

# POPULATION GENETICS OF GUNNISON SAGE-GROUSE: IMPLICATIONS FOR MANAGEMENT

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**Abstract:** The newly described Gunnison sage-grouse (*Centrocercus minimus*) is a species of concern for management because of marked declines in distribution and abundance due to the loss and fragmentation of sagebrush habitat. This has caused remaining populations to be unusually small and isolated. We utilized mitochondrial DNA sequence data and data from 8 nuclear microsatellites to assess the extent of population subdivision among Gunnison sage-grouse populations in southwestern Colorado and southeastern Utah, USA. We found a high degree of population structure and low amounts of gene flow among all pairs of populations except the geographically adjacent Gunnison and Curecanti populations. Population structure for Gunnison sage-grouse was significantly higher than has been reported for greater sage-grouse (*C. urophasianus*). Further, we documented low levels of genetic diversity in some populations (particularly Dove Creek/Monticello and Piñon Mesa with an average of only 3.00 and 2.13 alleles per locus respectively) indicating that translocations from larger, more genetically diverse populations may be warranted. Bayesian analysis identified 3 potential migrants (involving San Miguel, Dove Creek/Monticello, Crawford, and Curecanti). Further, this analysis showed that 4 individuals from Cerro/Cimarron were more closely related to birds from San Miguel than to its geographically closer neighbors Gunnison and Curecanti. This suggests the Cerro/Cimarron area may act as a stepping stone for gene flow between San Miguel and Gunnison and that habitat restoration and protection in areas between these 2 basins should be a priority in an attempt to facilitate natural movement among these populations. Conservation plans should include monitoring and maintaining genetic diversity, preventing future habitat loss and fragmentation, enhancing existing habitat, and restoring converted sagebrush communities.

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**Key words:** Colorado, *Centrocercus minimus*, gene flow, genetic diversity, Gunnison sage-grouse, microsatellites, mitochondrial DNA, Utah.

Loss, degradation, and fragmentation of habitat have negatively impacted sage-grouse (*Centrocercus* spp.; Braun 1998). In Colorado, the distribution and abundance of sage-grouse have been greatly reduced. Extirpations have occurred in 12 of the 27 counties in which they were thought to have occurred in the 1900s, and populations in 9 of the remaining 15 counties are thought to number <500 breeding birds (Braun 1995).

The newly recognized Gunnison sage-grouse, whose range is almost exclusively limited to the state of Colorado, have been severely impacted by loss and fragmentation of habitat (Oyler-McCance et al. 2001) and, as a result, populations are small and isolated (Fig. 1). The entire species range consists of 1 moderately sized population in Gunnison (~500 strutting males) that is surrounded by 7 small satellite populations, most of which have fewer than 50 strutting

males (Colorado Division of Wildlife, unpublished lek data).

There has been much concern about the viability of small populations and how they might be affected by demographic, environmental, and genetic stochasticity, as well as catastrophic events (Shaffer 1981, Soulé 1987). Although minimum viable population sizes vary enormously among species, it is generally thought that populations smaller than a few hundred individuals warrant careful scrutiny (Shaffer 1987). The persistence of wild populations is usually influenced more by the above-noted ecological effects than by genetic effects, but when these populations are reduced by artificial means such as habitat destruction, genetic factors and interaction with ecological factors become increasingly important (Lande 1995).

Previous genetic studies have used mitochondrial markers (Kahn et al. 1999) and both mitochondrial and nuclear markers (Oyler-McCance et al. 1999) to compare greater sage-grouse populations from northern Colorado with Gunnison

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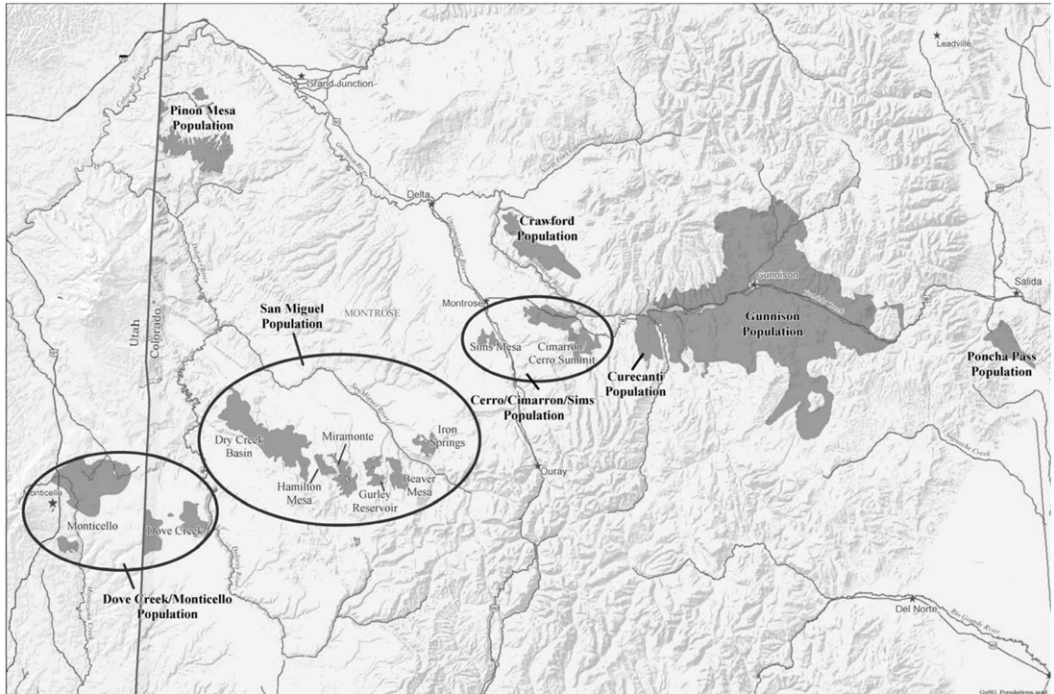


Fig. 1. Distribution of Gunnison sage-grouse and sample population locations.

sage-grouse. These genetic studies, as well as comparisons of morphology (Hupp and Braun 1991) and behavior (Young et al. 1994) led to the recognition of Gunnison sage-grouse as a new species (Young et al. 2000). Since Gunnison sage-grouse are now recognized as a new species, it is necessary to investigate their population structure to obtain a more comprehensive understanding of the species. This study expands upon previous work by increasing sample sizes in each population, obtaining samples from 4 additional populations, and including data from 4 new microsatellite loci. Our goals were to provide strong estimates of population structure, genetic diversity, and relatedness among populations and to apply this genetic data to management issues.

## STUDY AREA

Gunnison sage-grouse have a very limited range as they are restricted to southwestern Colorado and southeastern Utah (Fig. 1). Eight geographically isolated populations have been identified and are monitored during the breeding season by Colorado and Utah State Wildlife Agencies. Blood and feather samples were obtained from 6 of these populations (Gunnison, Curecanti, Crawford, San Miguel, Dove Creek/Monticello,

and Piñon Mesa), and 4 samples from the Cerro/Cimarron portion of the Cerro/Cimarron/Sims population were collected and used in some, but not all, analyses due to small sample sizes. The Poncha Pass population was not sampled because it is composed primarily of birds transplanted from the Gunnison population.

The largest area of contiguous habitat and the largest population occurs in Gunnison (Fig. 1), which supports approximately 500 strutting males (Colorado Division of Wildlife, unpublished data) and is thought to be the most stable. Curecanti is separated from Gunnison by the Lake Fork of the Gunnison River Canyon and in some instances is thought to be part of Gunnison. Because there was some question about whether it should be considered part of the Gunnison population, we considered it separately with the intention of clarifying the status of Curecanti in this study. There were approximately 60 strutting males in this area (Colorado Division of Wildlife, unpublished data). Crawford (Fig. 1) has declined to approximately 30 strutting males (Colorado Division of Wildlife, unpublished data). The San Miguel population (Fig. 1) is stable or declining with approximately 50 strutting males. Dove Creek/Monticello (Fig. 1) is declin-

ing with approximately 35 strutting males (Colorado Division of Wildlife, unpublished data) as is Piñon Mesa with approximately 30 strutting males (Colorado Division of Wildlife, unpublished data). Cerro/Cimarron is an area west of Curecanti and south of Crawford (Fig. 1) with approximately 10 strutting males (Colorado Division of Wildlife, unpublished data).

## METHODS

### Tissue Collection and DNA Extraction

A spotlight trapping method (Giesen et al. 1982, Wakkinen et al. 1992) was used to trap 197 Gunnison sage-grouse. Blood samples were obtained by clipping a toe nail and collecting 2–3 drops in a microfuge tube previously coated with EDTA (Brinkmann). Blood samples and feathers were frozen at  $-20^{\circ}\text{C}$ . DNA was extracted using either a phenol-chloroform method (Kahn et al. 1999) or the GenomicPrep Blood DNA Isolation Kit (Amersham Biosciences) using the manufacturers instructions for extraction of DNA from nucleated whole blood with the following modifications. Subsequent to the RNase A treatment, 20  $\mu\text{l}$  of 2.5 mg/ml proteinase K was added, and the reaction was incubated at  $50\text{--}55^{\circ}\text{C}$  for 2–3 hr. To ensure proper precipitation of the proteins, a 5-min incubation on ice was added after the protein precipitation solution was added and the mixture vortexed. The isopropanol precipitation of the DNA was incubated at room temperature for 30 min to increase the yield of DNA. Rehydration of the dried DNA pellets occurred in 25  $\mu\text{l}$  of DNA rehydration buffer.

### Microsatellite Analysis

Eight nuclear microsatellite loci were screened. Primer pairs for 4 of these loci (LLST1, LLSD3, LLSD4, LLSD8) were originally designed for red grouse, *Lagopus lagopus scoticus*, (Piertney and Dallas 1997). An additional 3 microsatellite loci (SGCA5, SGCA9, SGCA11) were targeted using primers designed for greater sage-grouse by Taylor et al. (2003). Primers for ADL0230 were designed for chicken (*Gallus gallus*) by Cheng et al. (1995) and were polymorphic in both species of sage-grouse.

A standard 25  $\mu\text{l}$  PCR (Quinn 1992) was performed in a PTC-200 thermal cycler (MJ Research) for each locus using a dye-labeled forward primer and an unlabeled reverse primer. Primers, thermal profiles, and fragment analysis methods for the SGCA5, SGCA9, and SGCA11 loci are described in Taylor et al. (2003). The thermal profiles for the red grouse and chicken primers were as follows:

denaturation,  $95^{\circ}\text{C}$  for 1 min; annealing (see temperature below) for 1 min; extension,  $72^{\circ}\text{C}$  for 1 min. The annealing temperatures varied by locus: LLST1 ( $58^{\circ}\text{C}$ ), LLSD3 ( $55^{\circ}\text{C}$ ), LLSD4 ( $63^{\circ}\text{C}$ ), LLSD8 ( $56^{\circ}\text{C}$ ), ADL0230 ( $60^{\circ}\text{C}$ ). Each PCR had 35 amplification cycles. Amplified products were run on the CEQ 8000 Genetic Analysis System (Beckman Coulter) using the Size Standard-400 and the default Frag 3 method.

Raw data were analyzed using the CEQ 8000 Genetic Analysis software package (Version 6.0). Allele frequencies were calculated for each locus across all populations.

### Mitochondrial DNA Sequencing

A 141 base pair portion of hypervariable control region I was amplified and sequenced as described previously (Benedict et al. 2003). This region was used because it was known to contain approx 92% of the variable sites in a larger 380 base pair region spanning control region I (Kahn et al. 1999).

### Data Analysis

Microsatellite genotypes were tested for departures from Hardy-Weinberg equilibrium within each population using the computer program ARLEQUIN 2.00 (Schneider et al. 2000). ARLEQUIN employs a Markov-chain random walk algorithm (Guo and Thompson 1992) that is analogous to Fisher's exact test but extends it to an arbitrarily sized contingency table. We tested for linkage disequilibrium for each pair of loci in each population in GENEPOP (Markov chain parameters: 5000 dememorization steps, 500 batches, 5000 iterations per batch). We documented the amount of genetic diversity per population by calculating mean heterozygosity, mean number of alleles per locus and number of unique alleles for each population.

Population genetic structure was investigated using Analysis of Molecular Variance (AMOVA) that takes into account not only the gene frequencies but also the number of mutations between molecular haplotypes/alleles (ARLEQUIN 2.00). In the case of microsatellite data, the size difference between alleles is considered. Pairwise population  $F_{ST}$  significance tests were conducted among all populations.

A different approach made use of the software STRUCTURE 2.00 (Pritchard et al. 2000). STRUCTURE employs a model-based clustering analysis that groups individuals into clusters without regard to the population of origin. We first estimated the number of populations (K) by conducting 5 independent runs each of  $K = 1 - 8$  with 500,000 Markov Chain Monte Carlo repetitions

Table 1. Polymorphism of microsatellite loci among 6 populations of Gunnison sage-grouse.

Population	Mean sample		Mean no. of alleles per population		% of polymorphic loci	Mean observed heterozygosity		Mean expected heterozygosity	
	size	SD	population	SD		heterozygosity	SD	heterozygosity	SD
Gunnison	83.13	4.45	5.00	3.85	100	0.38	0.22	0.40	0.20
Curecanti	25.00	1.46	2.88	1.25	88	0.37	0.17	0.37	0.18
Crawford	22.50	0.76	3.00	1.41	88	0.41	0.23	0.43	0.21
San Miguel	56.75	2.55	3.25	1.98	75	0.51	0.09	0.57	0.10
Dove Creek/Monticello	42.38	2.26	3.00	1.77	75	0.46	0.24	0.51	0.22
Piñon Mesa	19.50	0.93	2.13	1.55	50	0.36	0.24	0.42	0.29

with a 500,000 burnin period using the model with admixture, correlated allele frequencies, and no prior information. The optimal value of K was then chosen by calculating the posterior probability for each value of K using the estimated log-likelihood of K. This method allows for the estimation of the most likely number of “populations” given the data. We used GeneClass2 (Piry et al. 2004) to determine the presence of migrants in each population using the Bayesian approach of Baudouin and Lebrun (2001) and 10,000 simulations following Paetkau et al. (2004).

For all pairs of populations we calculated the proportion of shared alleles (Bowcock et al. 1994) and  $F_{ST}$ . Those 2 genetic distance measures were then used to construct a neighbor-joining tree in PHYLIP 3.57 (Felsenstein 1989) that was viewed using the program TREEVIEW (Page 1996). We used a Mantel (1967) test to look for a correlation between genetic distance ( $F_{ST}$ ) and geographic distance among all pairs of populations (ARELEQUIN 2.00, 1000 permutations). Probabilities for the Mantel test were calculated following Smouse et al. (1986).

For the mitochondrial DNA data we documented population subdivision in ARLEQUIN 2.00 using significance tests of pairwise population  $F_{ST}$  values. The molecular distances between haplotypes were modeled following Tamura (1992). We used AMOVA to describe the proportion of variation that could be explained by variation among versus within populations.

**RESULTS**

**Microsatellite Data**

We found several alleles for each of the 8 microsatellite loci included in the study (Table 1). Gunnison exhibited the highest level of polymorphism with an average of 5.0 alleles per locus. Piñon Mesa had the least amount of polymorphism with only 2.1 alleles per locus. Further,

Gunnison showed polymorphism at all loci while all other populations had 1 or 2 monomorphic loci. In Piñon Mesa, 4 loci were monomorphic. None of the 38 population-locus comparisons showed significant departures from Hardy-Weinberg equilibrium using a Bonferroni corrected  $P$ -value of 0.001, and we found that there were no significant cases of linkage disequilibrium using a Bonferroni corrected  $P$ -value of 0.0003 for the 168 possible comparisons.

Pairwise population  $F_{ST}$  significance tests showed significant levels of population subdivision using a Bonferroni corrected  $P$ -value of 0.003. All pairs of populations were significantly different except Gunnison and Curecanti ( $P = 0.0385$ ). AMOVA showed that 22.71% of the variance could be explained by variation among groups. Both genetic distance measures, proportion of shared alleles and  $F_{ST}$ , produced neighbor-joining trees with similar topologies (Fig. 2). The branching order on both trees was the same with the Curecanti and Gunnison populations grouping together and the Piñon Mesa and Dove Creek/Monticello populations having separate, relatively long-branch lengths. There was a positive correlation between genetic distance ( $F_{ST}$ ) and geographic distance ( $r = 0.61, P = 0.0168$ ). We calculated  $F_{ST}$  for the 6 Gunnison sage-grouse populations used in this study (excluding the Cerro/Cimarron birds) and found it to be much higher ( $F_{ST} = 0.2639, 95\% \text{ CI } 0.2108\text{--}0.3045$ ) than that found in 45 greater sage-grouse populations ( $F_{ST} = 0.1160, 95\% \text{ CI } 0.0972\text{--}0.1345$ ; S. Oyler-McCance, unpublished data). The same 7 microsatellite loci (LLSD4 excluded) were used for this comparison.

For the STRUCTURE analysis, we input all of our data including the 4 samples from Cerro/Cimarron. The program identified 6 discrete clusters based on our data set. Each individual and subsequently each population was assigned a probability of belonging to each cluster (corresponding to the 6 different colors on

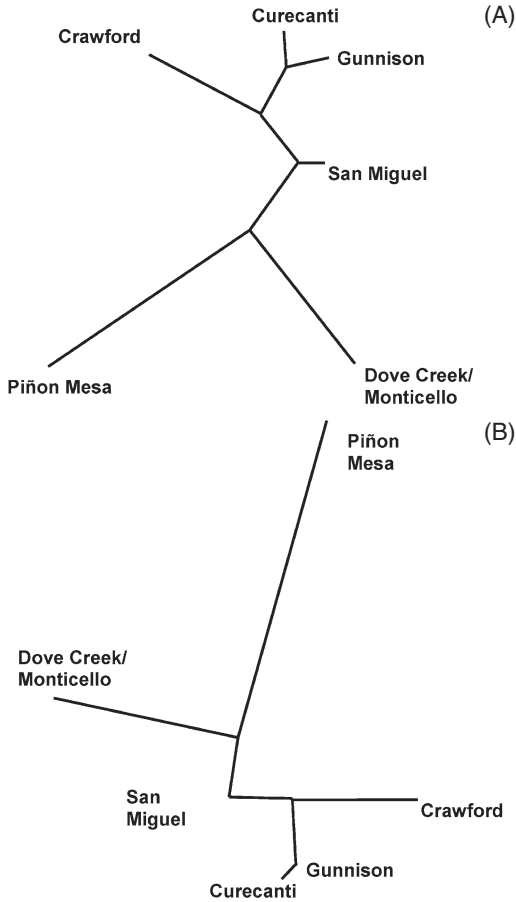


Fig. 2. Neighbor-Joining trees created from 2 genetic distances (A) proportion of shared alleles (B)  $F_{ST}$ .

Fig. 3). Some populations (Piñon Mesa, Dove Creek/Monticello) had a very high probability of belonging to only 1 cluster and thus are the most unique. Gunnison and Curecanti had an almost equal probability of belonging to 2 clusters, yet

(A) the highest probability in Gunnison was for the cluster coded blue. In Curecanti, the highest probability was for the cluster coded red, which suggests subtle differences between the 2 populations. The San Miguel population contained the largest mixture of individuals (i.e., some individuals had much different probabilities of belonging to certain clusters than others). The fact that we estimated that  $K = 6$  was most likely, however, reflects the substantial subdivision of our data (Fig. 3). The 4 samples taken from Cerro/Cimarron were assigned to the cluster dominated by individuals from the San Miguel population. We identified 3 potential migrants ( $P < 0.01$ ) using the GeneClass2 software. Each of those 3 individuals had been genotyped at all 8 loci. Two potential migrants were from San Miguel (1 into Dove Creek/Monticello and 1 into Crawford). The other potential migrant was from Curecanti into Crawford.

**Mitochondrial Data**

We found 5 different haplotypes across all individuals of Gunnison sage-grouse (Table 2). GenBank accession numbers were previously described in Kahn et al. (1999) and Benedict et al. (2003). Three of those haplotypes (A, D, DT) are also found in greater sage-grouse. The other 2 (AI, G) are unique to Gunnison sage-grouse. The greatest number of haplotypes per population was 3, which is less than that reported for populations of greater sage-grouse in northern Colorado (5–11 haplotypes per population with smaller sample sizes [Kahn et al. 1999]). The 5 closely related haplotypes found in Gunnison sage-grouse are all members of “clade I” described by Kahn et al. (1999). Among those 5 haplotypes, there are 6 variable sites (5 transitions and 1 transversion).

Pairwise population  $F_{ST}$  tests showed significant levels of population subdivision using a Bonferonni corrected  $P$ -value of 0.003 among all pairs of popu-

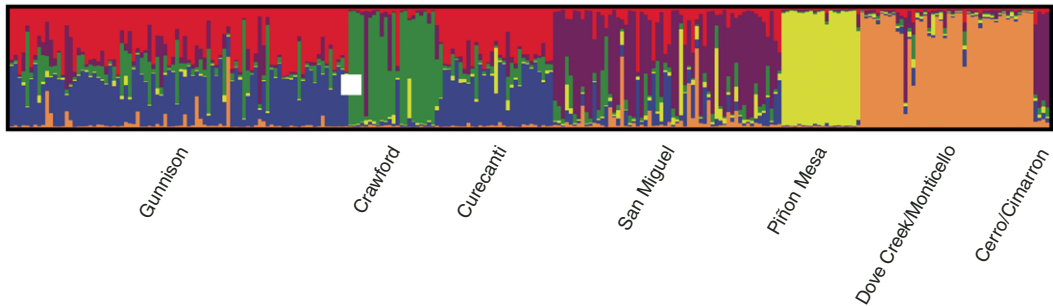


Fig. 3. Results of program STRUCTURE that found that the data best fit into 6 distinct clusters (represented by the 6 unique colors). Each vertical bar represents an individual Gunnison sage-grouse. Individuals are grouped into populations with the population names below each group of individuals. The colors on each vertical bar represent the probability of the individual belonging to a certain cluster.

Table 2. Mitochondrial DNA haplotype frequencies in 6 Gunnison sage-grouse populations.

Population	N	Haplotypes				
		A	D	G	AI	DT
Gunnison	86	79	4	3		
Curecanti	27	27				
Crawford	21	4		17		
San Miguel	55	21		14	20	
Dove Creek/Monticello	41	36			5	
Piñon Mesa	18	5		3		10

lations, except Gunnison and Curecanti ( $P = 0.364$ ) and Curecanti and Dove Creek/Monticello ( $P = 0.152$ ). AMOVA showed that 29.98% of the variance could be explained by variation among groups.

## DISCUSSION

Levels of genetic diversity (Table 1) were highest in Gunnison with an average of 5 alleles per microsatellite locus and 3 mitochondrial DNA haplotypes represented. This is consistent with the fact that this population is the largest and most stable. All other populations had much lower levels of diversity. For example, Piñon Mesa averaged only 2.13 alleles per locus. These lower levels of diversity of the other Gunnison sage-grouse populations are likely linked to small population sizes and a high degree of geographic isolation.

At the species level, Gunnison sage-grouse have low levels of genetic diversity particularly when compared to greater sage-grouse. Oyler-McCance et al. (2005) sequenced the same mtDNA region among 44 populations of greater sage-grouse from across the range and found an average of 6.9 haplotypes per population compared to an average of 2.33 found for Gunnison sage-grouse in this study. Oyler-McCance et al. (2005) also found an average of 5.88 microsatellite alleles per locus in greater sage-grouse, whereas Gunnison sage-grouse were found here to have an average of 2.9 alleles per locus.

Although the importance of maintaining substantial genetic variation in small populations is debated, most conservation biologists agree that genetic variation is relevant to the health and viability of populations and that it must be addressed and monitored in management plans (O'Brien and Evermann 1988, Quattro and Vrijenhoek 1989). Bouzat et al. (1998) and Westemeier et al. (1998) showed that fertility and hatching success of greater prairie chickens (*Tympanuchus cupido*) were reduced due to a bottleneck caused by habitat loss. The Gunnison sage-grouse, a close relative of greater prairie chicken (both are members of Tetraoninae), have experienced similar isolation

and reduction in population size resulting from the loss of habitat. Further, genetically depauperate populations face enhanced susceptibility to parasitic agents or infectious disease such as West Nile Virus, which has been shown to be a significant threat for greater sage-grouse (Naugle et al. 2004).

Pairwise population  $F_{ST}$  values showed congruent patterns of population genetic structure in both the microsatellite and the mitochondrial data suggesting that all populations are genetically discrete units and can be considered distinct populations with the exception of Gunnison and Curecanti, which are closely linked geographically. STRUCTURE analysis further substantiated our finding of a high degree of population structure and low amounts of gene flow by defining 6 populations, yet Curecanti and Gunnison were very closely related. Further,  $F_{ST}$  calculated among all 6 Gunnison sage-grouse populations was significantly higher than it was for greater sage-grouse. This is likely due to reduced gene flow among the 6 populations of Gunnison sage-grouse in conjunction with increased genetic drift that is characteristic of small populations.

Historically, Dove Creek/Monticello, San Miguel, Crawford, and Piñon Mesa all had much larger populations that were somewhat connected through more contiguous areas of sagebrush habitat. Oyler-McCance et al. (2001) quantified the loss and fragmentation of sagebrush habitat in southwestern Colorado. They documented that 20% of sagebrush habitat was lost between the late 1950s and the early 1990s and that sagebrush in 37% of the plots examined was significantly fragmented. The clearing of sagebrush for cultivated crops, highway construction, ranch development, powerline placement, reservoir construction, and other facets of human settlement has destroyed and fragmented sagebrush habitats in southwestern Colorado and led to the current isolation of these populations (Braun 1995), which is consistent with the relatively low amounts of gene flow and isolation by distance documented here.

Both neighbor-joining trees, constructed using different measures of genetic distance, showed similar topologies: Gunnison and Curecanti were closely linked, followed by Crawford and San Miguel. The Dove Creek/Monticello and Piñon Mesa populations were consistently set apart from all other populations and from each other. These neighbor-joining trees as well as a significant Mantel test showed that the geographic distances are correlated with genetic distances between populations.

It is interesting to note that a few individuals in the STRUCTURE analysis appear to have the

genetic characteristics of a population other than their own (Fig. 3) suggesting the possibility that they are dispersers from a different population. Using GeneClass2, we identified 3 potential dispersers. Two probable dispersers were individuals moving from San Miguel into Dove Creek/Monticello and Crawford. The San Miguel population itself appears to have a mixture of individuals with differing probabilities of belonging to different clusters (Fig. 3). This suggests that San Miguel may act as a conduit of gene flow among the satellite populations surrounding the larger population in Gunnison. Additionally, 1 other potential disperser involved movement into Crawford from Curecanti. This is not surprising given their geographic proximity. The 4 individuals from Cerro/Cimarron, however, are more closely related to individuals from San Miguel than from Gunnison or Curecanti, which are closer geographically. This suggests a linkage between San Miguel and the Cerro/Cimarron area that is surprising given the geographic distance between them and the fact that the city of Montrose sits between them. With a sample size of only 4 individuals it is hard to make strong conclusions about the genetic characteristics of Cerro/Cimarron/Sims, yet this suggests that the Cerro/Cimarron/Sims population may act as an important stepping stone that links the larger populations of Gunnison, Curecanti, and San Miguel.

### MANAGEMENT IMPLICATIONS

The low levels of genetic diversity found in Gunnison sage-grouse, particularly when compared to greater sage-grouse, should be of conservation concern. While there is nothing that can be done to increase genetic diversity within the species, steps may be taken to attempt to maximize the probability of maintaining the current levels of variation. This could involve translocations of individuals among populations to decrease the probability of losing alleles due to random genetic drift, which is a strong force in small populations. The Dove Creek/Monticello and Piñon Mesa populations have particularly low allelic diversity and the highest levels of monomorphism. This fact, coupled with their small population sizes, suggest that these 2 populations are most at risk of negative genetic impacts and may be the best candidates for translocations from the largest, most genetically diverse population in Gunnison. Further, characteristics of fitness as they relate to genetic diversity must be more closely examined. Research on reproductive features (sperm function, egg nor-

mality), parasite load, and disease resistance should be conducted, both among individual Gunnison sage-grouse and between the greater and Gunnison sage-grouse species.

We found that San Miguel may act as a conduit to gene flow among the small satellite populations. Surprisingly, we found a link between San Miguel and Cerro/Cimarron/Sims, suggesting gene flow between these areas. Cerro/Cimarron/Sims has not been well studied and deserves further attention. Additionally, habitat restoration and protection in areas between San Miguel and Gunnison should be a priority for conservation of the species in an attempt to facilitate natural movement among these populations.

While genetic concerns may be only 1 of several priorities for Gunnison sage-grouse conservation and management, we believe that they warrant consideration along with other issues (e.g., habitat loss and quality). Conservation activities should include monitoring and maintaining genetic diversity, preventing future habitat loss and fragmentation, enhancing existing sagebrush communities, and restoring sagebrush communities that have been converted.

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