The long-term rangewide decline of Greater Sage-Grouse (*Centrocercus urophasianus*) populations presents a major challenge to natural resource managers as they attempt to balance the needs of wildlife, agriculture, and industry. The recent expansion of energy development in sagebrush (*Artemisia* spp.) habitat is especially problematic for conservation efforts (Lyon and Anderson 2003, Holloran 2005, Walker et al. 2007, Naugle et al. 2011). This research demonstrated that sage-grouse occupying disturbed areas may alter their habitat use to avoid highly developed areas and that they exhibit increased mortality and reduced reproductive success relative to sage-grouse in undisturbed areas. Fragmentation associated with development may also isolate populations, causing genetic drift, inbreeding, and loss of genetic variability, thus reducing the likelihood of population persistence (Frankham 1995, Frankham et al. 1999, Reed and Frankham 2003, Allendorf and Luikart 2007).

Based on current leasing patterns, energy development in northeastern Utah is expected to increase dramatically over the next 10 years (BLM 2007, 2008). This trend is of concern because the area supports substantial populations of sage-grouse, as well as small, seemingly isolated populations that may be vulnerable to extirpation (Beck et al. 2003). Conservation...
strategies implemented in northeastern Utah during and after energy development will likely involve translocations to restore populations (UDWR 2009). To be effective, these translocations must consider genetic divergence and genetic diversity among populations (Oyler-McCance et al. 2005, Oyler-McCance and Quinn 2011). Currently, no genetic data exist for the small populations inhabiting areas slated for development in northeastern Utah. Heavier average weights, atypical habitat-use patterns, and confined seasonal movements of sage-grouse radio-collared from the Seep Ridge population of northeastern Utah in 2007 prompted the concern of UDWR biologists that local populations may be genetically unique (Smith 2009). Our objective was to assess the genetic divergence among local Greater Sage-Grouse populations in northeastern Utah. Understanding reference genetic patterns could help managers identify and sustain unique genetic lineages as a precursor for assisted migrations to restore extirpated populations to suitable habitats post–energy development (Oyler-McCance and Quinn 2011).

**STUDY AREA**

Our study focused on 3 Greater Sage-Grouse populations (Anthro Mountain, Deadman Bench, and Seep Ridge) in northeastern Utah. These populations are representative of major breeding complexes identified by the UDWR (2009) in northeastern Utah and were not genetically sampled in the Greater Sage-Grouse rangewide study conducted by Oyler-McCance et al. (2005). Additionally, energy development is anticipated or is currently present in areas that are designated as seasonal sage-grouse habitats (UDWR 2009). Historically, all of these populations may have been connected by sagebrush habitat (Beck et al. 2003, Schroeder et al. 2004). However, due to agricultural activities, residential development, and Utah juniper (*Juniperus utahensis*) woodland encroachment, populations were not believed to be connected by continuous habitat (Beck et al. 2003) at the time of our study.

Vegetation at the Anthro Mountain study site (Fig. 1) consisted of mountain big sagebrush
A. tridentata Nutt. ssp. vaseyana [Rydb. Beetle] intermixed with stands of quaking aspen (Populus tremuloides) at an elevation of 2600 m. The elevation and habitat was typical for sage-grouse (Schroeder et al. 1999, Connelly et al. 2000). In 2008, the population consisted of approximately 52 birds (R. Christensen, USDA Forest Service, personal communication, 2008). Population estimates were based on lek counts and assumed that maximum male attendance represented 75% of males, with females in the population constituting a 2:1 ratio to males (Connelly et al. 2003, UDWR 2009). Lek trends indicated that, as of 2009, the population was stable or increasing (Christensen 2007, UDWR 2009, UDWR unpublished data). Radio-collared sage-grouse from Anthro Mountain have been recorded using habitats located 32 km south, 35 km southeast, and 30 km north of the leks (Christensen 2007; B.D. Maxfield, UDWR, personal communication, 2011; Fig. 1). These movements occurred across nonhabitat areas dominated by Utah juniper (Juniperus utahensis).

The Deadman Bench and Seep Ridge sites (Fig. 1) consisted of Wyoming big sagebrush (A. t. Nutt. ssp. wyomingensis Beetle and Young) and black sagebrush (A. nova A. Nelson) intermixed with some stands of pinyon (Pinus edulis) and Utah juniper at an elevation of 1750 m. In 2008, the Deadman Bench and Seep Ridge population were estimated at 20 and 36 birds, respectively (B.D. Maxfield, UDWR, personal communication, 2008). Historically, sage-grouse densities in the Deadman Bench area have been low (UDWR 2009, UDWR unpublished data). However, the Seep Ridge population was estimated at 231 grouse in 1986, and lek counts conducted from 1990 to 2011 indicate that the population is declining. In 2008, sage-grouse radio-collared on Deadman Bench were recorded 25 km north of the site near another population of sage-grouse located on Blue Mountain (Fig 1; B.D. Maxfield, personal communication, 2009). The Blue Mountain sage-grouse population was previously genetically sampled by Oyler-McCance et al. (2005) and estimated at 600 grouse in 2007. Sage-grouse radio-collared on Seep Ridge were documented 28 km to the east of leks where they were captured (Smith 2009). These populations most likely used sagebrush areas intermixed with small stands of juniper woodlands during their movements.

Energy development plans for the Seep Ridge area include the construction of approximately 4000 natural gas wells in historic or occupied sage-grouse seasonal habitats (BLM 2007, 2008). Several of these well pads will be located 0.60 km from historic and existing leks (Smith 2009). Additional well pads are also proposed for the Anthro Mountain and Deadman Bench study areas.

METHODS

Capture and Sample Collection

We located and trapped grouse at night by spotlighting roost sites located near leks (Oyler-McCance et al. 2005). We also searched the study areas to ensure grouse were not congregating at undocumented satellite leks. We captured birds with a long-handled hoop net from the back of an all terrain vehicle and placed them in a small sack to minimize stress (Giesen et al. 1982, Wakkinen et al. 1992). Trappers collected blood samples from clipped grouse toenails on Nobuto blood filter strips (Advantec MFS, Inc., Dublin, CA). If bleeding did not stop after application of pressure with a cotton ball, silver nitrate was applied to the toenail. Additionally, we attached necklace-mounted radio-collars to each bird, thus avoiding sampling the same individual more than once. The capture and handling of sage-grouse was approved by the Utah State University Institutional Animal Care and Use Committee (USU IACUC Permit 1332).

Extraction and Amplification

DNA extractions were conducted using a salting-out extraction method modified from Sunnucks and Hales (1996). Blood samples were incubated with proteinase K in 300 μL TNES buffer (1 M Tris-HCl [pH 8.0], 0.5 M EDTA [pH 8.0], 5 M NaCl, and 10% SDS) overnight at 55 °C. After incubation, 85 μL of NaCl was added followed by centrifuging at 13,500 RPM for 10 min to pellet the proteins. An equal volume of cold 100% ethanol was added to the supernatant, and the sample was spun again for another 10 min to pellet the DNA. The ethanol was removed, and the DNA pellets were rinsed a final time using 75% ethanol and then centrifuged for 5 min. The pellets were dried and then suspended in 40 μL of 0.1X TE buffer.
Polymerase chain reactions (PCRs) were performed using previously described primers 16775L (Quinn 1992) and 521H (Quinn and Wilson 1993), followed by a nested PCR with primer 418H (Quinn and Mindell 1996) to amplify a 146-bp (base pair) section of mitochondrial control region I (Kahn et al. 1999). This region was chosen because it was estimated to contain approximately 92% of variable sites within a 380-bp section of control region I (Kahn et al. 1999). Additionally, reference data for this section of control region I was available for other populations. Amplifications were carried out on Applied Biosystems, Inc. (ABI) 2720 and 9700 thermal cyclers (Applied Biosystems, Inc., Forest City, CA) in 25 μL total reaction volume. PCR conditions consisted of preheating to 92 °C for 2 min, followed by 30 cycles of amplification (denaturing at 94 °C for 30 s, annealing at 56 °C for 30 s, and an extension at 72 °C for 2 min). A final extension was carried out for 10 min at 72 °C. The PCR product was purified using the Qiagen QIAquick PCR Purification Kit and following manufacturer instructions (Qiagen, Spin Handbook, 2006).

Mitochondrial Sequencing

Sequencing reactions were conducted with an ABI BigDye Terminator Kit v3.1, and reaction products were separated and visualized using an ABI PRISM 3730 Genetic Analyzer. Contiguous sequences for each individual were constructed using sequences from both directions and were aligned using SEQMAN and MEGALIGN software (DNASTAR Inc., Madison, WI).

Data Analysis

We used MEGA 4.0.2 (Tamura et al. 2007) software to compare sequenced haplotypes with previously described haplotypes available in GenBank and to construct neighbor-joining dendrograms. Previous research has shown that sage-grouse haplotypes fall into 2 distinct clades that are thought to represent ancestral isolation of populations that began 850,000 years ago during the Pleistocene (Kahn et al. 1999, Benedict et al. 2003, Oyler-McCance et al. 2005). Populations often contain haplotypes from both clades, complicating interpretation of distance-based haplotype trees in the context of current landscapes and populations. For this reason, we focused our examination of population structure on haplotype composition regardless of clade. We used ArcGIS 9.2 (ESRI, Redlands, CA) geographic information system (GIS) software to create maps.

RESULTS

We collected 17 samples from 3 populations (Anthro Mountain, n = 7; Seep Ridge, n = 7; and Deadman Bench, n = 3; Table 1). Given the estimated size of these populations at the time sampling was conducted, we sampled approximately 13% of the Anthro Mountain population, 15% of the Seep Ridge population, and 19% of the Deadman Bench population. Other surveys of northeastern Utah populations sampled 3% of the Diamond Mountain population and 7% of the Blue Mountain population (Oyler-McCance et al. 2005; percentages based upon UDWR lek count data).

We identified 6 unique mtDNA haplotypes among the 17 individuals assayed. The observed haplotypes fell into both of the monophyletic clades (clade I and clade II) as described by Kahn et al. (1999). We detected 2, 4, and 1 haplotypes in the Anthro, Seep Ridge, and Deadman Bench populations, respectively.

The most common haplotype observed in the Anthro Mountain population was DR (Clade I; n = 6 out of 7 samples). The haplotype is uncommon among sage-grouse populations and was previously found only in the Strawberry Valley population (n = 15 out of 23 samples; Oyler-McCance et al. 2005). The other haplotype observed in the Anthro Mountain population, DU (n = 1 out of 7 samples), is newly described here and differs from the DR haplotype by a single transversion. The Anthro Mountain population did not share any common haplotypes with the Seep Ridge and Deadman Bench populations.

Only 1 haplotype (B) was observed in the Deadman Bench population. By comparison,
the Seep Ridge population, in which the large-bodied grouse were observed in 2007, contained the greatest diversity of haplotypes, with 4 haplotypes observed in 7 samples (D, n = 1; B, n = 3; ER, n = 2; Z, n = 1). Haplotype B is widely distributed throughout the range of sage-grouse (Kahn et al. 1999, Benedict et al. 2003, Oyler-McCance et al. 2005). Haplotype types D and Z are less common at the rangewide scale, but they are found in neighboring populations located in northwestern Colorado (Kahn et al. 1999, Oyler-McCance et al. 2005). The haplotype ER is structurally similar to the haplotype B, but ER is rare, with only one copy observed in Weston, Wyoming (Oyler-McCance et al. 2005).

**DISCUSSION**

The haplotype composition of the Anthro Mountain population differed from other northeastern Utah sage-grouse populations, possibly due to geographic isolation and subsequent genetic drift. Comparisons of haplotype composition with previous studies suggest that the Anthro Mountain population may be more similar to the Strawberry Valley population, while the Seep Ridge and Deadman Bench populations may be more similar to Diamond and Blue Mountain, Utah, and Cold Springs, Colorado, populations (Oyler-McCance et al. 2005; Fig. 1). The Anthro Mountain and Seep Ridge populations were 80 km apart, but they did not share common haplotypes.

It is possible that Desolation Canyon of the Green River has acted as a long-term barrier between these populations, limiting gene flow and increasing genetic divergence. In northeastern Utah, Ruffed Grouse (*Bonasa umbellus*) occur only on the west side of the Green River, even though there is suitable habitat on the east side (B.D. Maxfield, personal communication, 2009). Alternatively, because mitochondrial DNA is maternally inherited, it is possible that haplotype distributions do not reflect current exchange of individuals or nuclear diversity, because movements of males could go undetected. Since differences between male and female grouse dispersal patterns are not well understood, it is difficult to determine whether this scenario is likely (Connell et al. 2004).

Due to the high mutation rate, lack of recombination, and mode of inheritance, mtDNA is often used to reconstruct intraspecific phylogenetic relationships (Avise et al. 1987, Kahn et al. 1999, Benedict et al. 2003, Oyler-McCance et al. 2005), and the differentiation of haplotypes in northeastern Utah may indicate a long-term separation of lineages reflecting divergent histories of adaptation. As indicated by previous research, adaptation of sage-grouse populations to local environmental conditions is likely (Oyler-McCance et al. 2005, Oyler-McCance and Quinn 2011). This likelihood has important implications for choosing source populations for translocations and identifying habitat for conservation. Regardless of the reason for the difference in haplotype composition, these populations should be managed separately until patterns of nuclear genetic diversity are described. We believe because we surveyed >10% of the estimated populations in our study areas (UDWR unpublished data), our results represent a valid depiction of the genetic composition of these populations. Other surveys of northeastern Utah populations surveyed 3% (Diamond Mountain, UT) and 7% (Blue Mountain, UT; Oyler-McCance et al. 2005; percentages based on UDWR lek count data).

Our study areas exhibited a high degree of fragmentation attributed to historic land uses. The effect of these land uses on sage-grouse habitat loss and fragmentation may be far exceeded by energy development. As energy development progresses in northeastern Utah, habitat will likely become more fragmented and populations more isolated (UDWR 2009). Assisted migrations through translocations may be necessary to artificially simulate gene flow and counteract the negative effects of inbreeding and genetic drift that can accelerate extinction of small populations. Baxter et al. (2008) demonstrated that translocations can be a viable tool for recovering declining sage-grouse populations. Translocation of birds to a declining Greater Prairie Chicken (*Tympanuchus cupido pinnatus*) population has also resulted in increased fitness and has been credited for the recovery of the population (Westemeier et al. 1998).

However, an inappropriate choice of a source population could be detrimental due to the effects of outbreeding depression (Frankham 1995, Edmands 2007). Although outbreeding depression is not as well understood as inbreeding depression, it can have serious
effects on small populations. For example, researchers monitoring descendants of natural immigrants to a population of song sparrows (*Melospiza melodia*) found that the descendants showed signs of outbreeding depression, including lower survival rates and reduced lifetime reproductive success (Marr et al. 2002).

To minimize the risks of outbreeding depression, we recommend translocations only when there is evidence of inbreeding. Common estimates of inbreeding in birds, such as fertility (fertile eggs/total eggs) or hatch success (hatched eggs/total incubated eggs; Westemeier et al. 1998, Crnokrak and Roff 1999), could be used to determine if translocations are necessary. If sufficient samples are available, inbreeding can also be detected via analysis of nuclear loci. Additionally, managers can minimize the risks of outbreeding depression by choosing source populations that are genetically and adaptively similar (Edmands 2007).

In northeastern Utah, we recommend that translocations to the Anthro Mountain or Strawberry Valley populations come from the Parker Mountain, Utah, population, because previous nuclear DNA analysis has shown that these populations cluster together, indicating that they have similar genetic compositions (Oyler-McCance et al. 2005). These populations are not currently connected by sagebrush habitat, but birds may have moved between populations historically (Schroeder et al. 2004). Based on comparative haplotype compositions, we recommend that if translocations to the Deadman Bench or Seep Ridge populations become warranted, the Diamond or Blue Mountain, Utah, populations be considered as source populations. However, we recognize the limitations of our data and recommend that our conclusions regarding gene flow patterns and diversity be confirmed by nuclear markers prior to consideration of a translocation.

In addition to radio-telemetry studies, we recommend that biologists routinely collect genetic samples when handling birds, or when carcasses are available, to better understand population structure. Genetic data can be used to identify unique groups, assess inbreeding depression, and identify appropriate sources for translocations. Genetic data may also be used to locate migratory corridors and (with sufficient polymorphic loci) to identify individuals and assess relationships among individuals (Oyler-McCance and Quinn 2011). We also recommend maintaining careful records of translocations to improve the understanding of the impacts of such translocations. However, even limited knowledge of the genetic composition of these populations will be valuable in planning conservation efforts. By preparing for impending development in northeastern Utah and other areas affected by development, it may be possible for managers to maximize the effectiveness of conservation efforts by choosing appropriate habitat for conservation and conducting informed translocations if necessary. We are hopeful that these efforts and future efforts will allow sage-grouse to coexist successfully with energy development.

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