COLORADO DIVISION OF WILDLIFE – AVIAN RESEARCH PROGRAM
PROGRESS REPORT
(AUGUST 20, 2010)

TITLE: Seasonal Habitat Use, Movements, Genetics, and Vital Rates in the Parachute/Piceance/Roan Population of Greater Sage-Grouse

AUTHOR: Anthony D. Apa, Colorado Division of Wildlife, 711 Independent Avenue, Grand Junction, Colorado 81505 tony.apa@state.co.us

PROJECT PERSONNEL: J.T. Romatzke, Area Wildlife Manager, John Broderick, Senior Terrestrial Supervisor, Brad Petch, Senior Wildlife Conservation Supervisor

Period Covered: March 1, 2006 – August 31, 2008

All information in this report is preliminary and subject to further evaluation. Information MAY NOT BE PUBLISHED OR QUOTED without permission of the author. Manipulation of these data beyond that contained in this report are discouraged.

ABSTRACT

Greater sage-grouse (*Centrocercus urophasianus*) historically inhabited sagebrush steppe habitat in at least 13 states and 3 Canadian provinces, and now occur in 11 states and 2 provinces. Habitat loss, fragmentation, and degradation are commonly suggested as reasons leading to the decline of sage-grouse and other sagebrush obligate avian species. The Colorado Division of Wildlife has been concerned with persistence of the Parachute/Piceance/Roan (PPR) sage-grouse population since at least the early-1990s. The PPR is one of several small, spatially fragmented populations of sage-grouse in Colorado. The specific objectives of this research project were to: 1) Obtain baseline information on genetic characteristics, 2) Acquire current estimates of reproductive parameters (nesting effort, apparent nest success, and renest success, and female success) and survival rates of adult and yearling females and males as well as juvenile sage-grouse up to 30 - 50 days of age, 3) Measure movements and seasonal habitat use patterns and, 4) Measure micro-habitat characteristics at nest and brood-rearing sites. The area occupied by the PPR population is located in Rio Blanco and Garfield county, Colorado, USA. During the spring of 2006, 2007 and 2008 and the fall of 2007, greater sage-grouse were captured and radio-marked. In 2007, day-old chicks were radio-marked. Blood samples were obtained from all captured sage-grouse for DNA analysis. In the spring and fall of 2006 and 2007 and the spring of 2008, 79 (12 M; 67 F) greater sage-grouse were captured and radio-marked. The mass of grouse capture varied by age and time of year captured. Nest initiation rates were 67%, 94%, and 63% for females in 2006, 2007, and 2008, respectively. Sixty nests were documented throughout the course of the study. Apparent nest success through the study period was 40%. Adult female annual survival was 0.65 and yearling female annual survival was 0.48. GRASSHT (grass height) and SAGEHT (big sagebrush height) were taller and PERGRASSCOV (perennial grass cover), TOTSHRUBCC (total shrub cover), and TOTSAGECC (big sagebrush cover) were higher at nest sites when compared to random sites. TOTSHRUBCC was lower (34 vs 46%) and SAGEHT was shorter at brood-rearing sites when compared to random sites. Sixty-nine percent of nests were located within 3.2 km (2 miles) of their lek of capture while 81% were located within 6.4 km (4 miles) of their lek of capture. Female survival was slightly higher and yearling female survival was dramatically lower than other reports previously documented. TOTSHRUBCC at nest sites exceeded recommendations in the Colorado Greater Sage-Grouse Conservation Plan (CCP). SAGEHT and TOTSAGECC both exceeded the CCP guidelines as well. Nearly 80% of females nested on westerly and easterly aspects on high or moderate slopes. Any management scenarios that decrease big sagebrush and non-big sagebrush cover should be avoided or

viewed with extreme caution even in a research scenario. Female survival (especially yearlings and chicks) needs further evaluation. Based on population viability analyses of the PPR grouse population in the CCP, the persistence of this species in the PPR could be problematic if yearling survival rates and chick survival rates documented during this study continue.

Acknowledgements

I would like to thank the individuals that worked on the project and the private landowners and companies that provided funding and/or access that made this project possible. A sincere thank you is extended to the most significant contributor in this project, Brandon Miller. Brandon was crew leader for the 2007 and 2008 portions of this project and was a success because of his hard work and dedication. The project was originally designed by J. Beck for field worked conducted in 2006. Seasonal habitat use maps were created by K. Eichhoff. Technicians that worked on the project included L. Dennis, A. Gmyrek, E. Phillips, A. Pratt, and A. Wiese. Funding for this project was provided by the Colorado Division of Wildlife, Encana Corporation, The Williams Companies Incorporated, and ConocoPhillips Company. Numerous individuals were instrumental in seeing this project in becoming a reality.

INTRODUCTION

Greater sage-grouse (*Centrocercus urophasianus*) historically inhabited sagebrush steppe habitat in at least 13 states and 3 Canadian provinces, and now occur in 11 states and 2 provinces (Schroeder et al. 2004). Sage-grouse are of particular conservation concern because populations have experienced dramatic range-wide declines over the past forty years (Connelly et al. 2004). In addition, some view sage-grouse as an umbrella species for sagebrush habitats (Rich and Altman 2002).

Habitat loss, fragmentation, and degradation are commonly suggested as reasons leading to the decline of sage-grouse and other sagebrush obligate avian species (Knick et al. 2003). Populations are migratory, moving >10 km to access seasonal habitats across large sagebrush landscapes, or are more sedentary, using the same habitats throughout the year to meet their life history requirements (Connelly et al. 2000). Impacts of human influences or other environmental perturbations may be more pronounced in populations that are small because persistence of small populations is affected by stochastic environmental, genetic, and demographic parameters that may overwhelm the natural variation of these parameters in small populations (Mills et al. 2005).

The largest, most persistent (>500 breeding birds) populations of greater sage-grouse in Colorado are found in Jackson, Moffat, Rio Blanco, and Routt counties (Braun 1995, Colorado Division of Wildlife 2008). Small (<200 males), isolated populations of sage-grouse are found in Colorado in the Parachute/Piceance/Roan (PPR) area in Garfield County, northern Eagle and southern Routt Counties (Schneider and Braun 1991, Colorado Division of Wildlife 2008), northwest Larimer County, and the Meeker/White River area in eastern Rio Blanco County (Colorado Division of Wildlife 2008). Oil and gas development activity is occurring in and/or planned for the Piceance Basin, and industry has expressed their interest in evaluating mitigation efforts and understanding the baseline habitat use, movements, and vital rates of this population.

Sage-grouse from Eagle County, North Park, and Middle Park, Colorado function as a genetically-related group. Birds within each group are genetically similar, while genetic relatedness differs between groups (Oyler-McCance et al. 2005a). The genetic relatedness of sage-grouse inhabiting the PPR area is unknown compared to other populations in Colorado or elsewhere (Oyler-McCance et al. 2005a). Genetic information is imperative in the event that future translocations of sage-grouse to and from the PPR population are needed.

The Colorado Division of Wildlife has been concerned with persistence of the PPR sage-grouse population since at least the early-1990s and discontinued hunting this population in the mid-1990s due to declining wing receipts and other indicators that the population may have been declining. Limited information is available for PPR sage-grouse including habitat use and seasonal movements (Krager 1977, Hagen 1999), lek complexes (Krager 1977), and harvest data used to compute sex and age ratios (Colorado Division of Wildlife 1995). However, the limited information that does exist does not provide a clear picture as to historical or current population levels or trends in vital rates.

The PPR is one of several small, spatially fragmented populations of sage-grouse in Colorado. The CDOW is interested in working with industry and other land owners and managers in the PPR area to sustain the PPR grouse population and plan for future management actions. This information will be useful in assessing the current population status and expected future trend of PPR sage-grouse, and for identifying alternative management strategies for this population.

The results of this 3-year study will provide important information that can be applied by land managers to enhance conditions to promote persistence and growth of the PPR sage-grouse population. This will be accomplished by collecting data that provide industry and agency managers a better understanding of the habitat use, seasonal movements, genetics, and vital rate demography of this small isolated population of greater sage-grouse.

The specific objectives of this research project were to:

1. Obtain baseline information on genetic characteristics of sage-grouse in the PPR population.
2. Acquire current estimates of reproductive parameters (nesting effort, apparent nest success, and renest success, and female success) and survival rates of PPR adult and yearling females and males

as well as juvenile sage-grouse up to 30 - 50 days of age.
3. Measure movements and seasonal habitat use patterns of PPR sage-grouse on a landscape level.
4. Measure micro-habitat characteristics at nest and brood-rearing sites.

Given the current status of this small population of sage-grouse and the landscape changes that are expected to occur over the next 5-10 years, there is a pressing need to obtain current, detailed baseline information on the population ecology of PPR sage-grouse and provide this information to managers.

STUDY AREA

The area occupied by the PPR population of sage-grouse is located in Rio Blanco and Garfield counties (Fig. 1). Hagen (1999:9) described the area: "The Piceance Basin-Roan Plateau is bordered on the north by the White River and on the south by the Colorado River. The Utah boarder is ~80 km to the west and the Grand Hogback borders the basin on the east. The study area encompasses approximately 1,400 km^2 of the ~ 3,000-km^2 region. The specific boundaries of the study area are the Dry Fork of Piceance Creek and Big Duck Creek to the north, and Skinner Ridge, Jack Rabbit Ridge, and Roan Creek to the southwest and south. Cathedral Bluffs defines the western limit and Colorado Highway 13 is the eastern boundary. Piceance Creek bisects the eastern third of the study site."

"The climate of the Piceance Basin is semiarid and exhibits extreme differential levels of monthly precipitation. Consecutive months often receive little precipitation. Mean annual precipitation was 35.3 ± 18.7 cm for eight weather stations in the region for 1951-70 (Cottrel and Bonham 1992) and snowfall comprised ~ 50% of the total precipitation. The mean annual temperature varies from7^o C at 1,800 m to -1^o C at 2,700 m." (Hagen 1999:9).

"The topography of the study areas has been described as a structural basin (Tiedeman and Terwilliger 1978) or a plateau that is dissected by narrow drainages. The sagebrush steppe consists of undulating north-south ridges parallel to each other. The ridge tops vary in width from 0.5 to 3 km, and 1 to 30 km in length. The ridges are gently rolling; however, the drainages that separate them are steep. Specifically, the ridges in the southern part of the study area are divided by canyons that drop nearly 1 km, vertically, in <500 m, horizontally; typically the elevation change is more gradual. Elevations vary from 1,800 m on Piceance Creek to 2,700 m at the upper reaches of the plateau. The higher elevation areas are known locally as the "summer range" as they are the location for summer grazing of livestock." (Hagen 1999:9).

Vegetation is dependent upon slope, aspect, and elevation. Three subspecies of big sagebrush (*Artemisia tridentata*) occupy the basin, and location of *Artemisia tridentata ssp*. is dependent upon soil type (Cottrell and Bonham 1992). Basin big sagebrush (*A. t. tridentata*) is the prevalent vegetation throughout the drainages at elevations of 1,800 – 2,000 m (Cottrell and Bonham 1992). Typically basin big sagebrush grows taller and denser than mountain big sagebrush (*A. t. vaseyana*) and Wyoming big sagebrush (*A. t. wyomingensis*) (Cottrell and Bonham 1992). *A. t. wyomingensis* is restricted to upland ridges at elevations of 1,900 – 2,000 m (Cottrell and Bonham 1992). *A. t. vaseyana* is confined to high mountain areas at elevations > 2,100 m (hereafter all references to big sagebrush will refer to *A. t. vaseyana*, unless otherwise noted)." (Hagen 1999:9).

"Pinyon pine (*Pinus edulis*) and juniper (*Juniperus spp.*) woodlands dominate the landscape until ~2,100 m. Big sagebrush, Utah serviceberry (*Amelanchier utahensis*), Gambel oak (*Quercus gambelii*), and antelope bitterbrush (*Purshia tridentata*) comprise most of the transition vegetation type. Low and rubber rabbitbrushes (*Chrysothamnus viscidiflorus, C. nauseosus*) are prevalent throughout the basin. Elevations of 2,400 to 2,600 are dominated by big sagebrush interspersed with bunchgrass meadows. North aspects often host substantial groves of quaking aspen (*Populus tremuloides*), serviceberry, and mountain snowberry (*Symphoricarpos oreophilus*). Big sagebrush and Douglas-fir (*Pseudotsuga menziesii*) dominate south and northwest aspects at elevations > 2,500 m, respectively. Free water can be scarce in dry years or late in the summer as most springs are in the bottom of steep canyons." (Hagen 1999:9).

METHODS

Capture and Marking of Grouse

During the spring of 2006, 2007 and 2008 and the fall of 2007, greater sage-grouse were captured and radio-marked. Sage-grouse were captured using night spot-lighting (Giesen *et al.* 1982, Wakkinen *et al.* 1994) techniques. Grouse captures were not randomly distributed throughout the study area, rather they were captured opportunistically on or near strutting grounds in the spring and by radiating away from the strutting grounds to appropriate capture locations. In the fall, grouse were captured using the same techniques in grouse concentration areas using marked females.

All grouse captured were weighed using an electronic scale (to the nearest 1 g) and uniquely marked with aluminum, uniquely numbered leg bands. The age and gender of each grouse captured was determined using wing (Dalke *et al.* 1963) and other plumage or morphological characteristics.

Female grouse were preferentially captured, although a sample of males was captured in 2006. A small sample of males and all females were equipped with a 17-g necklace-mounted radio transmitter with a 4-hour mortality circuit. Each transmitter had a nominal battery life of 18 months and had a 30 cm antenna that was placed dorsally between the wings and down the back of the grouse. The radio transmitters were 0.8% and 0.56% of the body weight of an adult and yearling male, respectively. The transmitter weight was only slightly heavier for females and consisted of 1.0% or 1.2% of the body weight for adult and yearling females, respectively.

Female grouse captured in the fall were also fit with radio-transmitters. A radio-tagged female was located at dusk to find her general use and roosting area and then re-located after sunset. Grouse associated with her were captured and radio-marked. Any juveniles captured were radio-marked if their body mass was > 900 g. Primary feather measurements and molting sequence were used to ascertain the gender of the juvenile. All fall captured grouse were weighed and banded the same as spring captured grouse.

In 2007, day-old chicks were radio-marked to estimate survival rates. Once nest monitoring revealed the successful hatch, all chicks in the brood were captured 1-2 days after hatching. Radio-marked brood females were located < 2 hours after sunrise in order to capture chicks while the female was brooding. Chicks were captured by hand and held in cotton bags for processing and to facilitate thermoregulation. All chicks within a brood were weighed and had a secondary feather collected. Two to four chicks/brood were randomly selected and a 1.4 gram, 60-day radio-transmitter was attached along the dorsal midline between the chick's wings (Burkepile *et al.* 2002). Chicks were released together at the capture location and monitored (<1 hr) to confirm the immediate survival of the chicks. In addition, broods were located latter in the day (> 2 hours after introduction) and < 2 hours before sunset to determine chick survival. Chick survival was monitored daily for at least 28 days, and every 2 – 3 days after 28 days up to 50 days.

Genetic Data

Blood samples were obtained by slightly over-clipping a toenail of all captured mature sage-grouse, and 2 - 3 drops of blood and were placed into a microfuge tube previously coated with EDTA (Oyler-McCance 1999). The blood samples in addition to feather samples were frozen at –20°C and stored at the Rocky Mountain Center for Conservation Genetics and Systematics in the Department of Biological Sciences at the University of Denver (Center) (S. Oyler-McCance, pers. comm.). All genetic analyses were conducted by Dr. Sara Oyler-McCance at the Center. DNA was extracted from blood samples using the GenomicPrep Blood DNA Isolation Kit (Amersham Biosciences) using the modifications of Oyler-McCance *et al.* (2005b). A 146 base pair portion of hypervariable control region I was amplified using the Polymerase Chain Reaction (PCR) and sequenced using a dye terminator cycle sequencing reaction (Beckman Coulter CEQ8000) as described by Benedict *et al.* (2003). This region was used because it was known to contain approximately 92% of the variable sites in a larger 380 base pair region spanning control region I (Kahn *et al.* 1999). A final report was prepared and delivered to CDOW.

Survival and Seasonal and Daily Movements

Movements and survival of radio-marked grouse were monitored 1-2 times/week. General locations were obtained by triangulation and radio-marked birds were not flushed. Hand-held Yagi antennas, attached to a receiver/scanner, were used to located radio-marked grouse. The loudest-signal method was used to locate grouse/transmitters (Springer 1979). Monitoring periods were distributed among 3 diurnal periods; morning (< 4 hours following sunrise), midday (> 4 hours after sunrise) and evening (< 4 hours before sunset). All grouse were circled at a 50 – 100 m radius (Apa 1998) to determine habitat type use. Precise Universal Transverse Mercator (UTM) locations were not possible at the time of location (the bird was not flushed), so the observer selected a location ≤ 50 m in one of the 4 cardinal directions from the estimated location of the bird. The observer collected a Global Positioning System (GPS) location and then manually corrected the UTM location. General cover types were recorded as shrub steppe (sagebrush), wet meadow, mountain shrub, oakbrush, grassland or agricultural field.

Females with radio-marked chicks were monitored daily to determine chick survival and brood location. Brood positions were determined by locating the female and circling to within 25 m. Position and relationship (i.e., distance) of radio-marked chicks in relation to the female were also recorded. In addition, cover type was determined at all locations. Daily observation of broods continued for 30 days or until death or transmitter failure. Efforts were made to find all chicks immediately after becoming separated or missing from broods to determine fate and/or cause of mortality. Brood locations were collected among 4 time periods: brooding (< 2 hour after sunrise or before sunset), morning (0800-1100), mid-day (1100-1400), and afternoon (1400-1800) throughout the study. After day 30, radio-marked chicks and females were located every 1-3 days.

Fixed-wing aircraft assisted to locate any grouse not located by ground monitoring or lost during ground monitoring efforts. General locations were identified aerially and ground locations were identified within 48 hours.

Microhabitat Characteristics

When a female was suspected of incubation, the nest location was determined using binoculars as described by Apa (1998). Once a female was identified as incubating, she was not disturbed during incubation. Diagrams of the nest location were drawn to assist in nest location after the completion of nesting. The precise UTM location was collected following the cessation of nesting. A nest was considered successful if ≥ 1 egg hatches (Rearden 1951).

In 2006, nest sites had vegetation measurements conducted as described by Beck (2006a). A slightly different approach was used in 2007. In 2007, all nest sites had four 10-m transects placed in the cardinal directions intersecting at the nest bowl. The nest shrub species and height was measured. The height of the lowest live and dead nest bush branch above the nest bowl was measured from the edge of the nest bowl. Canopy cover (foliar intercept) of the shrub species overstory was ascertained using line-intercept (Canfield 1941). Height of the of the nearest big sagebrush shrub within 1 m of the transect line was measured at 2.5 m, 5 m, and 10 m. Grass height was measured for the nearest grass part at the points where the edge of the nest bowl and the transect's intercept, and at the 1 m point on each transect.

The percent of forbs, annual and perennial grass cover, bareground, and litter horizontal understory cover was estimated using 50 x 25 cm microplots (Daubenmire 1959). Twelve cover classes were used and delineated as: < 1%, 1-10%, 11-20%, 21-30%, 31-40%, 41-50%, 51-60%, 61-70%, 71-80%, 81-90%, 91-99%, > 99%. The first 2 microplots were located on opposing sides of the nest bowl. Grass and forb height were also measured in subsequent plots placed systematically along the transects at 2.5, 5, and 10 m. In addition, the distance to nearest visible roadways, telephone poles, powerlines, and fence posts were determined.

The same vegetation data sampling techniques were conducted at least one random location for each nest. Random locations were obtained by using randomly selected UTM coordinates in the study area. Grouse movements delineated the study area boundary.

Females with broods, unsuccessful females, and males were located by the loudest-signal method 1-2 times per week. At each location, date, time, UTM coordinates, slope and aspect were recorded.

Unsuccessful females and males were located in the same manner as females with broods. When females with broods were circled, the intersection point of flags placed in the cardinal directions were used to identify the center of the brood location.

At the center of each brood location identified for vegetation sampling, the same vegetational structural characteristics were measured. One random site was selected for each brood vegetation site and the same vegetation sampling occurred. The aspect categories included northerly (315 – 45^{o}), easterly (46 – 135^{o}), southerly (136 – 235^{o}) and westerly (226 – 314^{o}).

The angle of inclination is measured and converted to percent slope. Categories include flat (0%), low slope (0 – 9%), moderate slope (10 – 18%), and high slope (> 19%).

Statistical Analyses

All statistical analyses were performed using statistical analysis software (SAS; SAS Institute 2003). Bird locations were entered into a geographic information system for analysis. Habitat selection and movements were evaluated with these data. The vegetation analysis includes 4th order selection (Johnson 1980) (nest or brood site) and 3rd order selection (nest or brood sites versus random site in the study area) (Johnson 1980). Additional analyses may include components of home range and other seasonal use components. Univariate and multivariate statistical approaches were used to characterize habitat and examine possible differences. Analysis of variance (ANOVA) and multivariate analysis of variance (MANOVA) were used. All variables were examined for univariate and multivariate normality. Those variables with non-normal distributions ($\alpha > 0.10$) were transformed using standard data transformation techniques (Zar 1984). Following data transformations, remaining variables were evaluated for their correlative nature and significant correlations ($\alpha > 0.10$) were removed from future analyses due to problems associated with colinearity and statistical power (Johnson and Wichern 1992). Other multivariate procedures such as principal components analysis and/or stepwise logistic regression will be used in future analyses to further evaluate habitat characteristics at nest and brood locations. An analyses of physiographic (slope, aspect, and elevation) characteristics was conducted using Chi-square or univariate analysis.

Annual and project-long survival rates for grouse were estimated by gender, age (adult, yearling, and fall juveniles) with the Kaplan-Meier product limit estimator (Kaplan and Meier 1958) modified for staggered entry (Pollock *et al.* 1989) where appropriate.

RESULTS

Capture and Marking of Grouse

In the spring and fall of 2006 and 2007 and the spring of 2008, 79 (12 M; 67 F) greater sage-grouse were captured and radio-marked (Table 1). Males were captured and radio-marked in 2006. Of the 67 females captured, 66% (n = 44/67) were captured in the spring on or near strutting grounds while and 34% (n = 23/67) were captured in grouse concentration areas in the fall. Sixteen, 44, and 7 females were captured in 2006, 2007, and 2008, respectively (Table 1).

Sixty-seven percent (n = 8/12) of the males captured were adults and 33% (n = 4/12) were yearlings. The age ratio of female captures varied by year. In 2006, 56% (n = 9/16) were adults and 44% (n = 7/16) were yearlings. "Yearlings" captured in the fall of 2006 could be juveniles from the 2006 juvenile cohort. Depending on the stage of molt, a yearling that attends a strutting ground in 2006 usually will have molted the #9 and #10 primary feathers, but this depends on the time of capture and nest success of the female. Therefore, the classification of yearlings in the fall can be misclassified as yearlings due to the primary feather shape and wear. The "yearlings" were captured between 14 August 2006 and 31 August 2006 by inexperienced crews and the age classification of these juveniles should be viewed with caution.

In 2007, adult females consisted of 50% (n = 14/28) of the captures, while yearlings were 50% (n = 14/28) of the captures (Table 1). No yearlings were captured in the fall of 2007 although 62% (n = 10/16) were adults and 38% (n = 6/16) were juveniles. In 2008, fewer captures occurred than all other years due to extreme weather conditions during the winter of 2007 – 2008. Only 7 females were captured

and 29% (n = 2/7) and 71% (n = 5/7) were adults and yearlings, respectively (Table 1).

The mass of grouse capture varied by age and time of year (Fig. 2). Although males were weighed, they were not included in the analyses because of the small sample size and obvious 2X mass difference when compared to females. Adult male mass ($\bar{x}$ ± SE) was 2,562 ± 86 g (n = 8) while yearling male mass was 2,437 ± 46 g (n = 4). Female mass by age class exhibited differences ($F_{3,57}$ = 22.37; P < 0.0001). Adult females weighed more (P = 0.0008) than yearling and juvenile (P = 0.0002) greater sage-grouse (Fig. 2). Adult female mass [1,503 ± 34 g (n = 31)] was over 350 g heavier than juveniles [1,143 ± 51 g (n = 6)] and approximately 100 g heavier than yearlings [1,408 ± 23 g (n = 24)] (Fig. 2). Spring captured yearling female mass was not significantly different from fall juvenile mass (P = 0.5081). The mass of female grouse also varied ($F_{2,52}$ = 17.34 P < 0.0001) when the season of capture was considered (Fig. 3). Spring female mass [1,509 ± 22 g (n = 38)] was nearly 300 g heavier than fall female mass [1,356 ± 45 (n = 17)] (Fig 3).

Nesting

We documented nest initiation with 67% (n = 6/9), 94% (n = 33/35), and 63% (n = 17/27) of the females in 2006, 2007, and 2008, respectively. We documented nest initiation with 84% (n = 37/44) of adult females and 81% (n = 22/27) of yearling females in the three years of the study.

Sixty nests were documented throughout the course of the study. Six nests were located in 2006, 37 in 2007 and 17 in 2008. Apparent nest success over the study period was 40% (n = 24/60). In 2006, apparent nest success was 0% (n = 0/6) and in 2007 and 2008 apparent nest success was 46% (n = 17/37) and 40% (n = 7/17), respectively. Throughout the study adult and yearling female apparent nest success was 40% (n = 16/40) and 40% (n = 8/20), respectively.

In contrast to nest success, female success (number of females having a successful nest rather than the number of successful nests) is a more useful demographic parameter. Over the course of the study, female success was 34% (n = 24/71). Female success in 2006, 2007, and 2008 was 0% (n = 0/9), 49% (n = 17/35), and 26% (n = 7/27), respectively. Four nests were located after a first nesting attempt failed (renests) and all were not unsuccessful.

Survival

Annual survival (12-month) as well as survival for the duration of the project (29-month) was estimated. Adult female annual survival was 0.65 (95% CI = 0.49 – 0.77; n = 27), while survival during the duration of the research project was 0.35 (95% CI = 0.17 – 0.52; n = 27). Yearling female annual survival was 0.48 (95% CI = 0.34 – 0.58; n = 32), while project duration survival was 0.17 (95% CI = 0.07 – 0.27; n = 32). Adult male annual survival was 0.58 (95% CI = 0.0 – 0.87; n = 8) and project duration survival was 0.27 (95% CI = 0.0 – 0.71; n = 8). Yearling male annual survival was 0.56 (95% CI = 0.0 – 0.85; n = 5) and project duration survival was 0.25 (95% CI = 0.0 – 0.67; n = 5). Juvenile survival was not calculated due to sample size issue and time of capture.

In 2007, we estimated chick survival from 1 – 30 days. Thirty-nine individual chicks were radio-marked at < 48 hours of age. The 39 chicks were sampled from 14 broods. The average number of chicks marked/brood was 2.8 (range 2 – 4 chicks). Survival ($\bar{x}$ ± SE) through 7 days was 0.56 ± 0.08 (n = 39). Survival through 14 days was 0.31 ± 0.08 (n = 39), and survival through 30 days was 0.12 ± 0.07 (n = 39) at 30 days (Fig. 4). Only 2 chicks remained radio-marked after 30 days of age. Apparent brood survival was 86% (n = 12/14) at 7 days, 62% (n = 9/14) at 14 days, and 14% (n = 2/14) at 30 days.

Microhabitat Characteristics

Nests

Variables without normal distributions were transformed using a $\log_{10}$ transformation. The transformed variables included forb cover (FORBCOV), annual grass cover (ANNGRASSCOV), perennial grass cover (PERGRASSCOV), and dead shrub cover (DEADSHUBCC). Each of these variables achieved normality following transformation. The variables of bareground (BARECOV), litter cover (LTRCOV), total shrub cover (TOTSHRUBCC), grass height (GRASSHT), forb height (FORBHT), big sagebrush height (SAGEHT), and big sagebrush cover (SAGECC) were normally distributed. To narrow the suite of variables describing nest and brood sites and reduce complications of colinearity, correlated variables were identified and one or both were removed from the analyses. The

variables included in the analyses were FORBCOV, PERGRASSCOV, TOTSHRUBCC, GRASSHT, SAGEHT, AND SAGECC.

I performed a MANOVA with the aforementioned variables to investigate potential differences between nest and random sites (3rd order selection). Differences between sites were detected (Wilks' λ = 0.69; $F_{6,97} = 7.38$; $P < 0.0001$). GRASSHT was taller ($F_{1,102} = 20.83$; $P < 0.0001$) and PERGRASSCOV ($F_{1,102} = 8.13$; $P = 0.0053$) was higher at nest sites compared to random sites. There was no difference in FORBCOV ($F_{1,102} = 0.01$; P = 0.9426). SAGEHT was 18 cm taller ($F_{1,102} = 15.70$; $P < 0.0001$) at nest sites compared to random sites. TOTSHRUBCC ($F_{1,102} = 33.88$; $P <0.0001$) and TOTSAGECC ($F_{1,102} = 19.66$; $P < 0.0001$) were higher at nest sites versus random sites (Fig. 5; Table 2).

Vegetation structure at the immediate nest site was also evaluated at 3rd order selection. The lowest branch of the nest bush above the nest bowl (LOWBRANCH), the grass height at the nest site (GRASSHTNEST), and the nest bush height (SAGEHTNEST) were compared. Differences (Wilks' λ = 0.69; $F_{3,54} = 8.19$; $P < 0.0001$) were detected. The LOWBRANCH was higher ($F_{1,56}$; $P = 0.0015$) from the ground at nest sites than nest bushes at random sites (22 vs 15 cm). In addition, SAGEHTNEST was 30 cm taller ($F_{1,56} = 23.00$; $P < 0.0001$) than random sites. GRASSHTNEST was the same ($F_{1,56} = 3.50$; $P = 0.0666$) between the nests and random sites.

At 4th order selection levels, female greater sage-grouse nested under nest bushes (all big sagebrush) that were 10 cm taller ($t_{2,68} = 2.15$; $P = 0.0352$) than the mean big sagebrush height within 10 m of the nest. In contrast, GRASSHTNEST had similar heights ($t_{2,68} = -0.3068$; $P = 0.7586$) at the nest bowl and mean grass heights within 10 m of the nest. The same set of variables were compared between successful and unsuccessful nests. All 6 variables were strongly similar between successful and unsuccessful nests (Wilks' $\lambda = 0.93$; $F_{6,30} = 0.38$; $P = 0.8832$).

The stepwise logistic regression included 7 variables. The variables include TOTSHRUBCC, GRASSHT, SAGEHT, TOTSAGECC, FORBCC, PERGRASSCOV, and SLOPE. Three of the variables were identified as significant contributors to the logistic regression model with 69% (n = 25/36) of the nests being correctly classified. SLOPE (Wald $\chi^2_3 = 22.12$; $P < 0.0001$) (Fig. 7), TOTSHRUBCC (Wald $\chi^2_3 = 13.76$; $P = 0.0002$) (Fig. 8), and TOTSAGECC (Wald $\chi^2_3 = 4.82$; $P = 0.0281$) (Fig. 9) were selected and retained in the model. The logistic models is:

Logit (P) = 8.0288 + (- 5.6286)(TOTSHRUBCC) + (- 5.2965)(SAGECC) + (- 0.2484)(SLOPE)

Physiographic Habitat Variables - There is significant evidence of an association between aspect and nest site use ($\chi^2_3 = 15.06$; $P = 0.0018$). Female use of nest sites was more prevalent (77.7%) on the westerly and easterly aspects. Females nested on westerly facing aspects more than expected and there were fewer than expected sites available at the random sites. Females nested on northerly aspects less than expected but more random sites were present than expected. There was also significant evidence that there was an association between slope and nest site use ($\chi^2_3 = 30.43$; $P < 0.0001$). Use was dominated (91%) on high and moderate slopes (> 10% slope). Nest sites were located on nearly twice the frequency as expected on high slope sites. No nests were found on sites with flat slope (0%) and the random sites suggest that there are only a small number of those sites available in this study area. No relationship ($F_{1,102} = 3.24$; $P = 0.0746$) was found with elevation when nest use sites and random sites were compared. Nest sites were located at 2,454 ± 10 m while random sites were 2,488 ± 12 m, a separation of only 30 m.

Brood-Rearing

A MANOVA of the aforementioned variables at brood sites was performed to investigate possible 3rd order differences between brood and random sites through the study area. Analysis was conducted on 29 brood locations. Significant (Wilks' $\lambda = 0.83$; $F_{6,89} = 3.01$; $P = 0.01$) results were detected and TOTSHRUBCC was lower ($F_{1,94} = 8.52$; $P =0.0044$) (34 vs 46%) and SAGEHT was shorter ($F_{1,94} = 5.81$; $P = 0.0179$) at brood-rearing sites when compared to random sites. In contrast, the remaining structural variables were not different between brood-rearing and random sites (Fig. 6; Table 3).

The stepwise logistic regression included 7 variables. The variables include TOTSHRUBCC, GRASSHT, SAGEHT, TOTSAGECC, FORBCC, PERGRASSCOV, and SLOPE. One variable was identified as a significant contributor to the logistic regression model with 14% (n = 4/29) of the nests being correctly classified. TOTSHRUBCC (Wald $\chi^2_3 = 9.05$; $P = 0.0026$) (Fig. 10, 11) was selected and retained in the model. The logistic models if as follows:

$$\text{Logit (P)} = - 0.8119 + (3.9958)(\text{TOTSHRUBCC})$$

Physiographic Habitat Variables - There was no significant ($\chi^2_3 = 2.71$; $P = 0.4381$) evidence of an association between brood-rearing sites and random sites with respect to aspect. Use was distributed across aspects as expected. There was no significant association with slope and brood use as well ($\chi^2_3 = 2.17$; $P = 0.5381$). Ninety percent of locations were found on 0 – 18% slope. Brood sites were located at 2,471 ± 10 m elevation while random sites were 2,488 ± 12 m, a separation of only 9 m with no differences ($F_{1,96} = 0.76$; $P = 0.3862$).

Breeding Season Movements

The distance moved from capture location to nest was summarized. Female grouse captured in the spring moved a median distance 956 m (25% and 75% Quartiles) (395, 3,392 m; $n = 48$) from the lek of capture to nest. Fall captured females moved a median distance of 1,211 m (916, 2,292 m; $n = 12$) from the capture location to nest. There were very few renests but the mean distance moved between consecutive nests within a breeding season was a median of 819 m (556, 2,690 m; $n = 4$). The distance in nest site fidelity between years was a median of 345 m (208, 851 m; $n = 13$). Sixty-nine percent of nests ($n = 33/48$) were located within 3.2 km (2 miles) of their lek of capture while 81% ($n = 39/48$) were located within 6.4 km (4 miles) of their lek of capture (Fig. 6).

Genetic Data

Genetic samples were collected from all birds captured (adults, yearlings, and chicks). A complete report of the genetic analyses is in Appendix A. Sixty-five individuals were genetically sequenced. They illustrated 8 different haplotypes and 5 of those haplotypes have been found in other GRSG populations in Colorado. The level of genetic diversity was also evaluated and it was found that the PPR population had levels of genetic diversity that were similar to other populations in Colorado. Although there was a unique haplotype found in the PPR population, other Colorado populations also had unique haplotyypes and as sample sizes increase it is likely that these haplotypes will no longer be unique.

DISCUSSION

This research project was developed to collect baseline information on the demography, genetics, seasonal movements for breeding (Fig. 12), summer (Fig. 13), and winter (Fig. 14) habitat, and 3rd and 4th order habitat use of greater sage-grouse in the PPR. The project was initiated by the CDOW in March 2006 (Beck 2006a, 2006b, 206c) and then continued by A. D. Apa from November 2006 through August 2008. At the on-set of the project, private land access was limited to localized portions of the PPR, but by March 2007, access issues were resolved and access was granted throughout most of the PPR.

Small populations of greater sage-grouse are difficult to obtain adequate sample sizes for rigorous statistical analyses. Therefore, several years of data must be collected and summarized to make meaningful conclusions. Therefore, this report only provides a "snap-shot" into population performance and seasonal movements. The PPR has exhibited all the challenges of small populations and additional years of data will be needed to have a more complete understanding of the dynamics of this population.

Small numbers of birds were captured in 2006 because of a naïve trapping crew and the understanding the logistics of a new study area. Many of those challenges were resolved and captures increased in 2007. Captures declined in 2008 due to weather logistics and physical access into the PPR to trap. Additionally, bird locations were limited due to weather access issues in 2008. Regardless of the challenges, 67 females were captured and radio-marked over the 3 years of this study.

Adult and yearling mass in the PPR (range 1,207 – 2,011 g) was similar to other studies

(Patterson 1952, Dalke et al. 1963, Wallestad 1975, Beck and Braun 1978, Autenrieth 1981, Hausleitner 2003). These authors also found yearling females weighing less than adults and a similar result is reported for the PPR where yearling female mass 100 g less than adults.

Connelly *et al.* (2004) reported female nest initiation rates of 79.9% with a range of 63 – 100%. The accuracy of this estimate is highly dependent upon research objectives and methodology and the skill of the investigators. Others have reported adult female nest initiation rates for adults are higher than yearling females (Connelly *et al.* 2001, Hausleitner 2003, Thompson 2007). The nest initiation rate in the PPR (84% adults; 81% yearlings) are on the lower end of what is reported but are within the range of other Colorado reports (range 79 – 92%).

Apparent nest success is a demographic parameter reported throughout greater sage-grouse literature. Nest success varies widely and has been reported to range from 14.5 – 86.1% (Connelly *et al*. 2004). The average for 16 studies summarized by Connelly et al. (2004) was 47.7%. Although the PPR is on the lower end of apparent nest success (40%), it is within the range reported across the range and in Colorado (Hausleitner 2003, Thompson *et al.* 2005, Thompson 2006, 2007).

Female success in Colorado ranges from 36% to 57% (Hausleitner 2003, Thompson *et al.* 2005, Thompson 2006, 2007) with an average of 43%. Female success in the PPR (34%) was well below the average reported in Colorado. This rate is of paramount interest due to the low renesting rates reported for GRSG in Colorado of 8 and 15% (Hausleitner 2003) and across the range (Connelly *et al.* 2004).

Female and male survival rates across GRSG distribution range from 55 – 75% (Connelly et al. 2004). Adult female survival ranges from 48 – 65% and yearling female survival ranged from 71 – 78% (Connelly *et al.* 2004). Zablan *et al.* (2003) found in Colorado that adult female survival was 0.59 (95% CI 0.57 – 0.61), yearling female survival was 0.78 (95% CI; 0.71 – 0.75), adult male was 0.36 (95% CI; 0.35 – 0.45) and yearling male survival was 0.63 (95% CI; 0.57 – 0.65). In the PPR female adult survival is slightly higher, yearling female survival is dramatically lower, adult male is slightly higher, and yearling male was about the same when compared to other reports. The samples sizes for males are very small and must be interpreted with caution. In contrast, yearling female survival in the PPR is 48% and the CI's do not overlap on the lower end with what is reported in the literature (0.57). This demographic parameter is concerning and must be further investigated as it may have long-term impact on population persistence. Female survival as well as clutch size, and juvenile female survival have been show to show the greatest degree of response when considering population growth and persistence (Colorado Division of Wildlife 2008).

Chick survival was investigated during the 2007 season. Therefore, the results must be interpreted with caution. Previous research in Colorado (Thompson *et al.* 2005, Thompson 2006, 2007) reported chick survival to 14 days ranged from 39% - 78% and survival through 28 days ranged from 14% - 73%. With one year of survival data, a 14-day survival rate of 0.31 is within, but on the lower end of other research in Colorado. At 30 days, the survival rate of 0.12 is lower that the lowest of 3 years in northwestern Colorado at 14%. Apparent brood survival in the PPR of 62% at 14 days and 14% at 28 days is also lower than reported in 2 years in northwest Colorado of 81% and 78% in 2005 and 85 and 74% in 2006. Further telemetry research on this subject is needed.

Numerous studies have described fourth order selection of nest habitat characteristics (Connelly et al. 2004). Nesting female sage-grouse in the PPR followed similar habitat use patterns, but in most cases used structural habitat characteristics that met or exceeded reported structural use characteristics, national guidelines, (Connelly *et al.* 2000) or Colorado developed guidelines (Colorado Division of Wildlife 2008).

TOTSHRUBCC at nest sites exceeded recommendations in the Colorado Conservation Plan (CCP). SAGEHT and TOTSAGECC both exceeded the CCP guidelines as well. GRASSHT and PERGRASSCOV both met the guidelines and only FORBCOV did not meet the guidelines by approximately 3% (12.2 vs 15%). Additionally, the mean of 12.2% is a mean of midpoints in the Daubenmire category of 10 – 20%. Therefore it is unlikely that there are FORBCOV issues in the PPR. Ironically TOTSHRUBCC and SAGEHT exceeded the guidelines at random sites as well, therefore even through randomly sampled sites exceeded the guidelines, female grouse used sites with higher

TOTSHRUBCC and taller SAGEHT than was available at random. This suggests that females are seeking very dense vegetation structure to nest in the PPR, even when less dense vegetation is available. In situations where the guidelines were also met by the random sites, females used nest sites that met the guidelines but exceeded what was available at random (GRASSHT and TOTSAGCC).

TOTSHRUBCC, TOTSAGECC, and SLOPE were good predictors of nest sites. As slope, total shrub and big sagebrush cover increased so did the likelihood that a site was a suitable nest site. Greater sage-grouse females seem to be using steep sites and as the aforementioned paragraph mentions, the total shrub cover and big sagebrush cover exceeds recommended guidelines.

With regards to brood-rearing habitat, although TOTSHRUBCC was higher and SAGEHT was taller than available at random, the random sites met or exceeded the CCP guidelines (Colorado Division of Wildlife 2008). All other variables measure for this study, met the CCP guidelines for brood-rearing (summer) habitat. TOTSHRUBCC illustrates an inverse relationship with brood-rearing use sites. As total shrub cover increases, the likelihood of a site being classified as a brood-rearing site increases. The presence of random sites in the low sagebrush cover range illustrates that there are many brood-rearing sites in the PPR and this is likely the explanation for the high misclassification of brood-rearing sites as random sites in the PPR.

Nearly 80% of females nested on westerly and easterly aspects on high or moderate slopes. There is very little flat or low slope available for use and the females use sites accordingly. Regarding brood-rearing sites, there was no association with aspect or slope, although 90% of locations were found on 0 – 18% slope.

The PPR is not a typical mildly undulating flat study area as is found in most of the GRSG range; it has deep canyons separate by narrow big sagebrush dominated ridges. This rough topography is not a barrier to movement and the PPR females which illustrated movements very similar to other females marked in Colorado (Colorado Division of Wildlife 2008). The median distance from the lek of capture to nest was approximately 1 km. Sixty-nine percent of females nested within 3.2 km of their lek of capture and 81% nested within 6.4 km of their lek of capture with some females nesting very close 57 m and very far from their lek of capture 14.7 km

MANAGEMENT RECOMMENDATIONS

Local scale micro-habitat use at nests and brood-rearing sites must be considered in overall management because PPR GRSG are nesting and raising broods in areas of shrub structure that exceed most reports across the range of GRSG. Habitat guidelines must be specific to the PPR and not extrapolated from other areas. The PPR is a high elevation mesic mountain big-sagebrush community interspersed with mountain shrub communities. Greater sage-grouse females nested and raised broods in sites that exceeded the CCP guidelines. They used nest sites that also exceeded what was available at random through the study area of the PPR. Nest sites were located on relatively steep and not always sagebrush dominated communities that provided excellent understories even though forb cover values seems somewhat marginal. Therefore any management scenarios that decrease big sagebrush or other shrub cover (Table 4) should be avoided or viewed with extreme caution even under a research scenario since this is a small isolated population.

Female survival (especially yearlings and chicks) needs further evaluation. Based on analyses of the PPR grouse population in the CCP, the persistence of this species in the PPR could be problematic if yearling survival rates and chick survival rates sampled in the short duration of this study continue. Precise and credible measures of chick survival need to be continued and validated with telemetry research to understand year to year variability.

PPR genetics do not suggest any anomalies although they do retain unique hapolotypes not observed in other GRSG populations in Colorado. Therefore, persistence of this population, as with all populations, is critical to retain genetic diversity throughout the isolated populations of Colorado.

LITERATURE CITED

Apa, A.D. 1998. Habitat use and movements of sympatric sage and Columbian sharp-tailed grouse in southeastern Idaho. Ph.D. Dissertation, University of Idaho, Moscow, Idaho.

Autenrieth, R. E. 1981. Sage grouse management in Idaho. Idaho Department of Fish and Game, Wildlife Bulletin 9, Boise, Idaho, USA.

Braun, C. E. 1995. Distribution and status of sage grouse in Colorado. Prairie Naturalist 27:1–9.

Beck, J. L. 2006a. Evaluating potential impacts of oil and gas development on small, isolated, greater sage-grouse populations in Colorado: a proposed pilot study (February 2006). Colorado Division of Wildlife, Grand Junction, Colorado, USA.

Beck, J. L. 2006b. Evaluating potential impacts of oil and gas development on small, isolated, greater sage-grouse populations in Colorado progress report (July 2006). Colorado Division of Wildlife, Grand Junction, Colorado, USA.

Beck, J. L. 2006c. A Preliminary Evaluation of Habitat Selection and Movements for the Parachute-Piceance-Roan Plateau Population of Greater Sage-Grouse Garfield and Rio Blanco Counties, Colorado – August 2006. Colorado Division of Wildlife, Grand Junction, Colorado, USA.

Beck, T. D. I., and C. E. Braun. 1978. Weights of Colorado sage grouse. Condor 80:241-243.

Benedict, N. G., S. J. Oyler-McCance, S. E. Taylor, C. E. Braun, and T. W. Quinn. 2003. Evaluation of the eastern (*Centrocercus urophasianus urophasianus*) and western (Centr*ocercus urophasianus phaios*) subspecies of Sage-grouse using mitochondrial control-region sequence data. Conservation Genetics 4:301-310.

Burkepile, N. A., J. W. Connelly, D. W. Stanley, and K. P. Reese. 2002. Attachment of radio transmitters to one-day-old sage grouse chicks. Wildlife Society Bulletin 30:93–96.

Canfield, R. H. 1941. Application of the line interception method in sampling range vegetation. Journal of Forestry 39:388-394.

Colorado Division of Wildlife. 1995. Piceance Basin sage grouse harvest data, 1977–94. Colorado Division of Wildlife, Fort Collins, Colorado.

Colorado Division of Wildlife. 2008. Colorado Greater Sage-Grouse Conservation Plan. Colorado Division of Wildlife. Denver, Colorado, USA.

Connelly, J. W., S. T. Knick, M. A. Schroeder, and S. J. Stiver. 2004. Conservation assessment of greater sage-grouse and sagebrush habitats. Western Association of Fish and Wildlife Agencies. Unpublished Report. Cheyenne, Wyoming.

Connelly, J. W., M. A. Schroeder, A. R. Sands, and C. E. Braun. 2000. Guidelines to manage sage grouse populations and their habitats. Wildlife Society Bulletin 25:967–985.

Connelly, J. W., W. L. Wakkinen, A. D. Apa, and K. P. Reese. 1991. Sage grouse use of nest sites in southeastern Idaho. Journal of Wildlife Management 55:521-524.

Cottrel, T. R. and C. D. Bonham. 1992. Characteristics of sites occupied by subspecies of *Artemisia tridentata* in the Piceance Basin, Colorado. Great Basin Naturalist 52:174-178.

Dalke, P. D., D. B. Pyrah, D. C. Stanton, J. E. Crawford, and E. F. Schlatterer. 1963. Ecology, productivity, and management of sage grouse in Idaho. Journal of Wildlife Management 27:811–841.

Daubenmire, R. 1959. A canopy-coverage method of vegetational analysis. Northwest Science 33:43-64.

Giesen, K. M., T. J. Schoenberg, and C. E. Braun. 1982. Methods for trapping sage grouse in Colorado. Wildlife Society Bulletin 10:224–231.

Hagen, C. A. 1999. Sage grouse habitat use and seasonal movements in a naturally fragmented landscape, northwestern Colorado. Thesis, University of Manitoba, Winnipeg, Canada.

Hausleitner, D. 2003. Population dynamics, habitat use and movements of greater sage-grouse in Moffat County, Colorado. Thesis, University of Idaho, Moscow, Idaho, USA.

Johnson, D. H. 1980. The comparison of usage and availability measurements for evaluating resource preference. Ecology 61:65–71.

Johnson, R. A., and D. W. Wichern. 1992. Applied multivariate statistical analysis, Third ed. Prentice-Hall, Englewood Cliffs, New Jersey, USA.

Kahn, N. W., C. E. Braun, J. R. Young, S. Wood, D. R. Mata, and T. W. Quinn. 1999. Molecular analysis of genetic variation among large- and small-bodied Sage-grouse using mitochondrial control-region sequences. Auk 116:819-824.

Kaplan, E. L., and P. Meier. 1958. Nonparametric estimation from incomplete observations. Journal of the American Statistical Association 53:457–481.

Knick, S. T., D. S. Dobkin, J. T. Rotenberry, M. A. Schroeder, W. M. Vander Haegen, and C. van Riper, III. 2003. Teetering on the edge or too late? Conservation and research issues for avifauna of sagebrush habitats. Condor 105:611–634.

Krager, R. 1977. Survey of sage grouse strutting ground complexes and seasonal use areas within the Piceance Basin Wildlife Habitat Area. Progress Report, Colorado Division of Wildlife, Denver, Colorado.

Mills, L. S., J. M. Scott, K. M. Strickler, and S. A. Temple. 2005. Ecology and management of small populations. Pages 691–713 *in* C. E. Braun, editor. Techniques for wildlife investigations and management. Sixth edition. The Wildlife Society, Bethesda, Maryland.

Oyler-McCance, S. J. 1999. Genetic and habitat factors underlying conservation strategies for Gunnison sage-grouse. Ph.D. Dissertation. Colorado State University, Fort Collins, Colorado.

Oyler-McCance, S. J., S. E. Taylor, and T. W. Quinn. 2005a. A multilocus population genetic survey of the greater sage-grouse across their range. Molecular Ecology 14:1293–1310.

Oyler-McCance, S. J., J. St. John, S. E. Taylor, and T. W. Quinn. 2005b. Population Genetics of Gunnison Sage-Grouse: Implications for Management. Journal of Wildlife Management 69:630-637.

Patterson, R. L. 1952. The sage grouse in Wyoming. Sage Books, Denver, Colorado, USA.

Pollock, K. H., S. R. Winterstein, C. M. Bunck, and P. D. Curtis. 1989. Survival analysis in telemetry studies: the staggered entry design. Journal of Wildlife Management 53:7–15.

Reardon, J. D. 1951. Identification of waterfowl nest predators. Journal of Wildlife Management 15:386 – 395.

Rich, T., and B. Altman. 2002. Under the sage grouse umbrella. Bird Conservation 14:10.

SAS Institute. 2003. SAS/STAT User's Guide, Release 9.1. SAS Institute, Incorporated, Cary, North Carolina.

Schneider, J. W., and C. E. Braun. 1991. Movements and habitat use by sage grouse, Eagle County, Colorado. Final Report, Colorado Division of Wildlife, Fort Collins, Colorado.

Schroeder, M. A., C. L. Aldridge, A. D. Apa, J. R. Bohne, C. E. Braun, S. D. Bunnell, J. W. Connelly, P. A. Deibert, S. C. Gardner, M. A. Hilliard, G. D. Kobriger, S. M. McAdam, C. W. McCarthy, J. J. McCarthy, D. L. Mitchell, E. V. Rickerson, and S. J. Stiver. 2004. Distribution of sage-grouse in North America. Condor 106: 363–376.

Springer, J. T. 1979. Some sources of bias and sampling error in radio triangulation. Journal of Wildlife Management 43:926–935.

Teidman, J. A. and C. Terwilliger. 1978. Phyto-edaphic classification of the Piceance Basin. Range Sciences Department, Range Science Series 31. Colorado State University, Forth Collins, Colorado.

Thompson, T. R. 2006. Dispersal ecology of greater sage-grouse in northwester Colorado: evidence from genetic and demographic data: 2006 Annual Progress Report. Colorado Division of Wildlife, Grand Junction, Colorado, USA.

Thompson, T. R. 2007. Dispersal ecology of greater sage-grouse in northwester Colorado: evidence from genetic and demographic data: 2007 Annual Progress Report. Colorado Division of Wildlife, Grand Junction, Colorado, USA.

Thompson, T. R., K.. P. Reese, and A. D. Apa. 2005. Dispersal ecology of greate sage-grouse in northwestern Colorado: Annual Report 2005. Colorado Division of Wildlife, Grand Junction, Colorado, USA.

Wakkinen, W. L., K. P. Reese, J. W. Connelly, and R. A. Fischer. 1992. An improved spotlighting technique for capturing sage grouse. Wildlife Society Bulletin 20:425–426.
Wallestad, R. O. 1975. Life history and habitat requirements of sage grouse in central Montana. Montana Fish and Game Department, Technical Bulletin, Helena, Montana, USA.
Zablan, M. A., C. E. Braun, and G. C. White. 2003. Estimation of greater sage-grouse survival in North Park, Colorado. Journal of Wildlife Management 67:144-154.
Zar, J. H. 1984. Biostatistical analysis, 2nd ed. Prentice-Hall, Inc. Englewood Cliffs, New Jersey, USA.

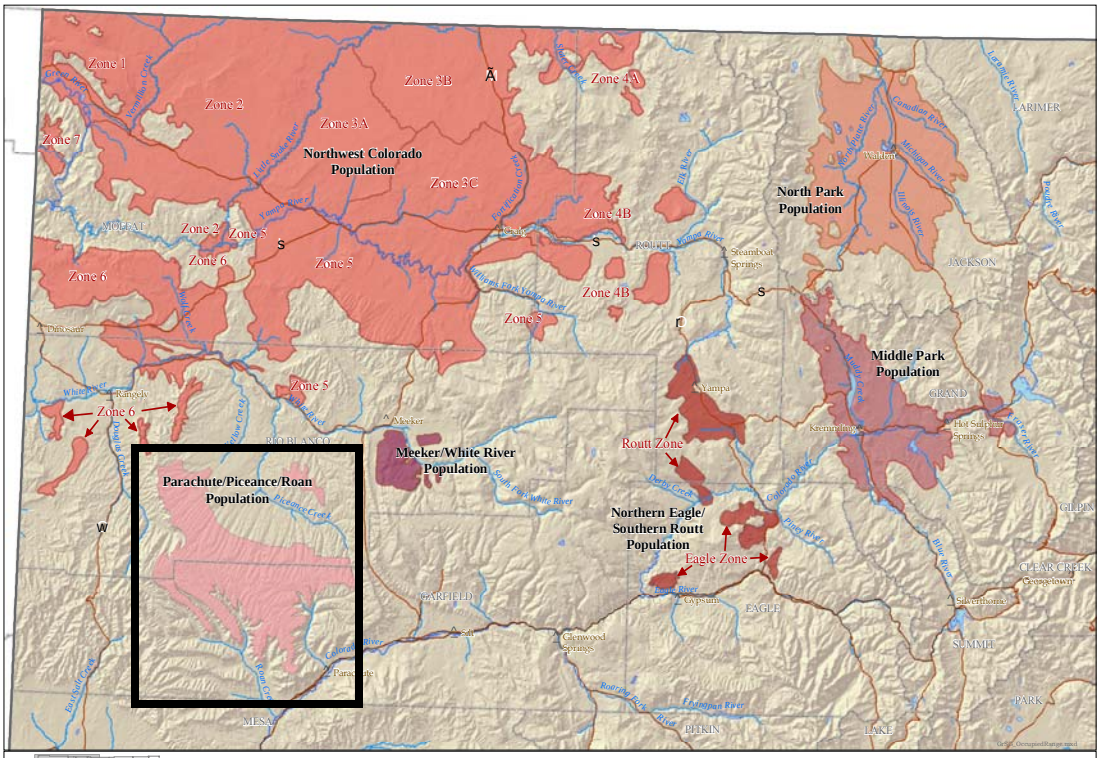

Figure 1. Location of the Parachute/Piceance/Roan (PPR) study area in relation to the overall statewide range of greater sage-grouse in northwestern Colorado, USA.

Table 1. Number, age, and gender of greater sage-grouse captured in the Parachute/Piceance/Roan (PPR) study area in west-central Colorado, USA, 2006 – 2008.

	Male		Female		
	Adult	**Yearling**	**Adult**	**Yearling**	**Juvenile**
			2006		
Spring	8	4	3	6	-
Fall	-	-	4	3	-
			2007		
Spring	-	-	14	14	-
Fall	-	-	10	-	6
			2008		
Spring	-	-	2	5	-
Total	**8**	**4**	**33**	**28**	**6**

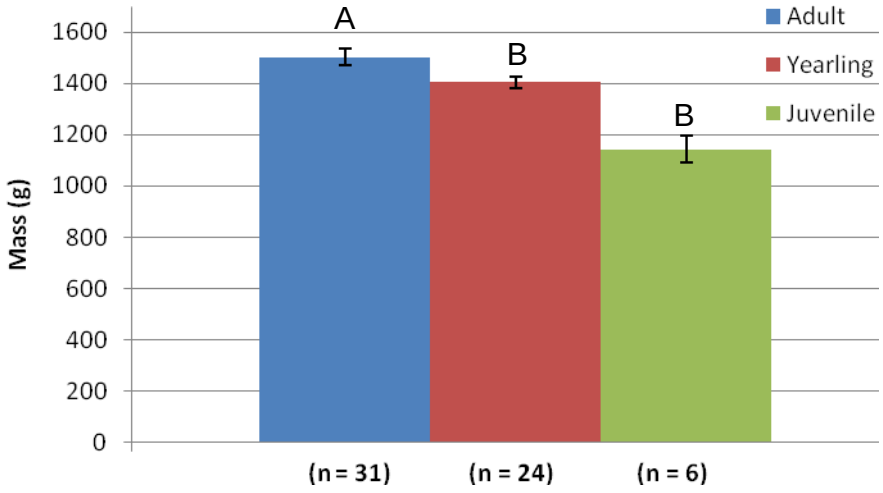

Figure 2. Mass ($\bar{x} \pm$ SE) of three age classes (adult, yearling, and juvenile) of greater sage-grouse captured in the in the spring and fall in the Parachute/Piceance/Roan (PPR) study area of west-central Colorado, USA, 2006 -2008. Means accompanied by the same letter are not significantly different (α = 0.05) (least square means analyzed and means reported).

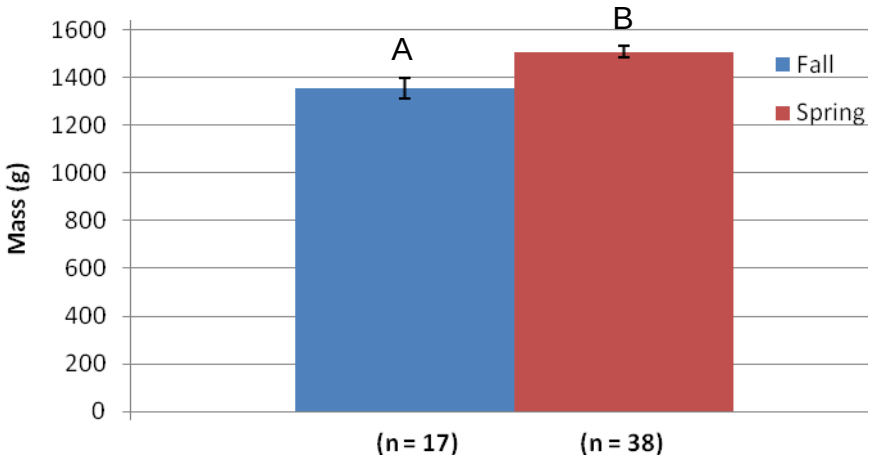

Figure 3. Mass ($\bar{x} \pm$ SE) of female greater sage-grouse captured in the in the spring and fall in the Parachute/Piceance/Roan (PPR) of west-central Colorado, USA, 2006 -2008. Means accompanied by the same letter are not significantly different ($\alpha = 0.05$) (least square means analyzed and means reported).

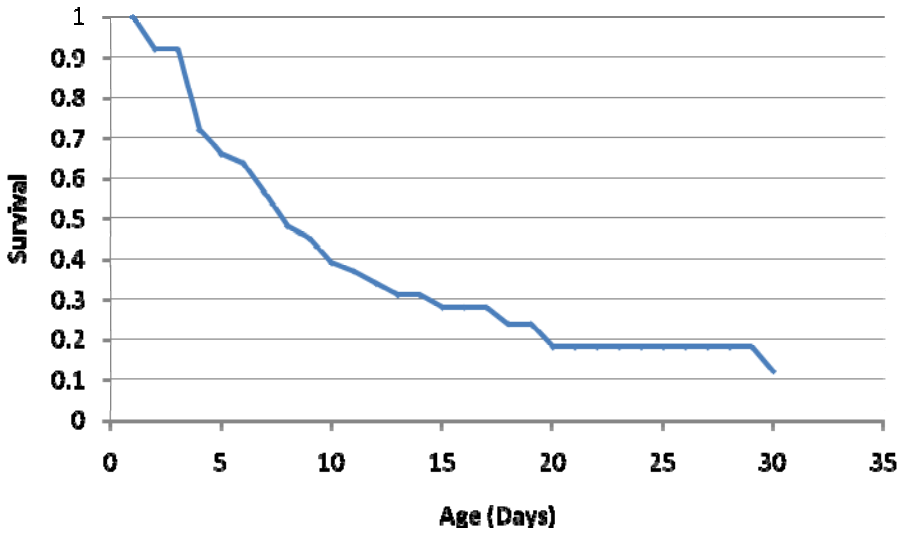

Figure 4. Greater sage-grouse chick survival from 1 – 30 days of age in the Parachute/Piceance/Roan (PPR) study area of west-central, Colorado, 2007.

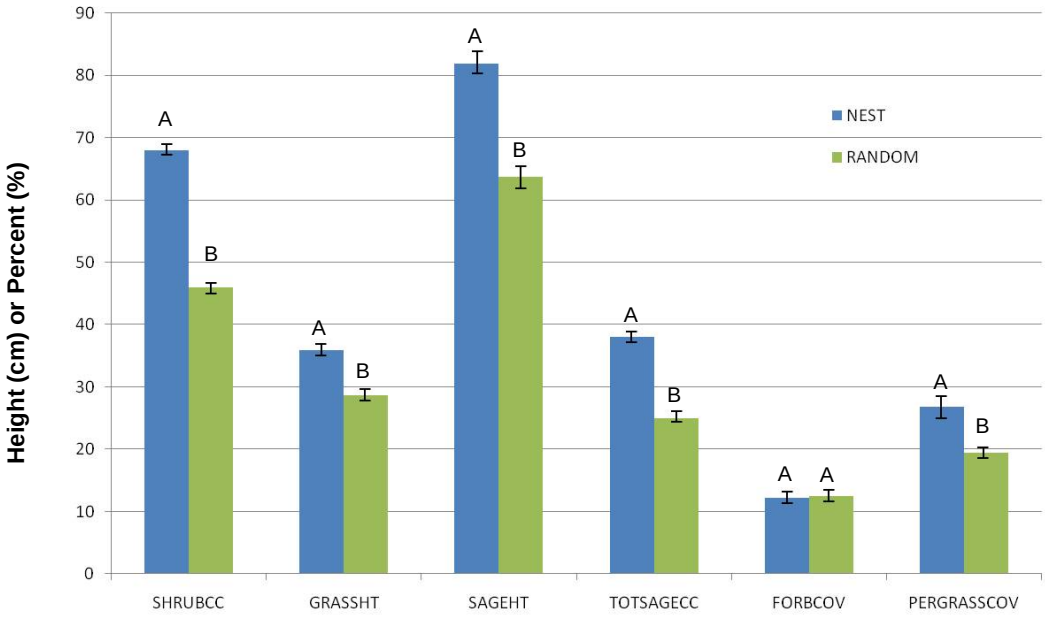

Figure 5. The percent ($\bar{x} \pm$ SE) of total shrub cover (SHRUBCC), big sagebrush cover (TOTSAGECC), forb cover (FORBCOV), and perennial grass cover (PERGRASSCOV) and height (cm) ($\bar{x} \pm$ SE) of understory perennial grass height (GRASSHT) and big sagebursh height (SAGEHT) at nest and random sites in the Parachute/Piceance/Roan (PPR) population of greater sage-grouse in west-central Colorado, USA, 2007). Like numbers are not different.

Table 2. Micro-habitat variables ($\bar{x} \pm$ SE) of total shrub cover (TOTSHRUBCC), grass height (GRASSHT), big sagebrush height (SAGEHT), total big sagebrush cover (TOTSAGECC), forb cover (FORBCOV), and perennial grass cover (PERGRASSCOV) at nest and random sites sampled in the Parachute/Piceance/Roan (PPR) study area in west-central Colorado, USA, 2006-2008.

	SITE TYPE				
	NEST		RANDOM		
VARIABLE	***n***	**Mean ± SE**	***n***	**Mean ± SE**	***P***
TOTSHRUBCC	37	67.7 ± 2.2	67	46.0 ± 2.4	< 0.0001
GRASSHT	37	35.9 ± 1.3	67	28.7 ± 0.9	< 0.0001
SAGEHT	37	81.8 ± 2.8	67	63.7 ± 3.0	< 0.0001
TOTSAGECC	37	37.6.± 2.1	67	24.8 ± 1.8	< 0.0001
FORBCOV[1,2]	37	12.2 ± 1.1	67	12.5 ± 0.8	0.9426
PERGRASSCOV[1,2]	37	26.8 ± 2.5	67	19.4 ± 1.6	0.0053

[1]Analysis conducted on transformed values, untransformed means reported.
[2]This value is a mean of midpoints for Daubenmire categories. FORBCOV mean is in Daubenmire category of 10 – 20% at nest sites and random sites. PERGRASSCOV is in Daubenmire category 20 – 30% for nest sites and 10 – 20% for random sites.

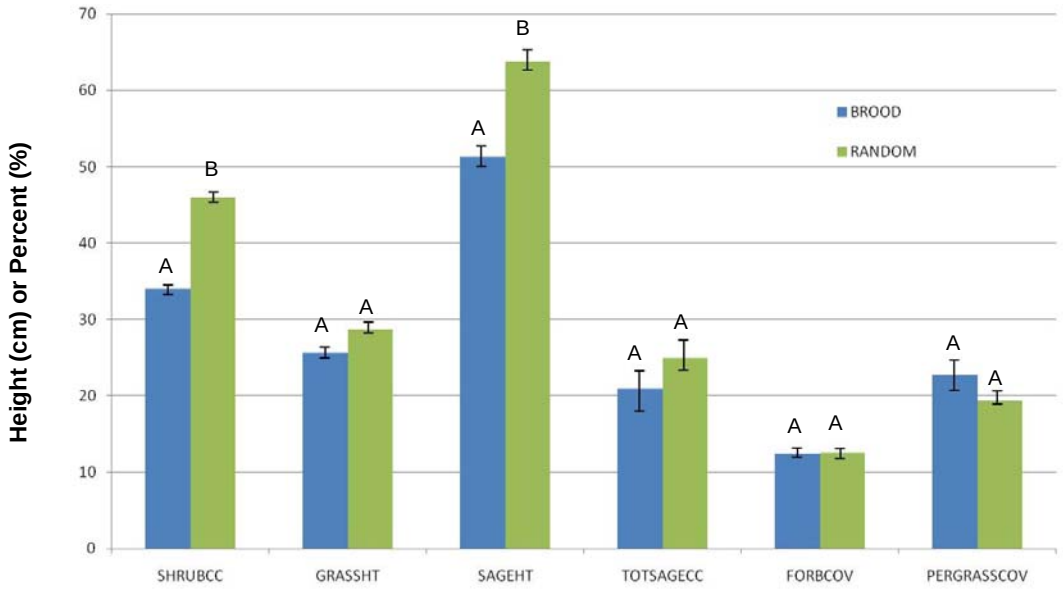

Figure 6. The percent ($\bar{x} \pm$ SE) of total shrub cover (SHRUBCC), big sagebrush cover (TOTSAGECC), forb cover (FORBCOV), and perennial grass cover (PERGRASSCOV) and height (cm) ($\bar{x} \pm$ SE) of understory perennial grass height (GRASSHT) and big sagebursh height (SAGEHT) at brood-rearing and and random sites in the Parachute/Piceance/Roan (PPR) population of greater sage-grouse in west-central Colorado, USA, 2007. Like numbers are not different.

Table 3. Micro-habitat variables ($\bar{x} \pm$ SE) of total shrub cover (TOTSHRUBCC), grass height (GRASSHT), big sagebrush height (SAGEHT), total big sagebrush cover (TOTSAGECC), forb cover (FORBCOV), and perennial grass cover (PERGRASSCOV) at brood-rearing and random sites sampled in the Parachute/Piceance/Roan (PPR) study area in west-central Colorado, USA, 2006-2008.

	SITE TYPE				
	BROOD		RANDOM		
VARIABLE	***n***	**Mean ± SE**	***n***	**Mean ± SE**	***P***
TOTSHRUBCC	29	33.6 ± 2.9	67	46.0 ± 2.4	0.0044
GRASSHT	29	25.7 ± 1.3	67	28.7 ± 0.9	0.0565
SAGEHT	29	51.3 ± 2.8	67	63.7 ± 3.0	0.0179
TOTSAGECC	29	20.9.± 2.5	67	24.8 ± 1.8	0.2319
FORBCOV[1,2]	29	12.4 ± 1.1	67	12.5 ± 0.8	0.8322
PERGRASSCOV[1,2]	29	22.7 ± 2.5	67	19.4 ± 1.6	0.1470

[1]Analysis conducted on transformed values, untransformed means reported.
[2]This value is a mean of midpoints for a Daubenmire category. FORBCOV is in the Daubenmire category of 10 – 20% for brood sites and random sites. PERGRASSCOV is in the Daubenmire category of 20 -30% for brood sites and 10 – 20% for random sites.

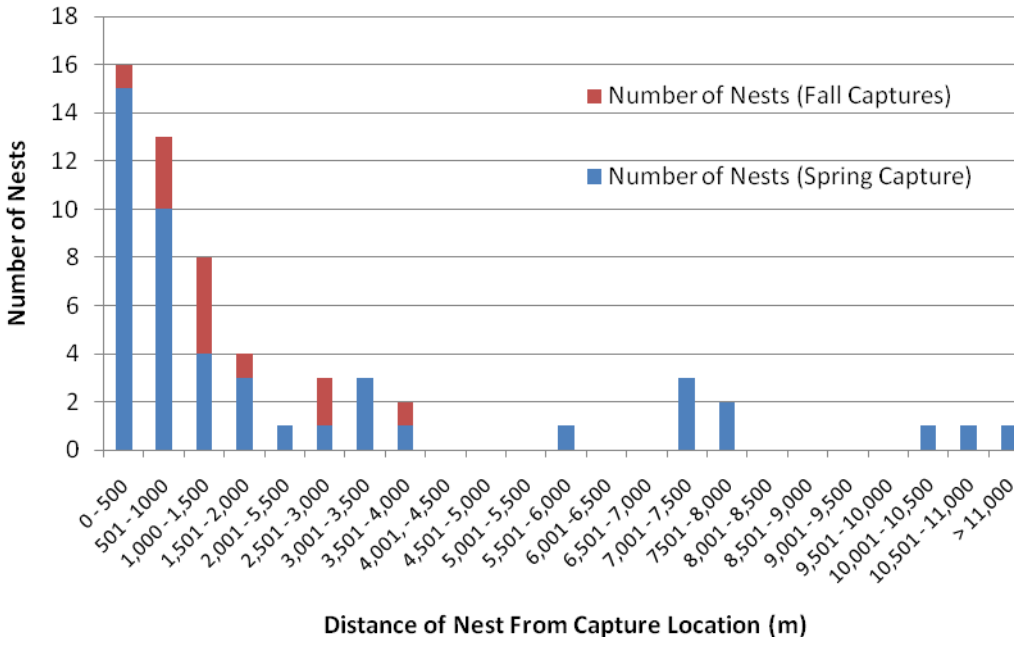

Figure 6. The number of nests and frequency of distribution for spring and fall captured locations to nest sites by female greater sage-grouse in the Parachute/Piceance/Roan (PPR) population in west-central Colorado, USA, 2006 – 2008.

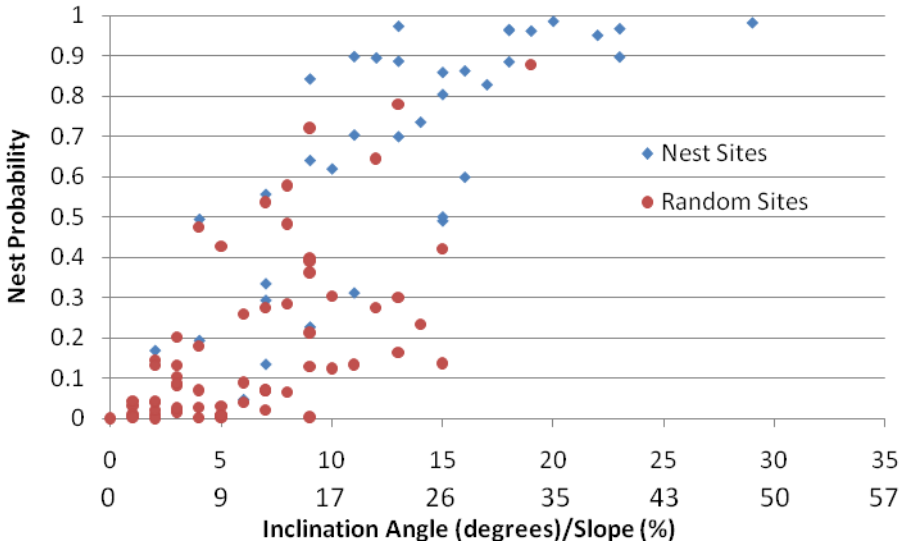

Figure 7. The estimated probability of a greater sage-grouse nest versus a random site when slope is entered into ghe logistic regression in the Parachute/Pieceance/Roan (PPR) population in west-central Colorado, USA, 2007.

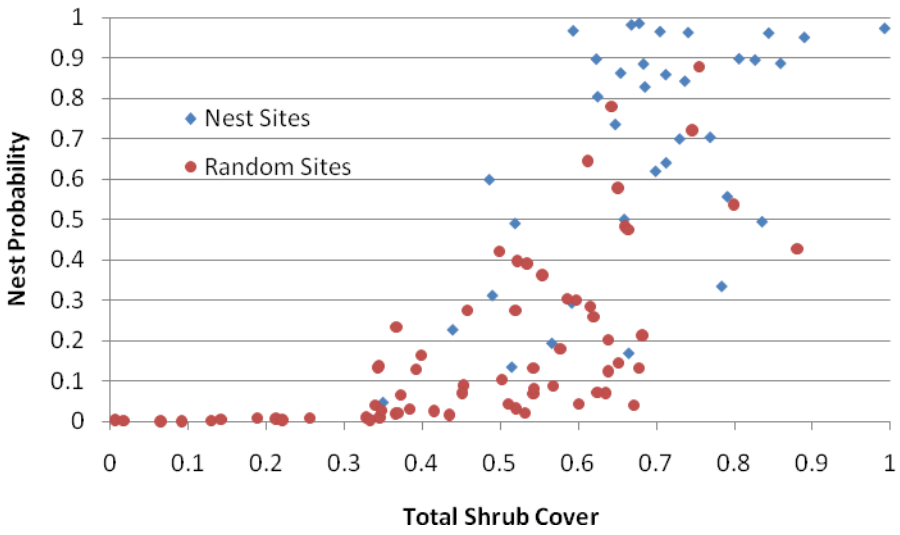

Figure 8. The estimated probability of a greater sage-grouse nest versus a random site when total shrub cover is entered into ghe logistic regression in the Parachute/Pieceance/Roan (PPR) population in west-central Colorado, USA, 2007.

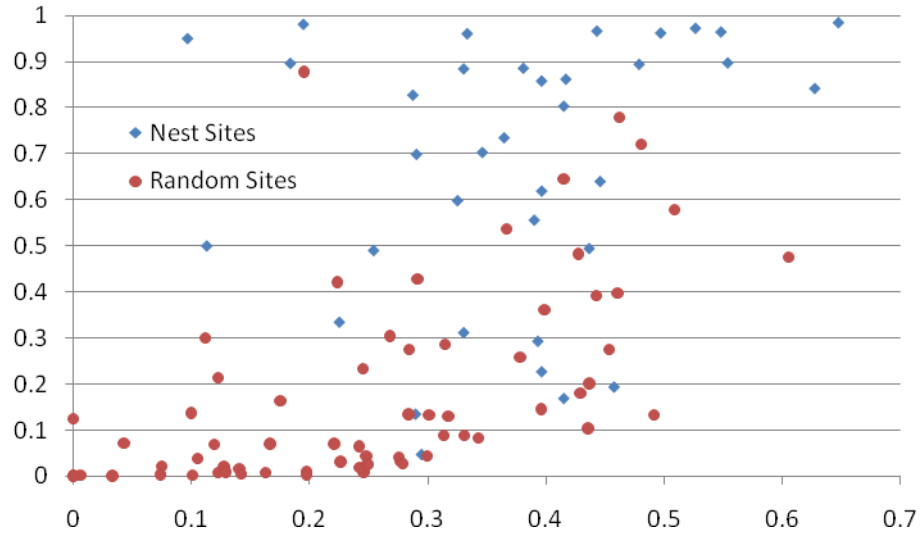

Figure 9. The estimated probability of a greater sage-grouse nest versus a random site when big sagebrush coverr is entered into ghe logistic regression in the Parachute/Pieceance/Roan (PPR) population in west-central Colorado, USA, 2007.

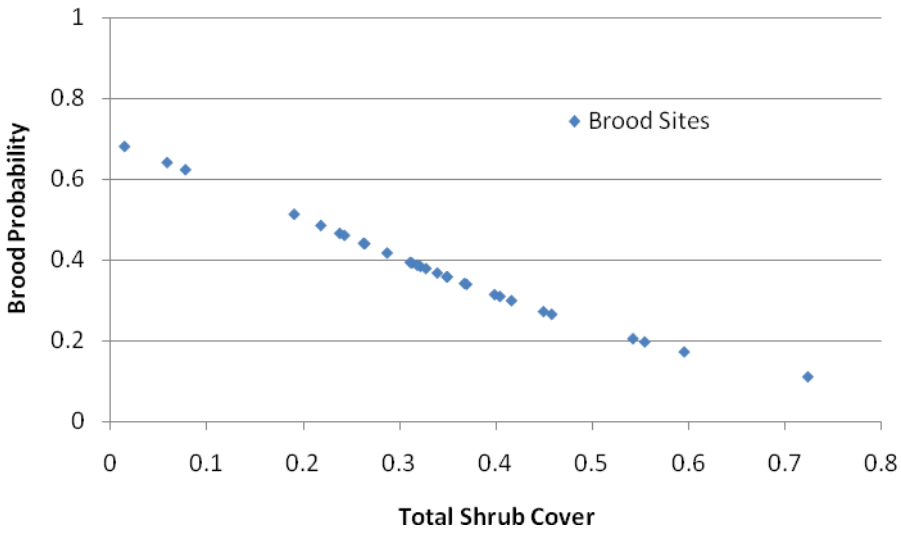

Figure10. The estimated probability of a greater sage-grouse brood sites when total shrub cover is entered into ghe logistic regression in the Parachute/Pieceance/Roan (PPR) population in west-central Colorado, USA, 2007.

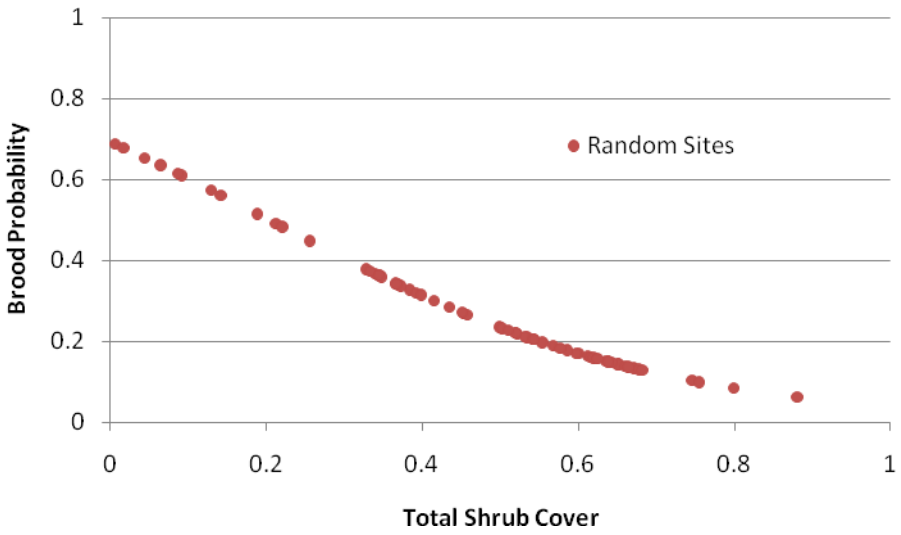

Figure11. The estimated probability of a random sites when total shrub cover is entered into ghe logistic regression in the Parachute/Pieceance/Roan (PPR) population in west-central Colorado, USA, 2007.

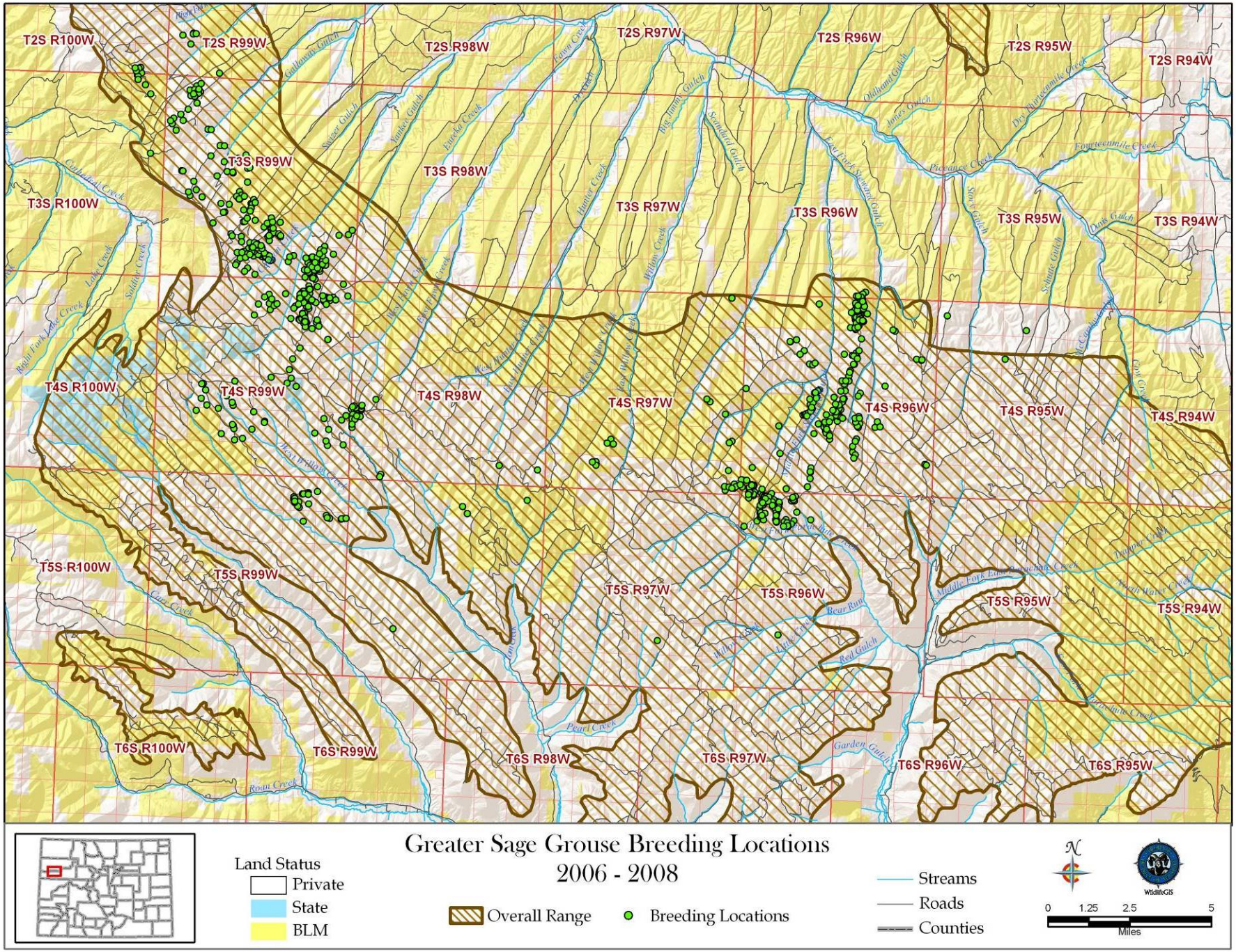

Figure 12. Breeding habitat (April – June) locations of male and female greater sage-grouse in the Parachute/Piceance/Roan (PPR) population in west-central Colorado, USA, 2006-2008).

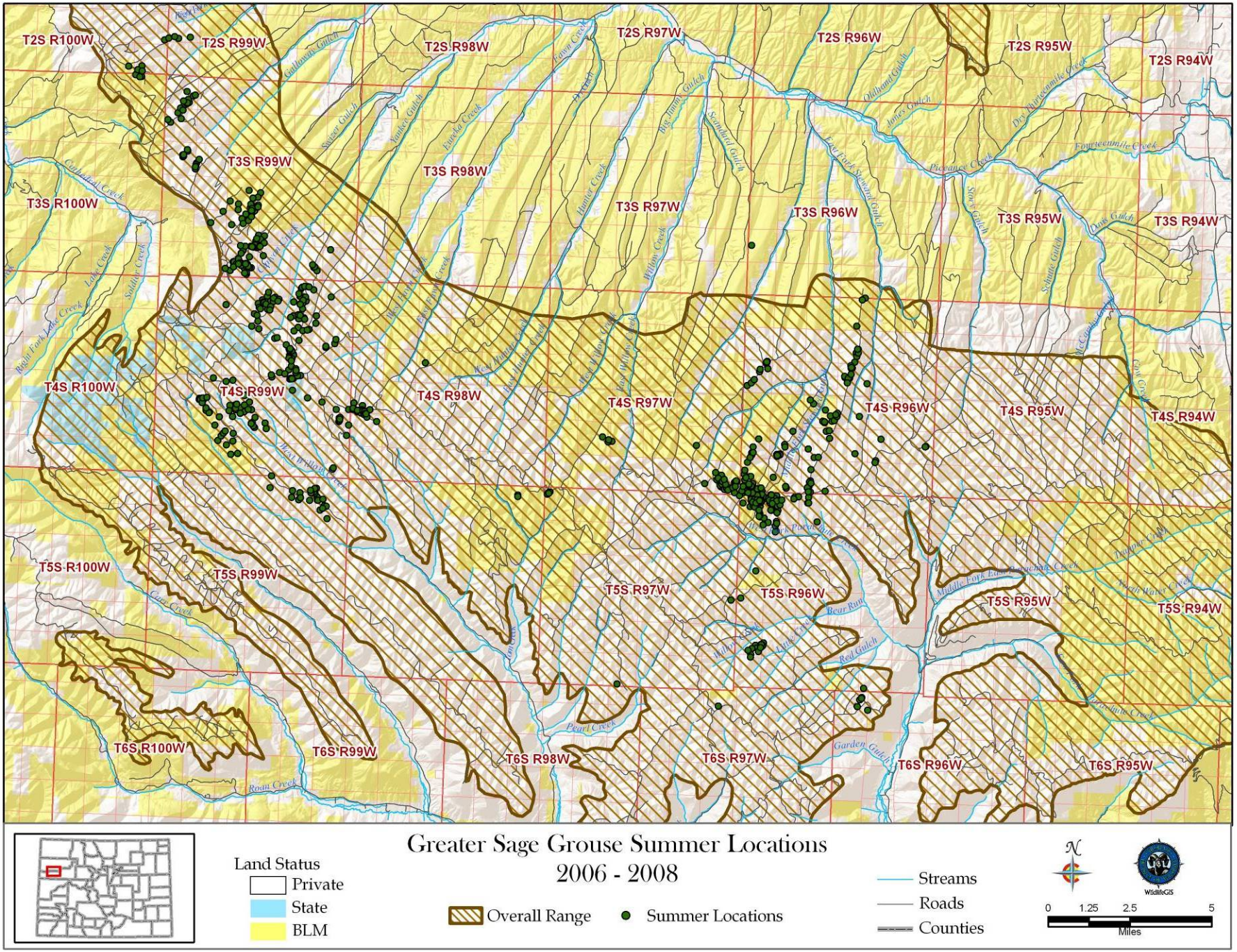

Figure 13. Summer habitat (July – September) locations of male and female greater sage-grouse in the Parachute/Piceance/Roan (PPR) population in west-central Colorado, USA, 2006-2008).

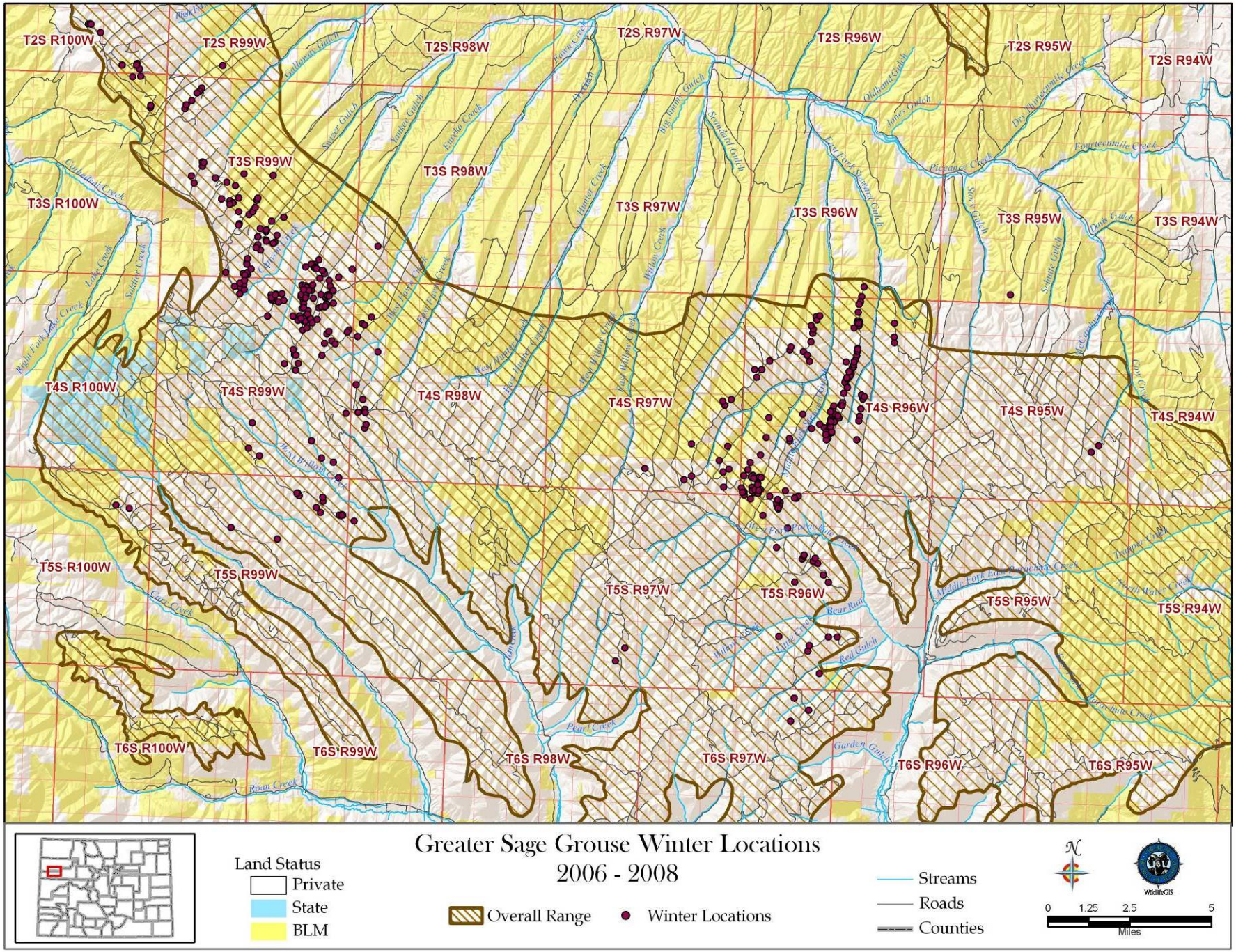

Figure 14. Winter habitat (October – March) locations of male and female greater sage-grouse in the Parachute/Piceance/Roan (PPR) population in west-central Colorado, USA, 2006-2008).

Table 4. Scientific and common name of shrubs encountered at nest, brood-rearing, and random sites in the Parachute/Piceance/Roan (PPR) study area in west-central Colorado, USA, 2007.

Scientific Name	Common Name
Amelanchier alnifolia	Serviceberry
Artemisia fridgida	Fringed Sage
Artemisia ludoviciana	Prairie Sagewort
Artemisia tridentata	Big Sagebrush
Artemisia tridentata vaseyana	Mountain Big Sagebrush
Ceanothus martinii	Martin's Ceanothus
Cercocarpus montanus	Alderleaf Mountain Mahogany
Chrysothamnus spp.	Rabbitbrush
Chrysothamnus nauseosus	Rubber Rabbitbrush
Chrysothamnus parryi	Parry's Rabbitbrush
Chrysothamnus vicidiflorus	Green Rabbitbrush
Gutierrezia sarothrae	Broom Snakeweed
Juniperus monosperma	Oneseed Juniper
Mahonia repens	Creeping Barberry
Pinus edulis	Twoneedle Pinyon
Prunus virginiana	Chokecherry
Purshia tridentata	Antelope Bitterbrush
Quercus gambelii	Gamble Oak
Ribes cereum	Wax Current
Ribes spp.	Current
Symphorocarpus albus	Snowberry
Tetradymia canescens	Spineless Horsebrush

APPENDIX A

Genetic Make-up of the Parachute/Piceance/Roan Population of Greater Sage-grouse

FINAL REPORT

Sara J. Oyler-McCance
Rocky Mountain Center for Conservation Genetics and Systematics
Department of Biological Sciences
University of Denver
Denver, CO 80208 USA

Introduction

The Parachute/Piceance/Roan (PPR) population of Greater Sage-grouse (*Centrocercus urophasianus*) is one of several small, isolated populations of Sage-grouse (*Centrocercus spp.*) in the state of Colorado. Habitat for Greater Sage-grouse in this area is naturally fragmented and is undergoing rapid oil and gas development. For this reason, it is important to identify baseline information on the genetic characteristics of this population, as it will be used to assess current population status and to help identify future management strategies for this population.

Previous genetic studies (Kahn et al. 1999, Oyler-McCance et al. 2005a) have characterized the genetic make-up of five Greater Sage-grouse populations in Colorado using mitochondrial DNA (mtDNA) sequence data and data from nuclear microsatellites. The populations used in these studies included North Park, Middle Park, Eagle, Cold Springs, and Blue Mountain. The objective of this study was to characterize the PPR population using the same mtDNA and nuclear markers as have been used previously (Kahn et al. 1999, Oyler-McCance et al. 2005a) so that a direct comparison could be made between PPR and the five other characterized Greater Sage-grouse populations in Colorado.

Materials and Methods

Tissue collection and DNA extraction

Seventy blood and feather samples were collected from the PPR population during various research projects. DNA was extracted from blood samples using the GenomicPrep Blood DNA Isolation Kit (Amersham Biosciences) using the modifications of Oyler-McCance et al. (2005b).

Mitochondrial sequencing

A 146 base pair portion of hypervariable control region I was amplified using the Polymerase Chain Reaction (PCR) and sequenced using a dye terminator cycle sequencing reaction (Beckman Coulter CEQ8000) as described by Benedict et al. (2003). This region was used because it was known to contain approximately 92% of the variable sites in a larger 380 base pair region spanning control region I (Kahn et al. 1999).

Microsatellite fragment analysis

Seven nuclear microsatellite loci (LLST1, SGCA5, SGCA9, SGCA11, LLSD3, LLSD8, and ADL0230) were screened using the methods described in Oyler-McCance et al. (2005b). Briefly, PCR reactions were performed using a dye-labeled forward primer and amplified products were then run on the CEQ 8000 Genetic Analysis System (Beckman Coulter). One locus, SGCA11, was dropped due to difficulty comparing it to previous data.

Data analysis

All mtDNA sequences were edited and aligned using Sequencher Version 4.1.4 and haplotypes were identified. Measures of genetic diversity were calculated in Arlequin 2.000 (Schneider et al. 2000) as were pairwise population F_{ST} tests. Populations were deemed to be significantly different using a Bonferroni corrected P value of 0.003. Pairwise F_{ST} values were then used to construct a neighbor-joining network in PHYLIP 3.57 (Felsenstein 1989) that was viewed using the program TREEVIEW (Page 1996).

The mean number of alleles for each population were calculated and the observed and expected levels of heterozygosity were estimated using Genalex (Peakall and Smouse 2006). Microsatellite loci were tested (by population) for departures from Hardy-Weinberg equilibrium (Guo and Thompson 1992) using the computer program Arlequin 2.000 (Schneider et al. 2000). Pairwise population genetic distances (R_{ST}) were calculated in Arlequin 2.000 (Schneider et al. 2000). Populations were deemed to be significantly different using a Bonferroni corrected P value of 0.003. Pairwise R_{ST} values were then used to construct a neighbor-joining network in PHYLIP 3.57 (Felsenstein 1989) that was viewed using the program TREEVIEW (Page 1996).

Population structure was also examined using STRUCTURE 2.00 software (Pritchard et al. 2000). In this program, individuals are grouped into clusters without regard to the assigned population

using a model-based clustering analysis. The number of "populations" (K) was initially estimated by conducting five independent runs each of K = 1- 10 with 100,000 Markov Chain Monte Carlo (MCMC) repetitions and a 100,000 burnin period using the model with admixture, correlated allele frequencies, and no prior information. An additional set of five independent runs was then conducted with K= 1 - 5 with 500,000 MCMC repetitions and a 500,000 burnin period using the above model.

Results

Mitochondrial Sequence Analysis

Of the 65 individuals sequenced, 8 different haplotypes were found (Table 1, Fig. 1). Of those 8 haplotypes, 5 were found elsewhere in Colorado. Three of those haplotypes (A, B, and C) were common in Colorado, found in at least 4 of the 5 other populations. Haplotypes E and H are also shared with Colorado populations (Table 1) yet with three or less populations. Haplotype W, which occurs in PPR and not elsewhere in Colorado, is found in Wayne and Rich counties in Utah and also in the Strawberry Valley population in Utah (Oyler-McCance et al. 2005a). Haplotype EU is also found in the Rawlins, Wyoming population (Oyler-McCance et al. 2005a). A new haplotype (labeled New3) was found in PPR and is not found elsewhere among Greater Sage-grouse (Oyler-McCance et al. 2005a). This haplotype is very closely related to haplotype B with only one substitution differing between them.

Levels of genetic diversity in PPR were similar to other populations in Colorado (Table 2). PPR had 8 haplotypes, which is well within the range of the other Colorado populations with the number of haplotypes ranging from 5 in Eagle to 11 in Blue Mountain. In terms of haplotype diversity, PPR also falls well within the range of the other populations (Table 2).

Pairwise population F_{ST} tests revealed that PPR was significantly different from three other Colorado populations (Blue Mountain, Cold Springs, and Eagle). The only other significant difference in Colorado was between Blue Mountain and Eagle. This metric, however, is influenced by comparisons using widely different sample sizes. It is possible that there are more significant comparisons with PPR due to the unusually high sample size in that population. The neighbor-joining network (Fig. 2) showed that PPR was associated most closely to North Park and did not appear to be more different than other populations in Colorado.

Microsatellite Analysis

Tests for departures from Hardy-Weinberg Equilibrium (HWE) within PPR showed that no locus was out of HWE. Levels of genetic diversity in PPR, measured using microsatellite data, were comparable to other populations in Colorado. The mean number of alleles per locus in PPR was 5.67 (Table 4), which again is well within the range of other populations in Colorado with a low of 5.33 in Eagle and a high of 5.83 in Cold Springs and North Park (Oyler-McCance et al. 2005a). The mean observed heterozygosity in PPR was slightly lower (0.55) than other values in Colorado, which ranged from 0.61 in Cold Springs to 0.69 in Middle Park.

Pairwise population R_{ST} significance tests revealed that most populations in Colorado are not significantly different. PPR was found to be significantly different from Blue Mountain and Cold Springs, however. Cold Springs was shown to be the most different as it was significantly different from PPR, Blue Mountain, Eagle, and Middle Park. The neighbor-joining network (Fig. 3) showed that PPR was most closely related to Middle Park, followed by Eagle and North Park.

The STRUCTURE analysis revealed that the most appropriate number of populations (K) given the data was 1. This suggests that there is little genetic structure among populations.

Discussion

This analysis of the PPR population compared with 5 other Greater Sage-grouse populations in Colorado revealed that the genetic make-up of PPR is generally consistent with the other 5 populations. Using mtDNA sequence data, 5 of the 8 haplotypes found in PPR (66% of the PPR birds) were also found

in the other populations in Colorado (Table 1, Fig 1.). Of the three PPR haplotypes not found in Colorado, 2 (EU and W) were found in the neighboring states of Utah and Wyoming. One haplotype was unique to PPR (New3) and at relatively high frequency (20%). Two other Colorado populations (Blue Mountain and Cold Springs) each also had a unique haplotype representing 10 and 8% of the populations respectively (Oyler-McCance et al. 2005a). The PPR population, had a much higher sample size (65 compared to ~ 20 in the other populations) and the sampling method was different (trapped birds in PPR vs. hunter killed birds in the rest of the Colorado birds), which may influence the potential for relatedness among samples. Additionally, the PPR population did have similar levels of genetic diversity (both in the number of haplotypes and in haplotypes diversity) as the other Colorado populations (Table 2) yet again, a higher sample size likely resulted in more haplotypes being identified. Nonetheless, it appears that the PPR population does not suffer from low diversity and appears to have diversity levels that are comparable to the other Colorado populations. The mtDNA neighbor-joining network (Fig. 2), which was constructed using F_{ST} genetic distances among populations, suggests that PPR is more closely related to North Park, Cold Springs, and Blue Mountain, than to Middle Park and Eagle. The fact that PPR is not shown to have branch lengths longer than the other Colorado populations suggests that it is not genetically distinct from all other Colorado Greater Sage-grouse populations.

The microsatellite data are relatively concordant with that of the mtDNA data. The STRUCTURE analysis found that the most appropriate number of discrete genetic clusters (K) was 1 given the data from these 6 populations, suggesting that there was little genetic structure within the data. Pairwise population R_{ST} tests (Table 5), based on allele frequencies of populations, revealed a few significant differences among populations yet these differences were primarily between Cold Springs and the other populations. This finding is highlighted with the microsatellite neighbor-joining network (Fig. 3) that shows Cold Springs as the most genetically distinct population. This network suggests that PPR is more closely related to Middle Park and Eagle, contrary to the network built with mtDNA data. This discrepancy is likely due to the different patterns of inheritance of these two types of genetic markers (maternal vs. biparental). An additional factor that could lead to minor differences between the two data sets has to do with the number of loci sampled (sampling error). While the mitochondrial genome represents one locus, multiple sites were sampled in the nuclear genome. Levels of genetic diversity in PPR (Table 4) were again similar to what had been previously been reported for populations in Colorado (Oyler-McCance et al. 2005a). The levels of mean observed heterozygosity in PPR were the lowest reported in Colorado (Table 4) yet the values are only slightly lower than those reported elsewhere (0.55 as opposed to 0.61-0.69). This could be due to a number of factors including smaller population sizes, increased fragmentation among sagebrush habitat resulting in sampled birds being more related, or merely due to the different sampling method used in this study (trapped birds vs. hunter killed birds).

In summary, the Greater Sage-grouse in PPR do not appear to be substantially different from other Greater Sage-grouse sampled in Colorado. There is some level of uniqueness (as represented by the new haplotype found in 20% of the PPR birds) yet this is not unusual as both Cold Springs and Blue Mountain also contained haplotypes that were unique to that particular population. Additionally, the levels of genetic diversity in PPR do appear to be comparable to other populations although they were reported to have the lowest levels of observed heterozygosity levels.

Literature Cited

Benedict, N. G., S. J. Oyler-McCance, S. E. Taylor, C. E. Braun, and T. W. Quinn. 2003. Evaluation of the eastern (*Centrocercus urophasianus urophasianus*) and western (Centr*ocercus urophasianus phaios*) subspecies of Sage-grouse using mitochondrial control-region sequence data. Conservation Genetics 4:301-310.

Felsenstein, J. 1989. PHYLIP- Phylogeny Inference Package (Version 3.2). Cladistics 5:164-166.

Guo, S. W., and E. A. Thompson. 1992. Performing the exact test of Hardy-Weinberg proportions for multiple alleles. Biometrics 48:361-372.

Kahn, N. W., C. E. Braun, J. R. Young, S. Wood, D. R. Mata, and T. W. Quinn. 1999. Molecular analysis of genetic variation among large- and small-bodied Sage-grouse using mitochondrial control-region sequences. Auk 116:819-824.

Oyler-McCance, S. J., S. E. Taylor, and T. W. Quinn. 2005a. A Multilocus Genetic Survey of Greater Sage-Grouse Across Their Range. Molecular Ecology 14:1293-1310.

Oyler-McCance, S. J., J. St. John, S. E. Taylor, and T. W. Quinn. 2005b. Population Genetics of Gunnison Sage-Grouse: Implications for Management. Journal of Wildlife Management 69:630-637.

Page, R. D. M. 1996. TREEVIEW: An application to display phylogenetic trees on personal computers. Computer Applications in the Biosciences 12: 357-358.

Peakall, R..and P. E. Smouse. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6:288-295.

Pritchard, J. K., M. Stephens, and P. J. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155: 945-959.

Schneider S., D. Roessli, and L. Excoffier. 2000. Arlequin version 2.000: A software for population genetics data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Table 1. Sampling locations and mtDNA haplotype frequencies of Sage-grouse in Colorado (from Kahn et al. 1999)

		Haplotypes																		
Location	*N*	A	B	C	D	E	H	L	S	W	X	Z	AA	AC	AD	AE	AF	AL	AM	EU	New3
PPR	65	1	10	13		6	13			1										8	13
Blue Mountain	21	1	8	1	1				1			3	1	1	1	2	1				
Cold Springs	25	3	7	10	1			2				1		1							
Eagle	26	2	2	15	4		3														
Middle Park	21		7	9	2	1	1											1			
North Park	23	4	5	6	3	2	1				1								1		

Table 2. Mitochondrial DNA genetic diversity measures of Greater Sage-grouse populations in Colorado. Standard errors are in parentheses.

Population	Sample size	Number of Haplotypes	Haplotype Diversity (SE)
PPR	65	8	0.85 (0.01)
Blue Mountain	21	11	0.85 (0.07)
Cold Springs	25	7	0.77 (0.06)
Eagle	26	5	0.64 (0.09)
Middle Park	21	6	0.72 (0.07)
North Park	23	8	0.86 (0.04)

Table 3. Pairwise population F_{ST} significance tests. F_{ST} values in bold represent significant differences using a Bonferroni correct P value of 0.003.

	Population				
	PPR	Blue Mountain	Cold Springs	Eagle	Middle Park
Blue Mountain	**0.09110**				
Cold Springs	**0.07643**	0.06103			
Eagle	**0.11458**	**0.20377**	0.03766		
Middle Park	0.07123	0.07353	-0.01906	0.03400	
North Park	0.04689	0.03997	-0.00657	0.05395	0.00509

Table 4. Microsatellite genetic diversity measures of Greater Sage-grouse populations in Colorado. Standard deviations are in parentheses.

Population	Sample size	Mean # of alleles per locus	Mean observed heterozygosity	Mean expected heterozygosity
PPR	70	5.67	0.55 (0.17)	0.61 (0.20)
Blue Mountain	25	5.50	0.68 (0.22)	0.65 (0.23)
Cold Springs	30	5.83	0.61 (0.13)	0.64 (0.17)
Eagle	26	5.33	0.66 (0.24)	0.67 (0.17)
Middle Park	21	5.50	0.69 (0.10)	0.66(0.15)
North Park	22	5.83	0.66 (0.15)	0.61(0.15)

Table 5. Pairwise population R_{ST} significance tests. R_{ST} values in bold represent significant differences using a Bonferroni correct P value of 0.003.

	Population				
	PPR	Blue Mountain	Cold Springs	Eagle	Middle Park
Blue Mountain	**0.09560**				
Cold Springs	**0.21178**	**0.08328**			
Eagle	0.01375	0.03431	**0.13454**		
Middle Park	-0.03364	0.01800	**0.11034**	-0.01182	
North Park	-0.01793	-0.00044	0.06848	0.00119	-0.01986

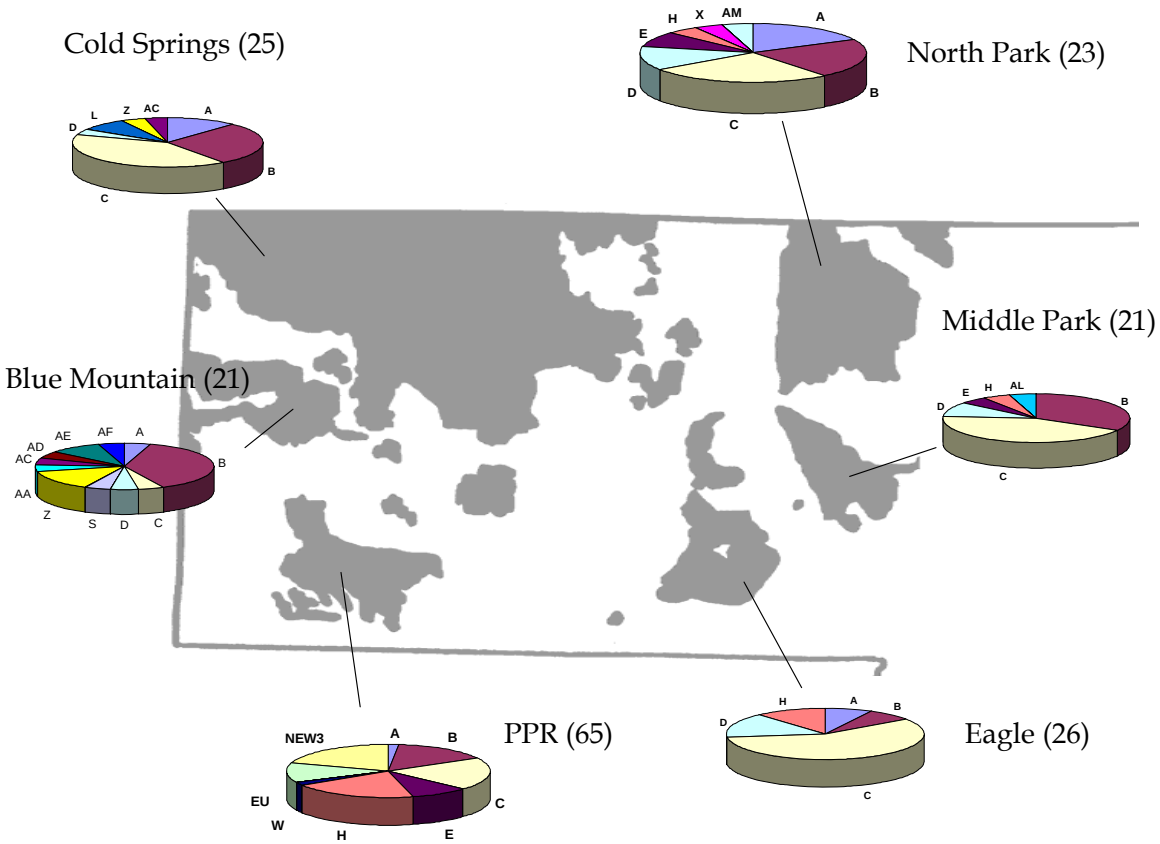

Figure 1. Distribution of mtDNA haplotypes found in PPR and 5 other previously studied populations of Greater Sage-grouse in northern Colorado (Kahn et al. 1999).

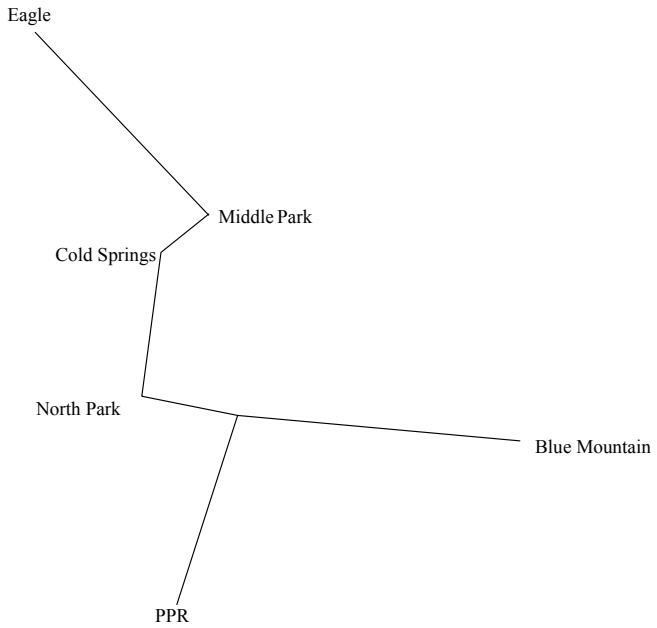

Figure 2. Mitochondrial DNA neighbor-joining network constructed using pairwise F_{ST} values as a genetic distance.

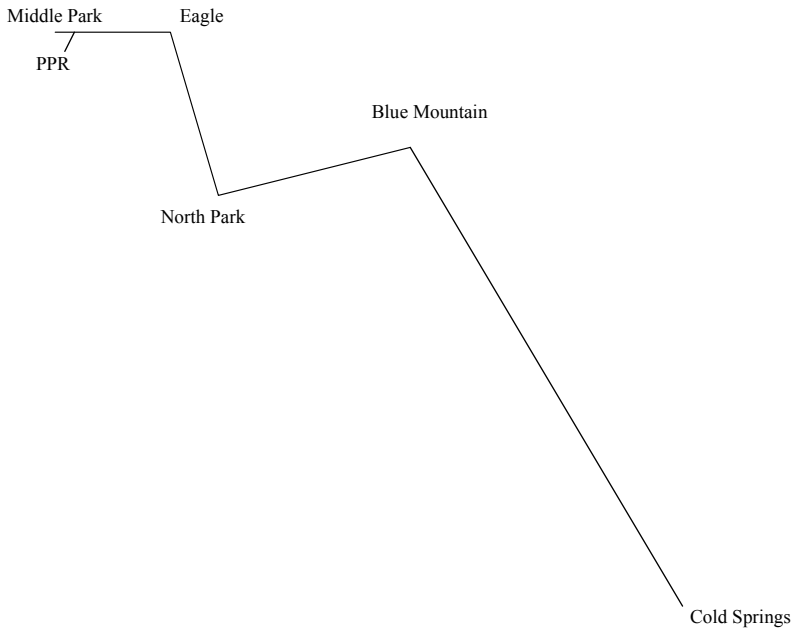

Figure 3. Microsatellite neighbor-joining network constructed using pairwise R_{ST} values as a genetic distance.

Appendix 1. Microsatellite alleles across 6 loci for PPR and the 5 other Greater Sage-grouse populations in Colorado included in this study.

		Loci											
Individual	Population	L1		S5		S9		L3		L8		ADL230	
PI 1	PI	143	146	265	275	322	332	137	145	139	139	109	111
PI 2	PI	143	143	259	265	318	332	137	137	139	139	107	113
PI 3	PI	143	143	259	265	318	318	137	137	139	139	109	113
PI 4	PI	143	143	273	275	340	340	137	137	139	139	105	111
PI 5	PI	143	146	263	265	318	340	137	137	139	139	105	111
PI 6	PI	0	0	0	0	0	0	0	0	139	139	109	109
PI 7	PI	143	143	265	275	328	332	0	0	145	145	105	107
PI 8	PI	0	0	265	273	0	0	137	137	139	139	107	111
PI 9	PI	143	143	261	265	326	342	137	145	139	139	111	113
PI 10	PI	143	143	259	275	326	342	137	145	139	145	111	113
PI 11	PI	0	0	0	0	0	0	0	0	139	139	0	0
PI 12	PI	146	146	265	265	0	0	0	0	139	139	105	111
PI 13	PI	143	146	259	259	318	332	137	145	139	145	105	107
PI 14	PI	143	143	261	265	340	342	137	141	139	139	105	111
PI 15	PI	143	146	265	265	318	364	0	0	139	139	105	113
PI 16	PI	0	0	265	265	338	364	0	0	139	139	109	109
PI 17	PI	143	143	265	275	326	340	0	0	139	145	105	113
PI 18	PI	143	146	265	265	318	342	137	147	139	145	109	109
PI 19	PI	143	143	265	275	0	0	137	145	139	139	109	109
PI 20	PI	143	143	255	275	340	364	137	141	139	145	105	105
PI 21	PI	143	143	259	265	318	318	0	0	139	139	111	113
PI 22	PI	143	143	265	271	332	366	137	141	139	139	0	0
PI 23	PI	143	143	259	265	332	366	137	137	139	139	105	109
PI 24	PI	143	143	261	275	318	338	137	141	139	139	105	107
PI 25	PI	143	146	261	275	0	0	0	0	139	159	111	113
PI 26	PI	143	146	265	275	0	0	137	137	139	159	107	107
PI 27	PI	143	146	265	271	318	358	145	145	139	159	109	109
PI 28	PI	143	146	265	271	318	318	0	0	139	159	109	109
PI 29	PI	143	143	271	275	318	360	137	145	139	159	109	109
PI 30	PI	143	146	265	271	318	322	0	0	139	139	109	113
PI 31	PI	143	143	265	265	0	0	137	137	139	139	105	105
PI 32	PI	0	0	261	273	318	332	0	0	0	0	109	109

PI 33	PI	143	143	259	261	318	340	137	137	145	145	109	109
PI 34	PI	143	143	259	273	318	340	0	0	139	145	109	109
PI 35	PI	143	146	263	265	0	0	137	137	139	139	105	109
PI 36	PI	143	146	265	265	318	318	137	137	139	139	0	0
PI 37	PI	143	143	265	265	318	360	0	0	139	139	109	111
PI 38	PI	143	143	263	265	318	340	137	137	139	139	105	111
PI 39	PI	143	143	0	0	0	0	0	0	139	145	111	113
PI 40	PI	0	0	271	271	318	332	0	0	139	145	105	113
PI 41	PI	143	143	263	275	0	0	145	145	159	159	105	109
PI 42	PI	143	146	261	273	0	0	141	145	139	145	111	113
PI 43	PI	143	143	0	0	318	358	137	145	139	139	109	109
PI 44	PI	143	143	265	265	0	0	137	145	139	139	109	113
PI 45	PI	143	143	271	273	0	0	137	145	139	139	109	113
PI 46	PI	143	143	261	273	322	332	137	147	139	145	107	109
PI 47	PI	143	146	273	275	0	0	145	145	139	139	0	0
PI 48	PI	0	0	261	265	0	0	0	0	145	159	0	0
PI 49	PI	143	143	0	0	326	364	137	137	139	145	109	109
PI 50	PI	143	146	265	273	0	0	137	147	139	139	109	109
PI 51	PI	143	143	271	275	318	318	137	145	139	139	109	109
PI 52	PI	146	146	0	0	0	0	137	141	139	139	0	0
PI 53	PI	143	146	261	265	318	326	137	137	139	139	109	109
PI 54	PI	143	143	265	265	322	332	137	137	139	139	109	109
PI 55	PI	143	143	261	271	322	322	0	0	139	139	107	109
PI 56	PI	143	143	259	261	326	326	137	137	139	139	0	0
PI 57	PI	143	143	261	265	326	326	141	141	139	145	109	113
PI 58	PI	143	146	263	263	326	326	137	145	139	139	109	113
PI 59	PI	143	143	0	0	0	0	137	137	0	0	109	109
PI 60	PI	143	146	0	0	326	326	137	137	139	159	107	109
PI 61	PI	143	143	261	265	326	326	0	0	139	145	105	105
PI 62	PI	143	146	261	271	0	0	137	141	139	159	109	111
PI 63	PI	143	146	0	0	322	322	0	0	139	145	107	109
PI 64	PI	143	143	271	273	332	332	0	0	139	139	109	111
PI 65	PI	143	146	261	265	326	342	0	0	139	139	109	113
PI 66	PI	143	143	265	265	340	340	0	0	139	145	109	109
PI 67	PI	146	146	0	0	326	332	145	145	145	159	109	111
PI 68	PI	143	146	265	275	326	332	137	137	139	145	105	109

PI 69	PI	143	146	259	261	322	332	137	141	139	159	109	109
PI 70	PI	143	143	265	271	326	326	137	147	145	145	109	109
BM1	BM	143	143	0	0	340	340	137	141	139	145	105	107
BM10	BM	143	143	259	265	322	342	137	145	145	145	105	109
BM11	BM	143	146	255	265	342	342	137	141	139	139	105	111
BM12	BM	143	143	259	273	340	342	137	145	139	159	107	107
BM13	BM	143	146	0	0	0	0	137	145	0	0	0	0
BM14	BM	143	146	259	265	318	340	137	145	139	139	105	113
BM15	BM	143	146	265	265	318	342	137	137	139	159	105	109
BM16	BM	143	146	259	263	340	340	137	137	139	159	109	109
BM17	BM	143	143	259	265	322	326	137	145	145	159	109	111
BM18	BM	143	143	265	273	318	342	137	157	139	159	105	107
BM19	BM	143	143	255	273	318	336	137	145	139	147	101	109
BM2	BM	143	143	263	273	318	328	137	145	139	145	101	109
BM20	BM	143	143	255	273	322	326	137	145	139	145	105	109
BM21	BM	143	143	255	259	318	340	137	141	139	159	101	113
BM22	BM	143	143	255	259	318	326	137	137	159	159	109	109
BM23	BM	143	143	261	265	318	340	137	137	139	159	107	111
BM24	BM	143	143	259	265	326	342	141	145	139	145	107	111
BM25	BM	143	143	255	265	322	326	137	141	139	159	105	107
BM3	BM	0	0	0	0	0	0	0	0	0	0	101	109
BM4	BM	143	143	259	259	326	326	145	145	159	159	101	111
BM5	BM	143	143	265	265	318	326	137	137	139	159	109	109
BM6	BM	143	146	261	271	322	340	139	141	139	139	109	111
BM7	BM	143	143	255	255	318	322	145	145	139	139	107	109
BM8	BM	143	143	273	275	318	326	145	145	139	165	105	111
BM9	BM	143	143	255	271	340	342	137	137	139	159	109	111
CS10	CS	143	143	0	0	318	342	137	141	139	145	105	105
CS11	CS	143	143	0	0	0	0	0	0	139	139	105	109
CS12	CS	143	146	259	273	338	340	137	137	139	159	105	109
CS13	CS	143	143	265	265	322	322	137	137	139	145	105	109
CS14	CS	143	146	273	273	318	318	137	137	139	159	105	113
CS15	CS	143	146	273	273	318	318	137	137	139	159	105	113
CS16	CS	143	143	259	265	318	318	139	145	159	159	105	111
CS18	CS	143	146	265	273	322	322	141	145	139	145	109	111
CS19	CS	143	143	259	265	322	322	137	145	139	145	105	105

CS2	CS	143	143	255	265	318	322	137	145	145	145	109	109
CS20	CS	143	146	259	277	318	324	137	137	159	159	105	109
CS22	CS	143	143	271	275	326	326	141	145	0	0	101	107
CS23	CS	143	143	255	265	318	318	141	157	145	145	101	107
CS24	CS	143	146	255	273	318	326	137	137	139	145	99	107
CS25	CS	0	0	259	265	318	324	145	157	159	159	0	0
CS26	CS	143	146	259	265	318	322	145	145	139	157	101	109
CS27	CS	143	143	259	259	318	322	137	137	145	159	101	101
CS28	CS	143	146	265	273	326	340	137	145	139	159	101	101
CS29	CS	143	143	0	0	318	340	137	145	145	159	107	109
CS3	CS	143	146	259	273	318	318	137	137	145	145	105	109
CS30	CS	143	143	255	275	322	322	145	145	145	159	103	105
CS32	CS	143	143	263	277	318	340	137	145	145	159	101	101
CS33	CS	143	146	255	263	326	340	137	137	139	159	105	109
CS34	CS	143	146	265	265	0	0	137	141	139	139	99	105
CS4	CS	143	143	265	275	318	322	137	137	145	145	105	105
CS5	CS	143	143	255	265	318	322	137	137	145	145	105	109
CS6	CS	143	143	0	0	318	342	137	137	145	145	105	105
CS7	CS	143	143	259	259	322	324	137	141	139	159	105	109
CS8	CS	143	146	259	261	318	322	137	145	139	145	105	109
CS9	CS	143	143	265	277	318	326	137	139	159	159	105	109
EG10	EG	143	143	265	265	326	342	137	145	145	159	105	111
EG11	EG	143	143	265	273	342	356	137	141	0	0	105	109
EG12	EG	143	146	265	275	318	326	145	157	139	139	109	111
EG13	EG	143	143	255	261	342	350	137	137	139	159	105	109
EG14	EG	143	146	259	273	350	350	137	141	139	159	109	109
EG16	EG	143	143	265	275	342	342	141	141	139	159	111	111
EG17	EG	143	143	261	265	326	326	137	145	139	145	111	111
EG18	EG	143	143	0	0	0	0	137	157	139	145	109	111
EG20	EG	146	146	265	273	326	326	141	141	139	159	105	109
EG21	EG	143	146	265	265	318	342	137	145	139	139	105	109
EG22	EG	143	143	265	275	318	318	137	141	145	159	109	111
EG24	EG	143	143	265	265	342	350	137	145	139	145	109	111
EG4	EG	143	146	259	273	350	350	137	141	139	159	109	109
EG5	EG	146	146	265	275	342	342	145	157	139	159	109	109
EG50	EG	143	143	265	265	344	352	141	157	139	159	103	107

EG51	EG	143	146	267	267	326	342	141	145	139	159	105	107
EG52	EG	143	143	275	275	318	318	137	141	145	159	105	107
EG53	EG	143	146	273	275	318	318	137	141	139	159	103	105
EG6	EG	143	143	265	275	318	326	137	141	139	159	0	0
EG7	EG	143	143	269	271	322	322	137	145	139	139	105	109
EG8	EG	143	143	269	271	322	322	137	145	139	139	105	109
EG9	EG	143	146	265	273	318	326	137	141	139	159	105	111
MEG1	EG	143	143	265	273	322	322	137	145	139	145	111	113
MEG2	EG	143	143	265	273	322	322	137	145	139	145	111	113
MEG3	EG	143	146	261	273	0	0	137	141	139	145	111	111
SEG1	EG	143	143	0	0	322	322	137	145	139	145	111	113
MP1	MP	143	143	259	265	328	328	137	157	139	139	105	111
MP10	MP	143	146	255	263	340	340	137	145	139	145	105	105
MP11	MP	143	143	261	277	326	328	137	137	139	145	105	113
MP12	MP	140	146	255	263	318	352	137	145	139	159	109	109
MP13	MP	143	146	271	277	318	326	137	141	139	159	105	111
MP14	MP	143	143	273	275	348	350	137	157	139	139	105	109
MP15	MP	143	143	265	265	326	350	137	137	139	139	105	105
MP16	MP	140	146	259	273	318	326	137	145	139	145	111	113
MP17	MP	143	146	273	275	342	348	137	157	139	159	109	109
MP18	MP	143	143	259	265	318	318	137	139	139	139	105	111
MP19	MP	140	143	259	273	318	326	137	141	139	145	105	111
MP2	MP	143	146	255	261	318	326	145	157	139	159	105	109
MP20	MP	143	143	255	261	0	0	137	137	139	145	109	113
MP21	MP	143	146	265	265	0	0	137	137	139	139	105	111
MP3	MP	143	143	265	265	328	342	137	145	139	139	105	111
MP4	MP	143	146	259	273	328	342	137	157	139	159	105	105
MP5	MP	143	143	265	265	0	0	137	137	145	159	109	113
MP6	MP	143	146	271	277	318	326	137	141	139	159	105	111
MP7	MP	143	143	261	265	326	328	145	157	139	145	105	105
MP8	MP	143	143	265	273	328	342	137	145	139	159	111	111
MP9	MP	143	152	275	275	318	318	141	145	139	139	105	109
NP1	NP	143	143	259	273	318	342	137	137	139	145	105	105
NP10	NP	143	143	259	271	318	318	137	145	139	139	107	111
NP11	NP	143	146	259	265	318	342	137	137	139	159	105	111
NP12	NP	143	143	259	261	318	322	145	157	139	159	105	111

NP13	NP	143	146	271	273	0	0	145	157	0	0	105	105
NP15	NP	143	143	259	265	0	0	137	145	139	145	105	105
NP16	NP	143	143	263	265	318	318	137	157	139	139	105	109
NP17	NP	143	146	265	273	322	328	153	157	145	145	105	109
NP18	NP	143	143	259	273	318	318	137	145	145	159	105	105
NP19	NP	143	143	259	259	328	328	137	137	139	159	105	109
NP2	NP	143	152	265	273	342	342	137	145	139	159	105	109
NP20	NP	143	143	265	273	318	360	137	145	139	145	105	111
NP22	NP	143	146	273	275	326	342	137	137	139	159	105	105
NP23	NP	143	146	265	271	318	350	137	137	139	145	105	105
NP24	NP	143	146	257	265	330	362	141	145	139	145	105	109
NP3	NP	143	152	259	265	318	326	137	147	139	139	105	111
NP4	NP	143	152	265	265	318	364	137	137	139	159	105	107
NP5	NP	143	143	259	273	342	342	137	137	145	159	107	111
NP6	NP	143	143	257	273	318	318	137	137	139	159	105	109
NP7	NP	143	152	265	273	318	342	137	145	139	145	105	107
NP8	NP	143	143	263	265	324	342	137	137	139	139	105	105
NP9	NP	143	143	265	265	318	328	137	137	145	159	105	109