. Isolation-by-distance was not detected for all birds combined across years or for either sex separately across years, which indicates that both sexes disperse. Overall within-lek relatedness for males and females was consistently close to zero in all years and for most leks, with the exception of lek 1/9. However, some lek-years had significantly positive or negative relatedness for both sexes, which suggests that although the overall pattern of kin association within leks was generally weak, there was considerable variation in the degree of relatedness detected for both sexes among lek-years. Although kin structure does not maintain leks in Alberta, it may be an indicator of lek health (recruitment of new individuals to leks) in specific years, because small or isolated leks had elevated relatedness.

Genetic diversity, differentiation, and gene flow.—We observed no population structure at the provincial scale, which is consistent with other analyses that have shown that birds north of the Missouri River (Alberta, Saskatchewan, and northern Montana) form a single genetic population with two subpopulations (north and south of the Milk River; Bush 2009). The lack of genetically differentiated lek clusters can be attributed to the geographic proximity of leks (Fig. 1) and high gene flow across the study area (Fig. 2 and Table 2).

Genetic diversity was high across Alberta despite the endangered status of sage-grouse and fragmentation of their habitat. Genetic diversity (H_O, AR, and F_{IS}) did not change over the 10 years, likely because the study leks are part of the larger, demographically stable northern Montana population. The exception to the high diversity was lek 1/9, which had low allelic richness and high relatedness (Table 1), which suggests that it was composed of highly related males (the average within-lek R of the 5 males sampled in 2004 was 0.79). Lek 1/9 was reestablished in 2001 after 25 years of inactivity (Alberta Fish and Wildlife unpubl. data). However, within-lek R of two other recently formed leks sampled in Montana and Wyoming did not differ from zero (K. L. Bush unpubl. data), which suggests that male relatives are not always the founders of new leks.

We compared our estimates of heterozygosity with published studies to assess the relative diversity of sage-grouse in Alberta. A common trend across all published grouse studies was that contiguous regions had the highest diversity and fragmented and peripheral regions the lowest (Table 5). Expected heterozygosity in Alberta was in the range detected in sage-grouse populations at the core of the species' current distribution (Montana, Wyoming, Nevada, Oregon, and Idaho) and was at the high end for peripheral

TABLE 5. Comparison of genetic diversity among grouse studies using average heterozygosity (H_E = expected, H_O = observed) for all loci and for the subset of loci used in common with the present study. Averages are given for single regions, populations, and leks, and ranges are given if multiple regions, populations, or leks were studied. Number of common loci among studies is given in parentheses.

Species, study	Location, heterozygosity type	Average *H* for study	Average *H* for common loci in study species	Average *H* for common loci in Alberta Greater Sage-Grouse
Greater Sage-Grouse, Oyler-McCance et al. 2005	Range-wide, H_E	0.29–0.86	0.45–0.75 (4)	0.75 (4)
Greater Sage-Grouse, Semple et al. 2001	California, H_O	0.64	0.62 (1)	0.75 (1)
Greater Sage-Grouse, Gibson et al. 2005	California (two periods), H_O	0.49–0.53	0.59–0.64 (3)	0.74 (3)
Black Grouse, Caizergues et al. 2003a	Alps, H_O	0.74	0.73 (6)	0.68 (6)
	Finland, H_O	0.75	0.79 (6)	0.68 (6)
Black Grouse, Lebigre et al. 2007	Finland, H_O	0.73	0.79 (5)	0.72 (5)
Rock Ptarmigan, Caizergues et al. 2003b	Norway, H_O	0.81	0.86 (2)	0.73 (2)
	Pyrenees, H_O	0.64	0.58 (2)	0.73 (2)
	Alps, H_O	0.84	0.86 (2)	0.73 (2)
Lesser Prairie-Chicken, Bouzat and Johnson 2004	New Mexican leks, H_O	0.53–0.55	0.60–0.89 (1)	0.66–0.83 (1)
Greater Prairie-Chicken, Bouzat et al. 1998	Illinois, Kansas, Minnesota, and Nebraska, H_O	0.57–0.65	0.75–0.89 (1)	0.72 (1)

and fragmented populations (Washington, California, Utah, Colorado, North Dakota, South Dakota, and Canada; Oyler-McCance et al. 2005; Fig. 1 and Table 5). H_O was higher in Alberta than in a peripheral and isolated sage-grouse population in California (Table 5), which suggests that sage-grouse in Alberta are not isolated. Alberta had lower levels of H_O than both fragmented (Alps) and contiguous (Finland) populations of Black Grouse and Rock Ptarmigan (*Lagopus muta*; Table 5). Diversity was likely higher in European grouse because many of the microsatellite loci were developed on these species (see microsatellite section of methods). H_O was comparable to levels in a peripheral population of Lesser Prairie-Chicken (*Tympanuchus pallidicinctus*) and isolated Rock Ptarmigan (Pyrenees; Table 5), but slightly lower than in all populations of Greater Prairie-Chicken (*T. cupido*). Heterozygosity in sage-grouse in Alberta was similar to that in fragmented populations of North American grouse and isolated populations of European Rock Ptarmigan in the Pyrenees (Table 5), which suggests that although diversity has not declined in Alberta, it may be lower by virtue of the birds' peripheral location in the species' range and smaller population size.

The absence of isolation-by-distance patterns across years and low relatedness within and among leks suggest extensive gene flow and little differentiation among leks in Alberta. Neither males nor females across years exhibited a correlation between genetic and geographic distances. Both sexes exhibited low average relatedness within leks, but lower relatedness in females suggests that they may have a greater predisposition to disperse. When analyzed separately, 3 of 10 years displayed significant IBD, which suggests a weak pattern of IBD varying among years, perhaps driven by population density, weather, or chance. Work on the entire northern Montana population revealed significant IBD for both sexes combined and separately, but IBD was not significant at the smaller regional scale (contiguous habitat <100 km across; Bush 2009). However, data from the northern Montana population was not analyzed on a year-by-year basis and lumping data across years may have masked a similar, weak pattern of IBD. Dispersal of sage-grouse in Alberta deviates from the typical avian pattern of male philopatry and female dispersal that was observed in sage-grouse in Colorado (Dunn and Braun 1985), because both sexes appeared to disperse at the regional scale.

Lek genetic structure.—Sage-grouse leks in Alberta were congregations of primarily unrelated males and females, with both sexes exhibiting limited kin association across years but a greater potential for kin association within years. An absence of strong male-biased kin structure in most leks suggests that males were not highly philopatric. Birds from all years and leks displayed higher relatedness within each of the nine study leks than among leks (Table 4) or in all leks combined (Table 3), which indicates weak familial associations within leks in both sexes. This variable pattern indicated that kin association by both sexes may play a role in the organization of some leks in some years. In Red Grouse, temporal variation in male kin structure was caused by delayed density-dependent changes in aggressiveness among males, which influenced recruitment to leks and regulated density (Piertney et al. 2008). It is possible that a similar mechanism operates in sage-grouse but that the cycle is obscured in Alberta because of the demographic decline. As for females, if productivity is high in the previous year, recruitment of siblings to individual leks, either via kin association or by chance, will be higher than in years following poor productivity. It is also possible that in a population as small as that in Alberta, relatedness varies from year to year strictly by chance, given that we documented no clear pattern of increasing, decreasing, or stable relatedness within leks or sexes over time. Most leks that exhibited elevated or more variable relatedness were either peripheral (22, 30, and 35) or small (1/9 and 2/24), which suggests that lek location and size may influence kin association. Small leks will have elevated relatedness even if they contain only a few relatives. By contrast, peripheral leks may have

increased relatedness by necessity. Birds in more isolated leks may be more philopatric because the costs of dispersing through inhospitable habitat or over long distances outweigh the benefits for most individuals.

Low overall within-lek male relatedness in Alberta sage-grouse resembles patterns seen in California leks (Gibson et al. 2005). With the exception of lek 1/9 ($R = 0.64$), within-lek male relatedness (–0.01 to 0.09) was considerably lower than in grouse populations studied over multiple years where leks were seen as a product of male kin selection ($R = 0.17$ to 0.36 for Lesser Prairie-Chicken leks [Bouzat and Johnson 2004]; $R = 0.11$ to 0.21 for kin clusters within Capercaillie leks [Segelbacher et al. 2007]). However, relatedness values were similar to those in other grouse leks that do not exhibit kin association ($R = -0.05$ to -0.11 for Lesser Prairie-Chicken [Bouzat and Johnson 2004]; $R = 0.003$ for California sage-grouse [Gibson et al. 2005]; $R = -0.02$ to -0.05 for Capercaille [Segelbacher et al. 2007]). None of these studies examined inter-annual variation in relatedness among males on individual leks, so it is difficult to determine whether kin association in other species fluctuates across multiple years or there is a consistent lack of kin association over years. Also, none of these studies considered females, which we found to display similar degrees of kin association as males. Our results suggest that some members of both sexes are philopatric in Alberta (Table 3 and Fig. 3), whereas others disperse (Table 2 and Fig. 2), and kin association does not play a major role in maintaining sage-grouse leks. Therefore, alternative mechanisms for the evolution or maintenance of leks deserve examination.

Conservation implications.—Sage-grouse in Alberta have maintained high genetic diversity over recent years. The lek system of sage-grouse should reduce effective population size, increase genetic structuring, and increase inbreeding potential if only one or a few males mate on a lek in a given year. However, we observed high diversity and low relatedness in both sexes. A relatively large effective population size and high levels of diversity may be maintained in Alberta via gene flow from other parts of the northern Montana population despite the recent demographic decline. This connection is positive for the conservation of Alberta birds, but habitat destruction in adjacent northern Montana and Saskatchewan is a continuing process. Sage-grouse exhibit evidence of gene flow (present study) and movement (Aldridge 2005) among Alberta leks, despite locally fragmented habitat, which indicates that these birds can traverse or circumnavigate unsuitable habitat. However, some leks appear to be more genetically isolated (leks 1/9 and 22), as indicated by elevated relatedness, and the population continues to decline. This suggests that connectivity of leks is not as great as it was in the past and that corridors or areas of critical habitat should be protected to minimize the impact of future fragmentation and isolation.

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