DISSERTATION

GENETIC AND HABITAT FACTORS UNDERLYING CONSERVATION STRATEGIES FOR GUNNISON SAGE GROUSE

Submitted by

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ABSTRACT OF DISSERTATION

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The newly recognized Gunnison sage grouse (*Centrocercus minimus*) has declined markedly with extirpations in 12 of the 17 counties in southwestern Colorado which supported them in the early 1900's. Populations that remain are small and isolated, and exist in degraded and fragmented habitats. As a result, conservation of this species has become a significant concern. Particular issues of concern involve habitat quality and quantity, and genetic isolation from other populations. I developed a habitat-based model to: (1) identify the relative importance of landscape and micro-level variables, (2) examine the suitability of any sagebrush (*Artemisia* spp.) patch in southwestern Colorado, and (3) identify which patches have the highest probability of occupancy by sage grouse. The best model to make inferences from the data included patch area and distance to the nearest paved road. I quantified loss and fragmentation of sagebrush-dominated habitat using aerial photographic analysis. Between the mid-50's and the mid-90's, 20% of habitat was lost and sagebrush in 37% of the plots was fragmented. The Gunnison Basin had the lowest rate of habitat loss. I examined whether genetic data supported the new species designation of Gunnison sage grouse, and documented relative amounts of gene flow and genetic diversity between Gunnison sage grouse populations and northern sage

grouse (*C. urophasianus*) populations from northern Colorado. My genetic data supported the species distinction, and I found that Gunnison sage grouse populations have less genetic diversity and gene flow than northern sage grouse. Incorporating data from the habitat and genetic studies, I developed a Geographic Information System (GIS) based model which consolidated current knowledge about Gunnison sage grouse so that managers could prioritize conservation strategies.

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INTRODUCTION

The Gunnison sage grouse (*Centrocercus minimus*) is a newly recognized species (Braun and Young 1995) whose range is restricted to southwestern Colorado and southeastern Utah. The distribution and abundance of Gunnison sage grouse in Colorado has declined markedly, with extirpations in 12 of the 17 counties in southwestern Colorado which once supported them (Rogers 1964, Braun 1995). Declines are thought to be the result of habitat loss (conversion of big sagebrush [*Artemisia tridentata*] into farmland or housing developments), habitat degradation (heavy grazing, sagebrush removal, road and powerline development through sagebrush areas), and habitat fragmentation (Braun 1995). The majority of populations that remain are small, and exist in isolated, degraded patches of sagebrush habitat. One large population does remain, however, in the Gunnison Basin. Because of its restricted range and small population size, the conservation of this species has become a significant concern.

The conservation of Gunnison sage grouse requires knowledge of certain issues which have not yet been addressed. First, little is known about landscape level habitat requirements of sage grouse living in fragmented habitats. It is not known how large a sagebrush patch must be to support sage grouse, or if patch edge, or distance to the nearest road affect the probability of sage grouse persistence. Second, it is not known how much sage grouse habitat has already been lost and how much might be lost in the

future, given human population growth and land development. This is essential information if a balance between human population growth and sage grouse conservation is to be achieved. Third, little is known of the dispersal movements of sage grouse, as only one study has addressed this issue. Dunn and Braun (1985) measured natal dispersal of sage grouse in contiguous but altered habitats of northwestern Colorado and found average dispersal distances of 8.8 km for juvenile females and 7.4 km for juvenile males. It is not known, however, whether Gunnison sage grouse move among fragmented habitats (across distances up to 300 km) or whether some populations in southwestern Colorado are truly isolated. Knowledge of movement among patches and the levels of genetic diversity would provide essential information and aid in any conservation plan which addresses translocations and reintroductions.

In this dissertation I address three issues for which information is lacking. In Chapter One, I develop a habitat-based model which can be used to identify the relative importance of landscape and micro-level variables (or combinations of them) in sustaining Gunnison sage grouse. This model can be used to examine the suitability of any sagebrush patch in southwestern Colorado using the variables deemed important by the model. This gives biologists information on which occupied patches are most at risk of extinction and also allows unoccupied sagebrush patches to be ranked in order to identify which patches have the highest probability of occupancy by sage grouse. This is important because sage grouse population expansion could involve translocation into unoccupied sagebrush patches.

Chapters Two and Three address genetic issues concerning Gunnison sage grouse. Chapter Two is a population genetic analysis of nine sage grouse populations in Colorado using two different molecular genetic markers. In this chapter I address the question of whether genetic data support the new species designation of Gunnison sage grouse, and I compare relative amounts of gene flow and genetic diversity between Gunnison sage grouse populations from southwestern Colorado and sage grouse (*C. urophasianus*) populations from northern Colorado. Management implications of the genetic data are addressed in Chapter Three.

In Chapter Four I document the loss and fragmentation of sagebrush-dominated habitat in southwestern Colorado using aerial photographic analysis. This is important because if this species is listed as threatened, quantitative documentation of habitat loss and fragmentation (thought to be a contributing factor in the species' decline) is essential. Also, rates of habitat loss and fragmentation can be used to make predictions about future habitat loss given current rates of human population growth and land development. This information is essential for managers attempting to protect current populations and perhaps establish new populations.

I develop a Geographic Information System (GIS) based model in Chapter Five which incorporates information from Chapters One - Four. The purpose of this model is to consolidate what is currently known about Gunnison sage grouse, represent it spatially, and make this information accessible to managers so that they can assess how their decisions might affect not only a specific population, but the entire group of populations. The basis for the model includes information on all Gunnison sage grouse populations,

information on all sagebrush patches in southwestern Colorado, and information on current and future human housing densities. Using the information in this model, mangers will be able to make more informed decisions about each population by considering not only the features of the population and the habitat, but also its relationship to other populations and habitats so that a network of highly connected patches could be created which could serve to stabilize populations of Gunnison sage grouse in southwestern Colorado.

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CHAPTER ONE

A HABITAT-BASED MODEL TO PREDICT GUNNISON SAGE GROUSE OCCURRENCE IN SOUTHWESTERN COLORADO

INTRODUCTION

Historically, sage grouse (*Centrocercus urophasianus*) occurred in at least 16 states and three provinces in North America (Aldrich 1963, Johnsgard 1973, Braun 1998). They have since been extirpated from five states and one province (Braun 1998) and, in those states and provinces where they still exist, their range has declined markedly (Braun et al. 1994). The distribution and abundance of sage grouse in Colorado have also been greatly reduced (Braun 1995). Sage grouse have been extirpated from 12 of the 27 counties in Colorado in which they occurred in the 1900's (Braun 1995) and populations in nine of the remaining 15 counties are thought to number less than 500 breeding birds.

Population declines have resulted from habitat loss (conversion of big sagebrush [*Artemisia tridentata*] into farmland or housing developments), habitat degradation (heavy grazing, sagebrush removal, road and powerline development through sagebrush, and human disturbance), and habitat fragmentation (Braun 1995). The Gunnison sage grouse (*C. minimus*), a newly recognized species (Braun and Young 1995) restricted to southwestern Colorado and southeastern Utah, has been severely impacted by these

processes. Most Gunnison sage grouse populations that remain in southwestern Colorado are small, widely scattered, and exist in degraded, fragmented habitats (Fig. 1.1).

The ecology of sage grouse is relatively well known (Patterson 1952). In winter, sage grouse are dependent solely on sagebrush leaves (primarily big sagebrush) for food (Patterson 1952, Wallestad et al. 1975). Because of the lack of a grinding gizzard, sage grouse cannot digest plant fiber well (Remington 1989) and, as a result, are dependent upon sagebrush because it retains nutritious leaves all winter. Thus, the loss, degradation, and fragmentation of sagebrush habitats pose serious problems for sage grouse. The loss of sage grouse habitat has historically been due to conversion of sagebrush steppe into farmland. With human population growth in Colorado, however, most of the current habitat loss is due to housing and ranchette developments. Habitat degradation can be caused by livestock grazing which depletes grasses and forbs which are essential for nest success and survival of juvenile sage grouse. Large expanses of sagebrush (needed by grouse for food and cover) have been degraded through chemical treatment to promote grass and forb growth for livestock grazing. Roads and powerlines crossing sagebrush patches can also be considered a form of habitat degradation because sage grouse can fly into powerlines and be hit by cars. They have also been documented to avoid powerlines (C. E. Braun, personal communication) because of increased predation risk from avian predators that use powerlines for perches.

The consequences of sagebrush fragmentation are much more than merely loss of habitat, as the quality of the remaining sagebrush can be diminished. Such fragmentation typically results in a few remnant sagebrush patches surrounded by a matrix of land that

is unsuitable for sage grouse use due to development and land-use changes. The distance from other remnants, time since isolation occurred, and the extent remnants are connected all are important factors in population persistence (Saunders et al. 1990). Successful movement among patches should be more likely if patches are close to one another and if they are relatively well connected (not separated by insurmountable barriers or "hostile" areas). Movement among remnant patches is important for inbreeding avoidance (Boecklen and Bell 1987), and recolonizing other remnant patches or augmenting small populations. Size of the remnant patch is also an important factor affecting population persistence (Verboom et al. 1991). In small patches, external factors (edge effects) are much more influential than in larger patches. External factors might include the presence of novel predators (such as northern goshawks [*Accipiter gentilis*]), invasion of plants (such as pinon [*Pinus* spp.] and juniper [*Juniperus* spp.] trees which provide perches for avian predators), and competition from other species which inhabit edge habitats. Also, potential population size in a small patch is much smaller than in a large patch making populations in small patches much more vulnerable to chance extinctions (Gilpin and Soule 1986).

In order to better understand persistence of Gunnison sage grouse in isolated and fragmented habitats, I developed a habitat-based model which can be used to identify the relative importance of landscape and micro-level variables (or combinations of them) in sustaining sage grouse. This model can be used to examine the suitability of any fragmented sagebrush patch by using habitat variables and landscape metrics. This will provide information for biologists on which occupied patches are most at risk of

as sagebrush height, cover, and density, health of the understory (evidenced by the percent cover of grass and forbs), presence or absence of other non-sagebrush species, and extent of invasion by pinon and juniper trees. Macro-scale (or landscape level) variables were used to describe the overall patch and its relation to landscape surrounding it. These variables included the area of each patch, the area/perimeter ratio, distance to the nearest paved road (from the centroid of the patch), and the presence of powerlines.

Data Collection

I used a cluster sampling technique in this study. Two slightly different methods of cluster sampling were used, depending on the size of the patch. If the patch was small (< 2 km^2) or if it consisted of a series of small islands of sagebrush surrounded by agricultural fields, transects covering the entire patch were established (or in the case of islands of sagebrush, transects were situated in the majority of islands). If the patch was large (> 2 km^2), the patch was divided into strata of equal size and transects were established in two or three of those strata.

In the first case (type one sampling), a corner of the patch was arbitrarily chosen and the location was recorded using a global positioning system (G.P.S.). Initial movement to the north or south was randomly decided and a corresponding distance (0 - 50 m) was randomly drawn. This was repeated for east or west with a corresponding distance (0 - 50 m). The starting point was defined by walking the chosen distance from the initial point in the north or south direction and again in the east or west direction. From the starting point, a transect extending either north/south or east/west was

established and vegetation measurements were taken every 200 m. The transect ended whenever the edge of the patch was reached. Another transect was then established in the opposite direction, parallel to the first, 200 m from the first transect. This was repeated until the entire patch was covered (Fig. 1.2).

When the patches were large and transects were set up within strata (type two), the patch was divided into sections of approximately equal size. Large patches were divided into at most five and at least three strata with each strata being > 1 km^2 in size. If a patch was < 3 km on a side, then only two strata were established in that patch. An initial point was then chosen arbitrarily (usually a corner) in each of the stratum, and the starting point was chosen randomly by the same method as with type one except the distances ranged from 0 to 500 m. The first transect extended 1 km in a chosen direction and vegetation measurements were taken every 200 m. At the end of the first transect, a second transect was established extending 1 km in the opposite direction, parallel to the first transect. 200 m away. This was continued until five transects were completed and data from 25 sampling plots were taken (Fig. 1.3).

The actual data collection for small and large patches was the same. At each stop along the transect, a 1-m^2 sampling plot was placed on the ground and the following variables were measured:

a) percent cover of: live sagebrush, dead sagebrush, grass, forbs, oakbrush, other brush (other than sage or oak), cactus, and pinon/juniper;

b) height of: live sagebrush, dead sagebrush, grass, forbs, oakbrush, other brush, and pinon/juniper;

c) density of live sagebrush > 20 cm for species of big sagebrush and >10 cm for black and low sagebrush surrounding the 1 m^2 plot (measured in a belt 1 m wide and 2 m long in each of the cardinal directions outside the plot);

d) species of sagebrush present in the plot and in the belt outside the plot;

e) distance to nearest oakbrush, pinon/juniper, wet meadow, and fence post (within 100 m); and

f) number of sage grouse pellets in the plot and the belt outside of the plot.

Observations of sage grouse or grouse sign along the transect between stops were recorded. Occupancy or vacancy of a patch was based on whether or not sage grouse pellets were seen or whether sage grouse were flushed. Sage grouse pellets last for up to a year (C.E. Braun, Colorado Division of Wildlife, personal communication). The area of each patch, the area/perimeter ratio, distance to nearest occupied patch, and the distance to the nearest road (paved and unpaved) were determined from satellite data in a GIS operated by the Western Region of the Colorado Division of Wildlife.

Data Analysis

A logistic regression framework (Proc GENMOD; SAS® Institute Inc.1993) was used for analysis since the dependent variable (occupancy) was binary. The general form of logistic regression is

$$\theta = \frac{1}{1 + e^{-\alpha}}$$

where

$$\alpha = \text{logit}(\theta) = \beta_0 + \beta_1 x_1 + \ldots + \beta_{k-1} x_{k-1},$$

$x_1 \ldots x_{k-1}$ are variables in the model, $\beta_0 \ldots \beta_{k-1}$ are coefficients fit by the model, and $\hat{\theta}$ is the predicted probability given the model.

Because the number of actual data points (patches) was small, only a limited number of candidate models should be considered for model selection (Burnham and Anderson 1998). Thus, I developed a number of composite variables from the raw data. The habitat requirements of sage grouse are well known and are generally categorized into winter, breeding and nesting, and summer habitat. I created composite variables representing the percent of a patch in winter habitat, breeding and nesting habitat, and summer habitat. Further, I created a variable representing the area of habitat in a patch that was preferable (meaning that it represented either winter, breeding and nesting, or summer habitat). Winter habitat was defined by greater than 20% cover of live sagebrush taller than 20 cm (Eng and Schladweiler 1972, Beck 1977). Breeding and nesting habitat was defined by 20 - 40 % cover of live sagebrush between 17 and 119 cm in height, 7 - 10 % cover of grass, and > 4% cover of forbs (Patterson 1952:114, Kebenow 1969, Wallestad and Pyrah 1974, Connelly et al. 1991, Gregg et al. 1994, Musil et al. 1994, Young 1994). Summer habitat was characterized by 14 - 30 % cover of live sagebrush, 1 - 17% forb cover, and 1 - 22% grass cover (Martin 1970, Wallestad 1971, Klebenow 1969, Klott and Lindzey 1990, Young 1994).

Inspection of the parameter estimates for the four top models (Table 1.3), however, revealed that the parameter estimate for area of suitable habitat was negative in one case (technically meaning that the less area of suitable habitat, the more likely it would be occupied). This obviously makes no biological sense. There are several reasons why this may have occurred. First, I estimated area of suitable habitat by calculating the percentage of plots with either winter, or breeding and nesting, or summer habitat and multiplying it by the patch area, which may not estimate this parameter well. Second, the definition of winter, breeding and nesting, and summer habitats, came from other studies of sage grouse (in most cases large-bodied *C. urophasianus,* not with small-bodied *C. minimus*) in other areas. The habitat requirements for the small-bodied Gunnison sage grouse may be somewhat different than for the large-bodied sage grouse. Finally, area of suitable habitat was highly correlated with patch area. Adding area of suitable habitat to the model already containing patch area and distance to the nearest road did not improve the model and, because of its high correlation with patch area, may cause a spurious parameter estimate.

As a result, I eliminated any models containing the variable area of suitable habitat and recalculated Akaike weights for the remaining three models (Table I.4). The top model with patch area and distance to the nearest paved road received more weight than models with either variable alone. The model with distance to the nearest paved road, however, was a close second. Because the Akaike weights of the top two models were similar (0.507, 0.486), suggesting that both models were competitive, I concluded that none of the three remaining models alone was sufficient to make predictions about

occupancy given patch area and distance to the nearest paved road. Instead, I used model averaging (which accounts for uncertainty in model selection), to estimate the probability of occupancy given a patch size and distance to road (Burnham and Anderson 1998).

I calculated the model averaged prediction $\hat{\theta}_a$ as:

$$\hat{\theta}_a = \sum_{i=1}^{R} w_i \hat{\theta}_i$$

where $\hat{\theta}_i$ is the predicted value for occupancy from model *i*, and w_i is the Akaike weight for model *i*. Variance was calculated as:

$$\hat{\text{var}}(\hat{\theta}_a) = \left[\sum_{i=1}^{R} \sqrt{\hat{\text{var}}(\hat{\theta}_i | M_i) + (\hat{\theta}_i - \hat{\theta}_a)^2} \right]^2$$

and confidence intervals were calculated using:

$$\hat{\theta}_L = \frac{\hat{\theta}_a}{\hat{\theta}_a + (1 - \hat{\theta}_a)C}$$

and

$$\hat{\theta}_U = \frac{\hat{\theta}_a}{\hat{\theta}_a + (1 - \hat{\theta}_a)/C},$$

where

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Table 1.1. Candidate models used for model selection, within a logistic regression framework to predict the probability of occupancy of Gunnison sage grouse in southwestern Colorado.

Model	Model structure
Area	$\beta_0 + \beta_1 \text{(area)}$
Distance to road	$\beta_0 + \beta_1 \text{(distance)}$
Area of suitable habitat	$\beta_0 + \beta_1 \text{(area suitable)}$
Area, distance to road	$\beta_0 + \beta_1 \text{(area)} + \beta_2 \text{(distance)}$
Area, area of suitable habitat	$\beta_0 + \beta_1 \text{(area)} + \beta_2 \text{(area suitable)}$
Distance to road, area of suitable habitat	$\beta_0 + \beta_1 \text{(distance)} + \beta_2 \text{(area suitable)}$
Area, distance to road, area of suitable habitat	$\beta_0 + \beta_1 \text{(area)} + \beta_2 \text{(distance)} + \beta_3 \text{(area suitable)}$

Table 1.2. Selection of models using AICc as a model selection criterion. Models in this logistic regression framework predict the probability of occupancy of Gunnison sage grouse in southwestern Colorado. K represents the number of parameters.

Model	K	AICc	ΔAIC	Akaike weight
Area, distance to road	3	23.530	0.000	0.335
Distance to road	2	23.614	0.085	0.321
Distance to road, area of suitable habitat	3	24.578	1.048	0.198
Area, distance to road, area of suitable habitat	4	25.340	1.811	0.135
Area, area of suitable habitat	3	32.050	8.520	0.005
Area	2	32.321	8.791	0.004
Area of suitable habitat	2	36.974	13.448	0.000

Table 1.3. Parameter estimates and model conditional standard errors for the four best logistic regression models to predict the probability of occupancy of Gunnison sage grouse in southwestern Colorado.

Parameter	Estimate	SE
Model with area, distance to road		
Intercept	-5.8699	2.8930
Area	0.0845	0.0609
Distance to road	0.0016	0.0008
Model with distance to road		
Intercept	-4.5271	1.8817
Distance to road	0.0015	0.0006
Model with distance to road, area of suitable habitat		
Intercept	-5.3861	2.5611
Distance to road	0.0016	0.0007
Area of suitable habitat	0.1089	0.0950
Model with area. distance to road, area of suitable habitat		
Intercept	-5.9005	2.9389
Area	0.3025	0.2785
Distance to road	0.0016	0.0008
Area of suitable habitat	-0.3643	0.4145

Table 1.4. Three logistic regression models remaining after model selection using AICc with updated Akaike weights. These models can be used to predict the probability of occupancy of sagebrush patches by Gunnison sage grouse in southwestern Colorado.

Model	K	AICc	ΔAIC	Akaike weight
Area, distance to road	3	23.530	0.00	0.507
Distance to road	2	23.614	0.085	0.486
Area	2	32.321	8.791	0.006

Table 1.5. Model averaged predictions of patch occupancy by Gunnison sage grouse for a series of different theoretical patch areas and distances to the nearest paved road.

Area (km^2)	Distance to nearest paved road (m)	Predicted probability of occupancy	95% CI
1	10	0.0086	0.0002 - 0.3118
1	100	0.0096	0.0002 - 0.3235
1	1000	0.0310	0.0014 - 0.4227
1	2000	0.1194	0.0157 - 0.5362
1	3000	0.3694	0.1152 - 0.7251
1	4000	0.7208	0.2989 - 0.9399
1	5000	0.9196	0.4070 - 0.9948
10	10	0.0113	0.0002 - 0.3610
10	100	0.0126	0.0003 - 0.3721
10	1000	0.0405	0.0021 - 0.4562
10	2000	0.1561	0.0280 - 0.5433
10	3000	0.4588	0.2082 - 0.7321
10	4000	0.7953	0.3956 - 0.9584
10	5000	0.9442	0.4913 - 0.9966
50	10	0.0942	0.0005 - 0.9519
50	100	0.1055	0.0007 - 0.9539
50	1000	0.2771	0.0058 - 0.9619
50	2000	0.5077	0.0405 - 0.9618
50	3000	0.7181	0.1500 - 0.9735
50	4000	0.8916	0.3273 - 0.9929
50	5000	0.9693	0.4169 - 0.9993
90	10	0.4434	0.0094 - 0.9853
90	100	0.4529	0.0112 - 0.9838
90	1000	0.5175	0.0255 - 0.9778
90	2000	0.5906	0.0454 - 0.9777
90	3000	0.7375	0.1364 - 0.9804
90	4000	0.8958	0.3331 - 0.9933
90	5000	0.9704	0.4922 - 0.9991
500	10	0.5183	0.0218 - 0.9811
500	100	0.5190	0.0220 - 0.9811
500	1000	0.5346	0.0258 - 0.9803
500	2000	0.5942	0.0447 - 0.9786
500	3000	0.7382	0.1357 - 0.9806
500	4000	0.8960	0.3320 - 0.9934
500	5000	0.9704	0.4901 - 0.9991

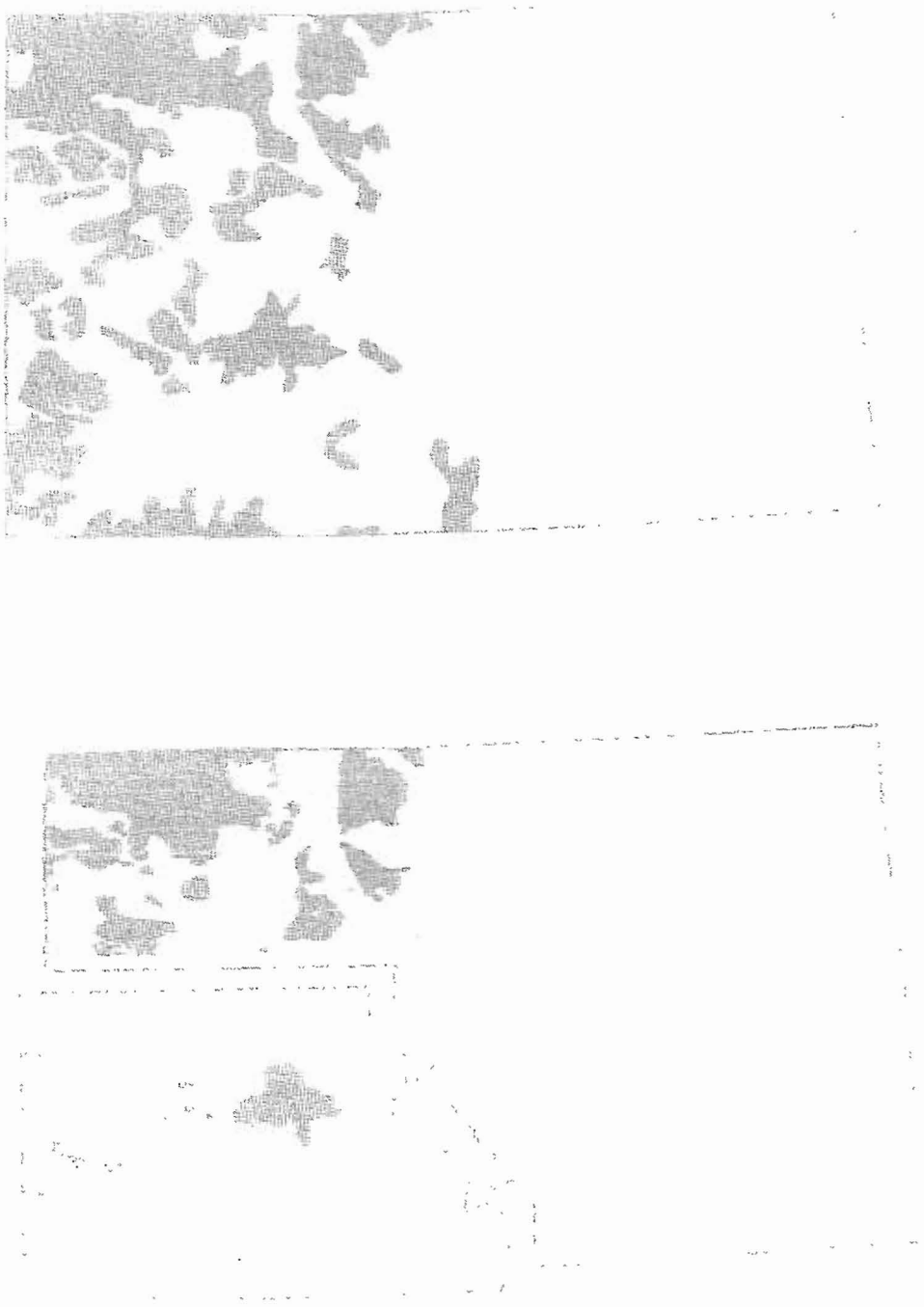

Figure 1.1. Historic (top) and current (bottom) distribution of sage grouse and Gunnison sage grouse (lower left cut out) in Colorado.

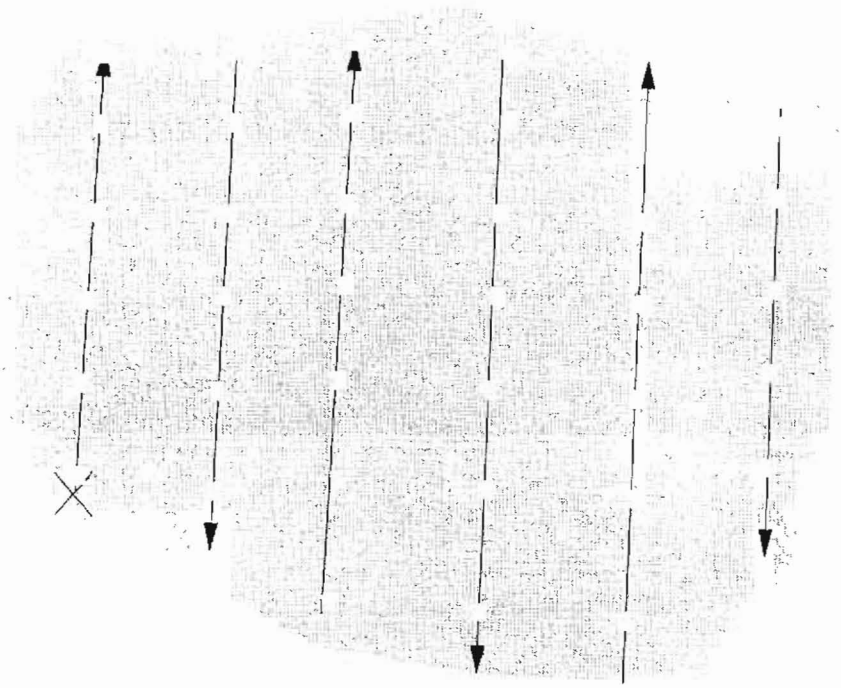

Figure 1.2. Sampling scheme for micro-scale variables in a small patch. Starting points were chosen randomly (represented here by X), transects were run in north/south directions, measurements were taken from 1-m^2 sampling frames (represented by the white box) every 200 m.

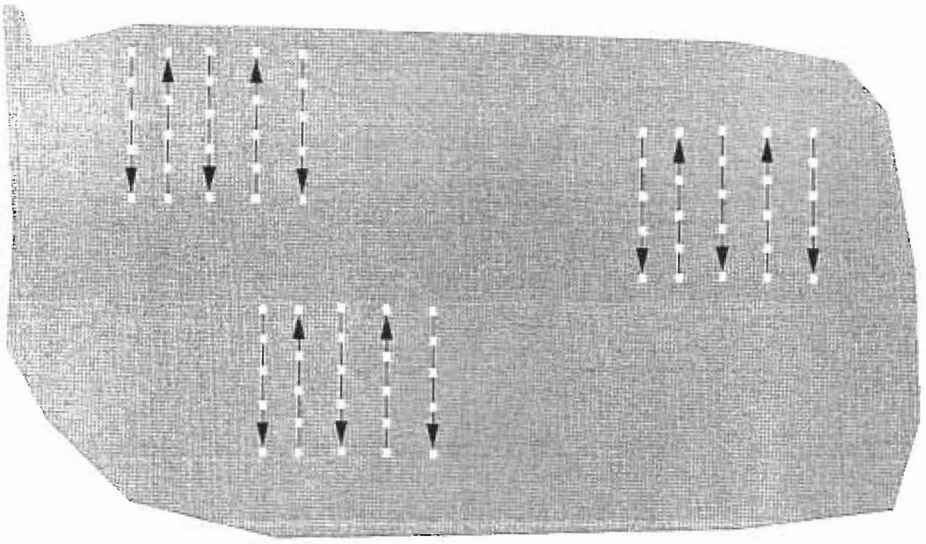

Figure 1.3. Sampling scheme for measurements in a large patch. Starting points were randomly chosen, transects were established in north/south directions, and measurements were taken in a 1-m^2 sampling plot (represented by the white boxes) every 200 m.

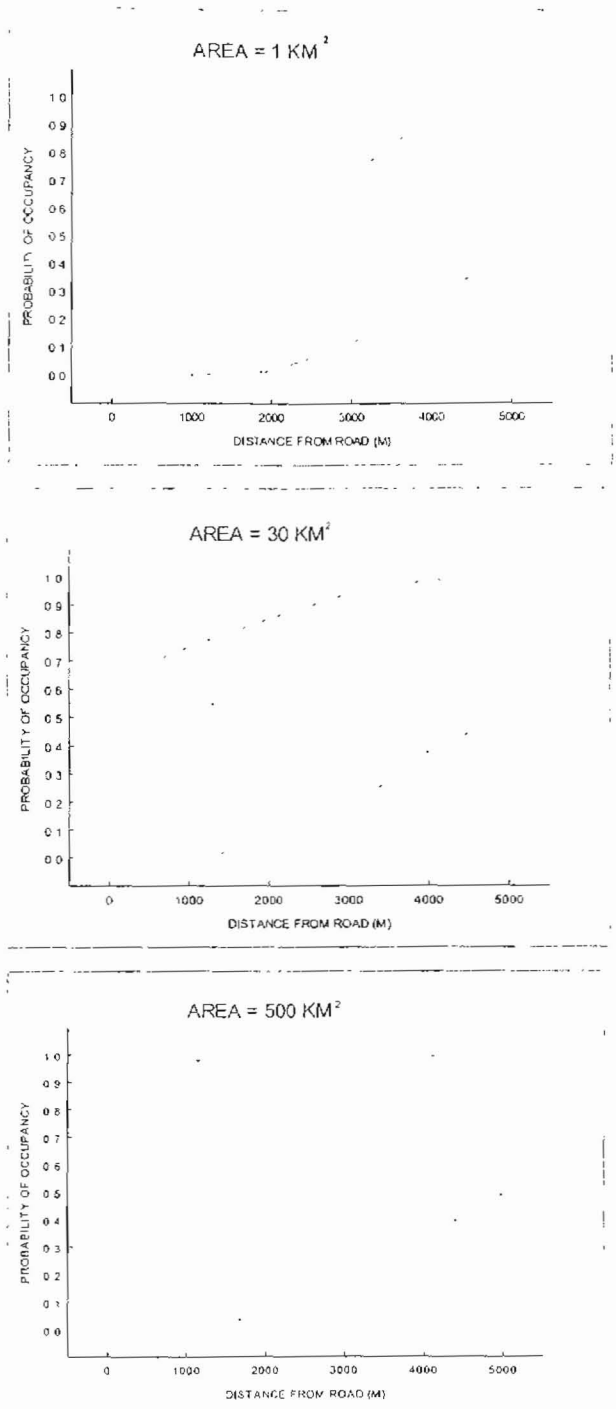

Figure 1.4. Theoretical relationship between the probability of occupancy and distance to the nearest paved road from the centroid of the patch for three different patch areas. Dotted lines represent upper and lower 95% confidence intervals for the predicted value (non-dotted line).

CHAPTER TWO

A POPULATION GENETIC COMPARISON OF LARGE AND SMALL-BODIED SAGE GROUSE IN COLORADO

INTRODUCTION

Sage grouse (*Centrocercus urophasianus*) have experienced marked declines in their distribution and abundance throughout their entire range (Braun et al. 1994). Their historic distribution included at least 16 states and three provinces in North America (Aldrich 1963, Johnsgard 1973, Braun 1998) and has since been extirpated from five states and one province (Braun 1998). In Colorado, the distribution and abundance of sage grouse have also been greatly reduced (Braun 1995) as they have been extirpated from 12 of the 27 counties in Colorado in which they occurred in the 1900's (Braun 1995) and populations in nine of the remaining 15 counties are thought to number less than 500 breeding birds. Because of this marked decline, sage grouse have become the focus of management and conservation concerns.

Sage grouse have historically been classified into two subspecies: *C. u. urophasianus* (Eastern sage grouse) and *C. u. phaios* (Western sage grouse). This subspecies distinction was based on plumage and coloration differences (Aldrich and Duvall 1955), yet its validity has been questioned (Johnsgard 1983). Studies in

southwestern Colorado (Hupp and Braun 1991) and southeastern Utah (Barber 1991) found sage grouse to be approximately 33% smaller than sage grouse from northern Colorado and throughout the rest of the species' range. Further, these "small-bodied" sage grouse have longer filoplumes and different tail banding patterns. Young (1994) and Young et al. (1994) compared strutting displays from the small-bodied sage grouse in southwestern Colorado to "large-bodied" sage grouse populations in northern Colorado and in California and found that many of the ritualized components of the strutting display differed. Further, Young (1994) found that small-bodied females avoided tape-recorded vocalizations of large-bodied males. Based on morphological and behavioral differences between large and small-bodied sage grouse, Braun and Young (1995) proposed that small-bodied sage grouse from southwestern Colorado and southeastern Utah be recognized as a new species, based on the biological species concept.

To determine whether genetic evidence is consistent with this new species designation, Kahn et al. (1999) compared the genetic variation among five populations of large-bodied sage grouse from northern Colorado, one population of large-bodied sage grouse from Utah, and one population of small-bodied sage grouse from southwestern Colorado. To document this variation, they sequenced 141 base pairs of a rapidly evolving portion (region I) of mitochondrial DNA (mtDNA) and showed that sequences from the seven populations included 21 haplotypes that formed two monophyletic clades. Several different haplotypes from both clades were found in all six large-bodied populations, while within the small-bodied population, all but one of the 31 individuals analyzed were genetically identical, and both observed haplotypes were members of the

same clade. They concluded that the unusually low level of genetic variation and absence of several haplotypes that were common in the large-bodied populations in Colorado provided evidence of a lack of gene flow between the two proposed species.

While their study provides evidence that can be construed to support the new species designation, I expanded it to include individuals from three additional small-bodied populations not included in Kahn et al.'s (1999) study, and supplemented their mtDNA data with data from the nuclear DNA. This was done to more completely characterize the mtDNA data and to eliminate any concern that male biased gene flow would not be elucidated using the maternally inherited mtDNA. The nuclear molecular markers that I chose were microsatellite markers which are areas in the nuclear genome characterized by short, tandem repeats with a high rate of variation in copy number among individuals. Microsatellites are highly variable and are generally considered to be among the most powerful molecular genetic markers for population genetic studies (Goldstein and Pollock 1997).

METHODS

Tissue Collection

Extracted DNA from 20 birds from the Gunnison Basin and from the five large-bodied populations that were used in Kahn et al.'s (1999) study, were also used in this study. These five northern Colorado populations include Cold Springs, Blue Mountain, Eagle, Middle Park, and North Park (Fig. 2.1). Blood samples and feathers were obtained from small-bodied sage grouse which were captured using a spotlight trapping method

(Giesen et al. 1982) in the following populations in Colorado: Dove Creek (N = 15), Dry Creek Basin (N = 22), Crawford (N = 20), and Gunnison Basin (N = 9) (Fig. 2.1). Blood samples were obtained by clipping a toe nail of each sage grouse and placing 2-3 drops of blood into a microfuge tube previously coated with EDTA. These blood samples, as well as feather samples from each sage grouse were frozen at -20°C. The nine Gunnison Basin samples were from the same area sampled by Kahn et al. (1999) and were used to augment the 20 Gunnison Basin samples collected by Kahn et al. (1999).

DNA Extraction and Microsatellite Genotype Scoring

DNA extractions from blood or the bottom 2 cm of the feather shaft, followed the procedure of Quinn and White (1987). Over 30 microsatellite primers from the chicken genome project were used to screen for polymorphism of microsatellites as well as 12 primers developed for red grouse (*Lagopus lagopus scoticus*). I found four microsatellites with clean, scorable products that were polymorphic in both the large and small-bodied sage grouse. These four loci proved to be informative and allowed me to sufficiently address the objectives of this study. Primers for those four microsatellites (LLST1F, LLST1R, LLSD3F, LLSD3R, LLSD4F, LLSD4R, LLSD8F and LLSD8R) were developed by Piertney and Dallas (1997). One primer (either the forward or reverse primer) was chosen and radioactively labeled for later visualization on autoradiography film using the T4 Polynucleotide Kinase (PNK) labeling procedure. In a 0.5 µl microfuge tube, 1 µl 10 µM primer,1 µl 10X Buffer, 0.25 µl T4 PNK (10 U/µl), 0.25 µl

λ-^{33}P-ATP(10.0 mCi/ml), and 7.5 μl H_20 were mixed and incubated at 37°C for 15 minutes. The reaction was stopped by heating to 70°C for 10 minutes.

Polymerase chain reactions (PCR) were performed in a Perkin-Elmer DNA thermal cycler. Approximately 30 ng of genomic DNA (in a 1μl volume) was used as template in each 25 μl PCR (as described in Quinn 1992), using one forward and one backward primer, with the following thermal profile: 2 min denaturation at 94°C followed by 35 cycles of "touchdown" ramping: 30 seconds denaturation at 94°C and 30 seconds annealing while stepping from 60°C to 50°C. A 20 minute extension at 74°C was performed at the end of the 35th cycle.

PCR products and a size standard were electrophoresed at 55 watts for two hours through 6% denaturing poly-acrylamide gels as described in Sambrook et al. (1989). Autoradiographs were made of each dried acrylamide gel by exposure to X-ray film (Fuji RX). Individuals were assigned genotypes (corresponding to microsatellite fragment length) based on banding patterns on the autoradiographs. In some cases samples containing alleles of similar sizes were rerun in adjacent lanes. The distribution of allele frequencies for each population was recorded.

mtDNA Sequencing

The procedures were described in detail previously (Kahn et al. 1999). I identified new haplotypes by comparison to those designated previously by Kahn et al. (1999).

Data Analysis

Microsatellite genotypes were tested for departures from Hardy-Weinberg equilibrium within each population at each locus using the computer program Arlequin (Schneider et al. 1997). Arlequin uses a Markov-chain random walk algorithm (Guo and Thompson 1992) which is analogous to Fisher's exact test but extends it to an arbitrarily sized contingency table. Population genetic structure was investigated using pairwise population F_{ST} significance tests. F tests (Tjur 1998) for each locus were conducted to determine whether the distributions of alleles were significantly different between the large and small-bodied birds. An F test is a ratio of mean squares (analogous to ANOVA) which is used here because it is robust to overdispersed data.

Genetic distance for all pairs of populations was estimated using two different distance metrics. Both metrics assume an infinite alleles model of mutation. Although Goldstein and Pollock (1997) advocate using stepwise mutation models to estimate genetic distances for phylogenetic reconstruction using microsatellite data, D. B. Goldstein (personal communication) suggests that population genetic studies using microsatellites should use genetic distances based on the infinite alleles model (specifically the proportion of shared alleles [Bowcock et al. 1994]) because they are linear over short periods of time and have a low variance. I calculated the proportion of shared alleles (Bowcock et al. 1994) and also Cavalli-Sforza and Edwards' (1967) chord distance because Takezaki and Nei (1996) showed it to have a higher probability of obtaining correct tree topologies than other distance measures with microsatellite markers. Chord distance, Dc, was calculated as

$$Dc = (2/\pi r)\sum_{j}^{r}\sqrt{2(1-\sum_{i}^{m_j}\sqrt{x_{ij}y_{ij}})}$$

where x_{ij} and y_{ij} are the frequencies of the ith allele at the jth locus in populations X and Y respectively, m_j is the number of alleles at the jth locus, and r is the number of loci examined. The proportion of shared alleles, Ps, was calculated as

$$Ps = s/(2l)$$

where s is the number of shared alleles summed over loci, and l is the number of loci compared. I calculated genetic distance between all pairs of populations and constructed neighbor joining trees describing the relationship among populations using the microsatellite data and both distance measures.

For the mtDNA analysis, I documented population subdivision in Arlequin (Schneider et al. 1997) using significance tests of pairwise population F_{ST} values. An F test was calculated to determine whether the distribution of haplotypes among the large and small-bodied birds differed. I conducted an analysis of molecular variance (AMOVA) as described by Excoffier et al. (1992) which produces estimates of variance components to reflect haplotype diversity at different levels of a hierarchy. I documented the variation due to large vs. small bodied birds as one level of hierarchy, the variation among populations within the two body sizes as a second level, and the variation among individuals in a population as the third level. The molecular distances between haplotypes were modeled following Tamura (1992) because my haplotypes had unequal frequencies of A, C, T, and G and because my transition/transversion ratio was

much higher than the expected (mathematically) ratio of 1:2. I calculated pairwise population genetic distances which incorporate both the Tamura (1992) corrected molecular distance between haplotypes and the haplotype frequencies in each population. Neighbor joining trees were constructed showing the relationship of the nine populations.

RESULTS

Microsatellite Data

I found high levels of polymorphism among the four microsatellite loci (Table 2.1) particularly among the large-bodied sage grouse (Appendix 2.A). The small-bodied sage grouse exhibited much less polymorphism with the average number of alleles per locus ranging from 1.8 to 3.8 compared to the large-bodied sage grouse with an average of 5.5 to 6.5 alleles per locus. Further, all loci among the large-bodied birds were polymorphic, while in some small-bodied populations either one or two loci were monomorphic. Only two of the 33 population/loci combinations showed significant departures ($P < 0.05$) from Hardy-Weinberg equilibrium (Dry Creek locus LLSD 3, $P = 0.008$ and Eagle locus LLSD3, $P = 0.0004$) (Appendix 2.A). Because I made 36 comparisons I might expect to get a P value of 0.008 by chance so I set my significance level to 0.001 leaving only one significant departure from Hardy-Weinberg equilibrium (Eagle, LLSD3).

Pairwise population F_{ST} significance tests showed significant population subdivision (Table 2.2). Due to multiple comparisons within the analysis I decreased my P value by a factor of ten to 0.005 to indicate statistical significance. All possible

pairwise comparisons between small and large-bodied sage grouse populations showed significant differences. Within the large-bodied sage grouse, no two populations were significantly different, while among the small-bodied sage grouse, only two population pairs were not significantly different (Gunnison and Dry Creek, $P = 0.0073$; Dry Creek and Dove Creek, $P = 0.025$). Further, I calculated F_{ST} values separately for the large-bodied and small-bodied populations. I found that large-bodied birds had much less population subdivision ($F_{ST} = 0.0266$, 95% CI -0.0016 - 0.0528) than did the small-bodied birds ($F_{ST} = 0.2153$, 95% CI 0.1230 - 0.3339).

I compared the distribution of alleles between the large and small-bodied birds for each microsatellite locus and found that three loci showed a significant difference between the two groups of birds (LLSD3 $F_{6,30} = 5.95$, $P < 0.001$; LLSD4 $F_{32,146} = 2.51$, $P < 0.001$; LLSD8 $F_{3,15} = 102.05$, $P < 0.001$) and one did not (LLST1 $F_{3,15} = 0.983$, $P > 0.05$). While the topologies of the trees from the two different distance measures differ slightly (Fig. 2.2), the main pattern of the distinction between the large and the small-bodied birds is evident.

mtDNA Data

There were 19 different haplotypes across all individuals. Kahn et al. (1999) found that the five large-bodied populations all had at least five different haplotypes in each population. They found four dominant haplotypes (A, B, C, and D) with haplotypes B, C, and D common in all large-bodied populations and haplotype A found in all but one. In the small-bodied populations, I found only two or three haplotypes per

population (Fig. 2.3). Only one of the haplotypes dominant in the large-bodied birds, A, was found and haplotype G was found to be unique among the small-bodied birds (Appendix 2.A). I found significant population subdivision using population pairwise F_{ST} significance tests (Table 2.3). As with the microsatellite data, all possible pairwise comparisons between small and large-bodied sage grouse populations showed significant differences. Further, I found that within the large-bodied sage grouse, no two populations were significantly different and among the small-bodied sage grouse, only one population pair was not significantly different (Dry Creek and Dove Creek, $P = 0.072$) (Appendix 2.A). To test whether the distribution of haplotypes from the large-bodied populations differed from the distribution of haplotypes from the small-bodied populations, I used an F test. There was a statistically significant difference between the distribution of haplotypes in the large and small-bodied populations ($F_{18,70} = 3.82$, $P < 0.001$). Further, I used AMOVA to examine components of variance between the large and small-bodied groups, among populations within groups, and among individuals within populations. I found that 65% of the variance could be explained by the large vs. small-bodied group distinction, only 2% of the variance was explained by between population variation within body size, and the remaining 33% of the variance was explained by within population variation (Table 2.4). The pattern noted in the trees from the microsatellite data is similar to the mtDNA tree (Fig. 2.4) suggesting a separation between the large and small-bodied sage grouse.

DISCUSSION

In all four microsatellites and in the 141 bp control region of the mtDNA high variability was found even at my smallest sampling level (within populations) which provided me with a powerful tool to detect population subdivision. The only significant departure from Hardy-Weinberg equilibrium (Eagle locus LLSD3) was a case of heterozygote deficiency which could be the result of many factors including null alleles, Wahlund effect, and inbreeding. Null alleles occur when a mutation causes one oligonucleotide primer not to amplify one allele which is manifested by a deficiency of heterozygotes (Pemberton et al. 1995). Null alleles are also sometimes detected when PCR products cannot be amplified for certain individuals (Lehman et al. 1997). I doubt null alleles were the cause for the heterozygote deficiency in Eagle because I had no problem getting PCR products from Eagle individuals for any loci. Also, I had two family groups of known mother and offspring which I tested over all loci and found no evidence of null alleles. Further, a heterozygote deficiency was found only in one population and I might expect to find deficiencies in other populations if null alleles were the cause. The heterozygote deficiency in Eagle might be the result of the Wahlund effect of pooling separate populations into one population or of inbreeding. However, if either was the case I would expect to find this effect among the three other loci which I did not.

Pairwise population F_{ST} significance tests showed similar patterns in the microsatellite and mtDNA analyses (Tables 2.2, 2.3). Both markers revealed significant differences between all large vs. small-bodied population comparisons supporting a

distinction between these two groups of birds. Both markers also revealed there were no significant differences among any of the large-bodied bird populations suggesting substantial gene flow among them. Within the small-bodied bird populations, most pairwise population comparisons showed significant differences among populations with a few exceptions (Gunnison and Dry Creek $P = 0.007$, Dry Creek and Dove Creek $P = 0.025$ for microsatellites; Dry Creek and Dove Creek $P = 0.054$ for mtDNA). Also, the F_{ST} value calculated among the large-bodied populations ($F_{ST} = 0.0266$, 95% CI - 0.0016 - 0.0528) is significantly smaller than the value calculated among the small-bodied populations ($F_{ST} = 0.2153$, 95% CI 0.1230 - 0.3339). This suggests there is some amount of subdivision among the small-bodied birds likely due to their small population sizes (~2600 birds in Gunnison Basin, ~ 175 birds in Crawford, ~ 75 in Dove Creek, and ~ 300 in Dry Creek, (C. E. Braun, Colorado Division of Wildlife, unpublished data)) and isolation (Fig. 2.1). This is consistent with Braun's (1995) assertion that clearing of sagebrush for cultivated crops, highway construction, ranch development, powerline placement, reservoir construction, and other facets of human settlement have resulted in the fragmentation and loss of sagebrush habitats such that sage grouse populations in southwestern Colorado are small and isolated (also see Chapter Four). This reduction of habitat is evident when comparing the historic range of sage grouse in Colorado with its current distribution (Fig. 2.1). A comparison of these two distributions reveals that the majority of fragmentation and loss of habitat has occurred in southwestern Colorado resulting in small, isolated populations, and that populations in northern Colorado remain relatively large and contiguous, all of which is supported by my genetic data.

The three of four significant *F* tests for the microsatellite loci and the significant *F* test for the mtDNA data reveal that the distribution of allele and haplotype frequencies are different for the large and small-bodied sage grouse populations. Further, in both the microsatellite and mtDNA data there are alleles and a haplotype unique to the small-bodied sage grouse thereby supporting the idea that gene flow between the two groups is likely absent and some amount of divergence has occurred. This supports Braun and Young's (1995) recognition of small-bodied sage grouse as a new species based on the biological species concept. In addition, the mtDNA AMOVA (Table 2.4) indicates that 65% of the total variation in the mtDNA data can be explained by the large vs. small-bodied sage grouse distinction and that only 2 % of the variation can be attributed to differences among populations within the large or small-bodied group. Kahn et al. (1999) discuss the ancestry of the mtDNA haplotypes and profess two different explanations for the establishment of the small-bodied sage grouse. They believe that either a founder population of large-bodied birds diverged rapidly from other large-bodied populations likely due to sexual selection or that the small-bodied sage grouse evolved across a widespread portion of the southwestern range (remaining unnoticed as a separate taxon) and underwent a severe bottleneck recently due to habitat fragmentation and habitat loss. My data are consistent with the founder hypothesis because in the microsatellite analysis the majority of the alleles present in the small-bodied populations are also present in the large-bodied populations, yet the diversity in the small-bodied populations (17 alleles) is much less than in the large-bodied populations (44 alleles). The mtDNA analysis also supports this hypothesis in that the dominant haplotype in the small-bodied populations

(A) is well represented in the large bodied birds. The haplotype unique to the small-bodied birds is close to the A haplotype (one transition) representing a recent mutation. As in the microsatellite analysis, the genetic diversity in the large-bodied populations is much higher (17 haplotypes) than in the small-bodied populations (three haplotypes).

All genetic distances from both markers show a similar broad pattern of a distinction between the large and small-bodied populations. From the mtDNA tree I can conclude that within the large-bodied group populations are more closely related (shorter branch lengths) than within the small-bodied group (longer branch lengths). This was also apparent from the pairwise population F_{ST} significance tests (Tables 2.2, 2.3) in which populations within the large-bodied group were not significantly different whereas, within the small-bodied group they were different.

This study has provided valuable additions to the study conducted by Kahn et al. (1999) in that there is now nuclear data to corroborate the mtDNA data. Further, I expanded the survey of small-bodied sage grouse to include information from three additional populations which is essential to the conservation of the small-bodied sage grouse. I not only extended Kahn et al.'s (1999) picture of the distinction between large and small-bodied sage grouse, but I documented the isolation and low genetic diversity of the small-bodied sage grouse populations. This is important information for the management of the small-bodied sage grouse as a species. Future research on sage grouse should include more microsatellite loci and population surveys throughout the entire range of sage grouse. This would provide a much deeper knowledge base for the understanding and management of sage grouse.

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Young, J. R., J. W. Hupp, J. W. Bradbury, and C. E. Braun. 1994. Phenotypic divergence of secondary sexual traits among sage grouse, *Centrocercus urophasianus*, populations. Animal Behavior 47:1353-1362.

Table 2.1. Polymorphism of microsatellite loci among nine populations of sage grouse in Colorado.

Population	Mean sample size per locus (SD)	Mean # of alleles per locus (SD)	Polymorphic loci (%)	Mean Heterozygosity	
				Observed (SD)	Expected from HdyWbg (SD)
Small-bodied					
Gunnison Basin	28.5 (0.5)	3.8 (1.4)	75	0.386 (0.123)	0.374 (0.120)
Crawford	17.3 (0.6)	2.3 (0.6)	75	0.299 (0.138)	0.297 (0.151)
Dry Creek	17.5 (1.6)	2.5 (0.6)	50	0.179 (0.135)	0.283 (0.177)
Dove Creek	14.5 (0.3)	1.8 (0.5)	50	0.193 (0.135)	0.221 (0.142)
Large-bodied					
Cold Springs	20.5 (0.6)	5.5 (2.5)	100	0.631 (0.118)	0.611 (0.114)
Blue Mountain	21.5 (1.2)	6.5 (3.2)	100	0.596 (0.120)	0.600 (0.144)
North Park	22.8 (1.0)	5.5 (2.2)	100	0.643 (0.080)	0.619 (0.098)
Middle Park	19.3 (0.8)	5.5 (1.6)	100	0.701 (0.089)	0.639 (0.078)
Eagle	20.3 (0.8)	5.5 (2.5)	100	0.748 (0.145)	0.636 (0.103)

Table 2.2. Significance ($P < 0.005$) of pairwise population F_{ST} tests for microsatellite data from sage grouse in Colorado. Pairs of populations significantly different are shown by + and those not significantly different are shown by -.

	Small-bodied				Large-bodied			
	Gunnison Basin	Crawford	Dry Creek	Dove Creek	Cold Springs	Blue Mountain	North Park	Eagle
Crawford	+							
Dry Creek	-	-						
Dove Creek	+	+	-					
Cold Springs	+	+	+	+				
Blue Mountain	+	+	+	+	-			
North Park	+	+	+	+	-	-		
Eagle	+	+	+	+	-	-	-	
Middle Park	+	-	+	+	-	-	-	-

Table 2.3. Significance ($P < 0.005$) of pairwise population F_{ST} tests for mtDNA sequencing data from sage grouse in Colorado. Pairs of populations significantly different are shown by + and those not significantly different are shown by -.

	Small-bodied				Large-bodied			
	Gunnison Basin	Crawford	Dry Creek	Dove Creek	Cold Springs	Blue Mountain	North Park	Eagle
Crawford	+							
Dry Creek	+	+						
Dove Creek	+	+	-					
Cold Springs	+	+	+	+				
Blue Mountain	+	+	+	+	-			
North Park	+	+	+	+	-	-		
Eagle	+	+	+	+	-	-	-	
Middle Park	+	+	+	+	-	-	-	-

Table 2.4. AMOVA design and results for mtDNA analysis of nine populations of sage grouse in Colorado.

Source of variation	df	Sum of Squares	Variance components	Percentage of Variation
Among groups	1	584.06	5.88	64.84
Among groups, within populations	7	43.57	0.15	1.63
Within populations	192	584.14	3.04	33.53
Totals	200	1211.77	9.07	

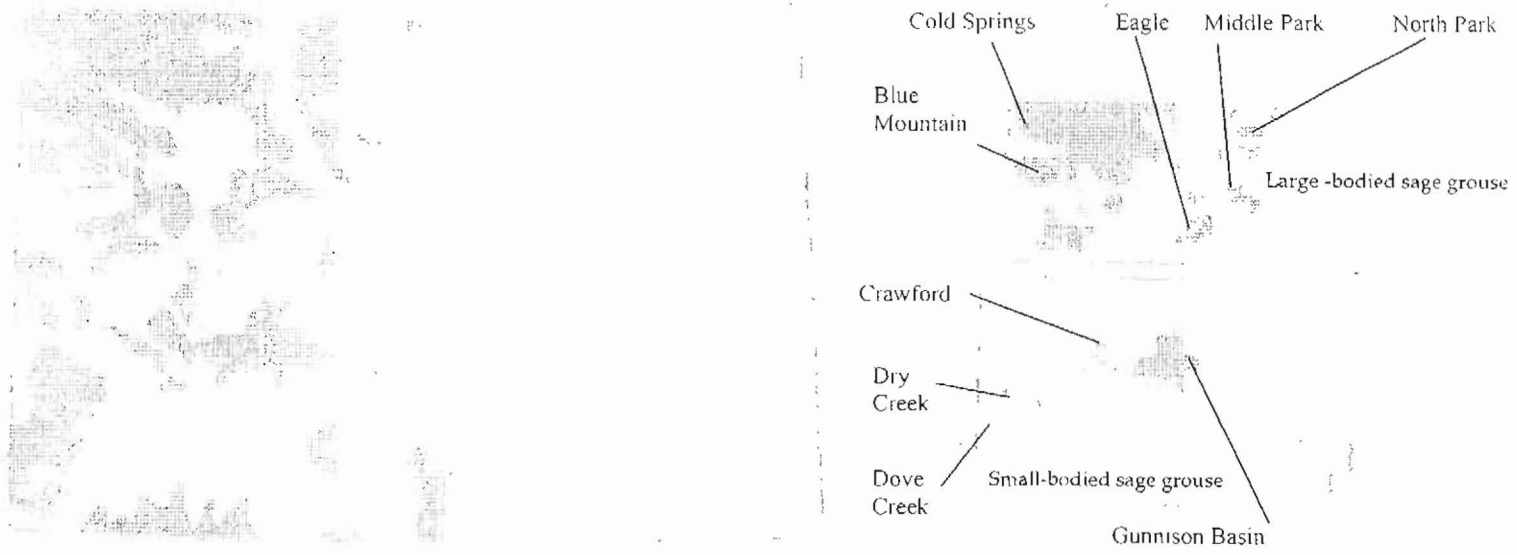

Figure 2.1. Historic (left) and current (right) distribution of large and small-bodied sage grouse and sample locations in Colorado. The boundary between the ranges of large and small-bodied birds is shown on the right.

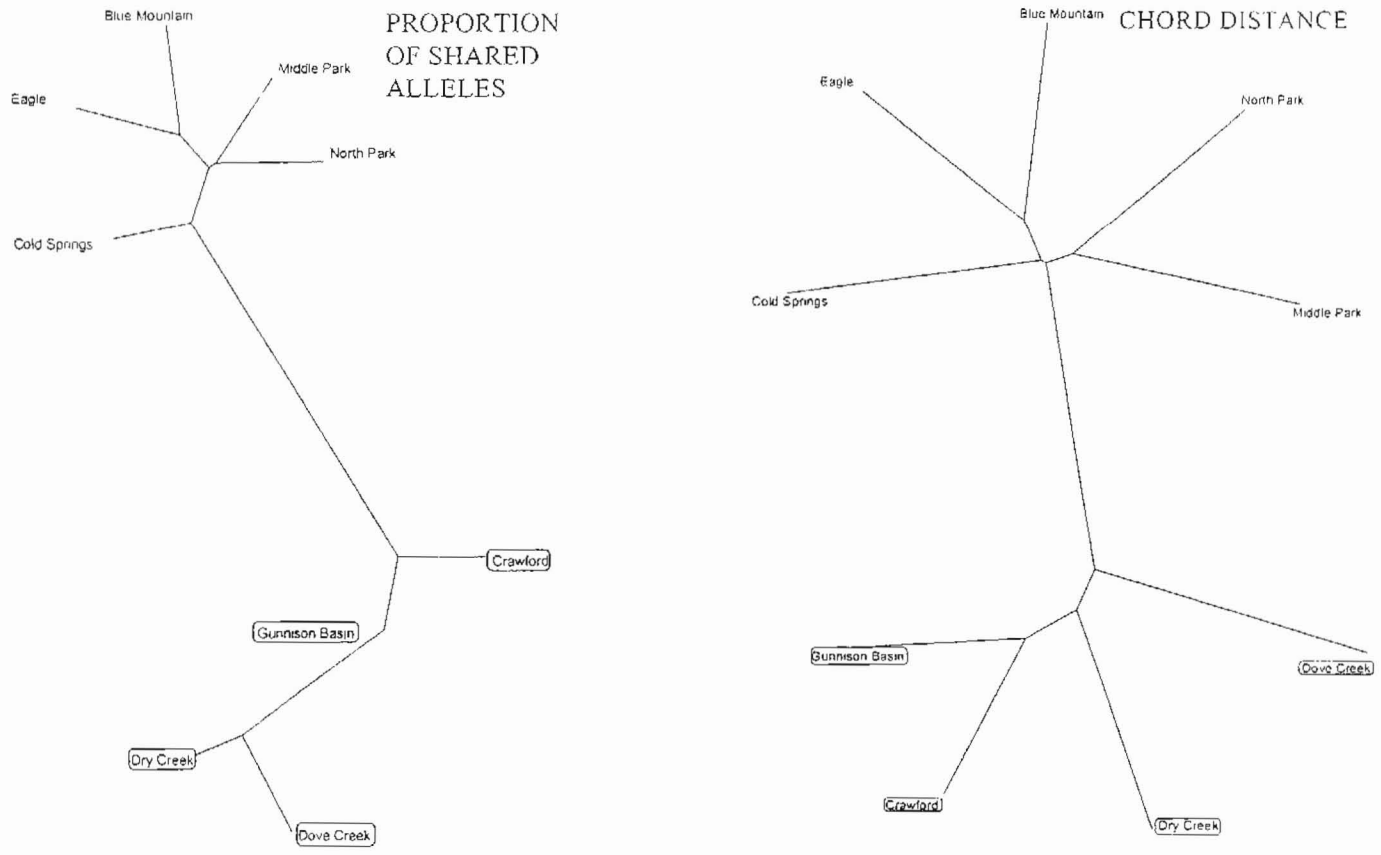

Figure 2.2. Neighbor joining trees of microsatellite data using two different genetic distance measures. Small-bodied populations are identified by a box around the name.

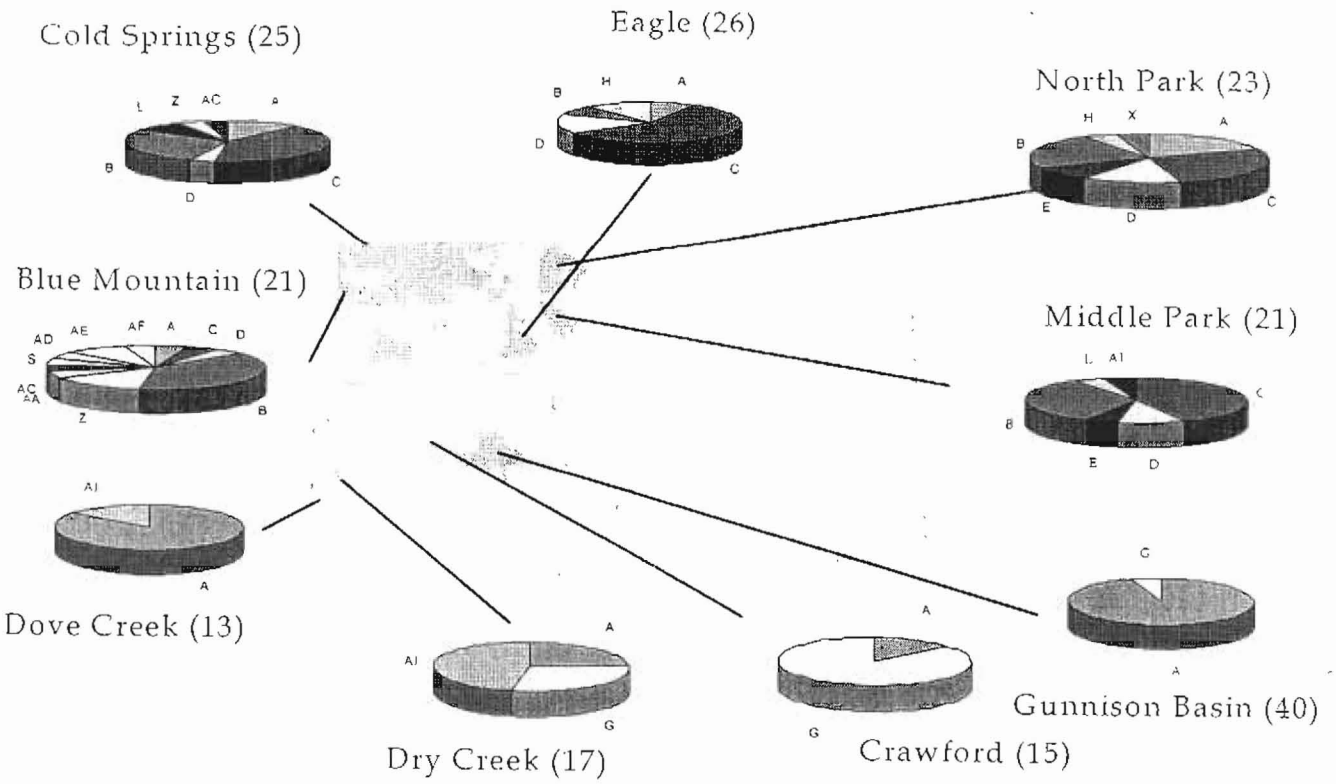

Figure 2.3. Distribution of 19 mtDNA haplotypes among nine populations of sage grouse in Colorado. Number in parentheses represents sample size for each population.

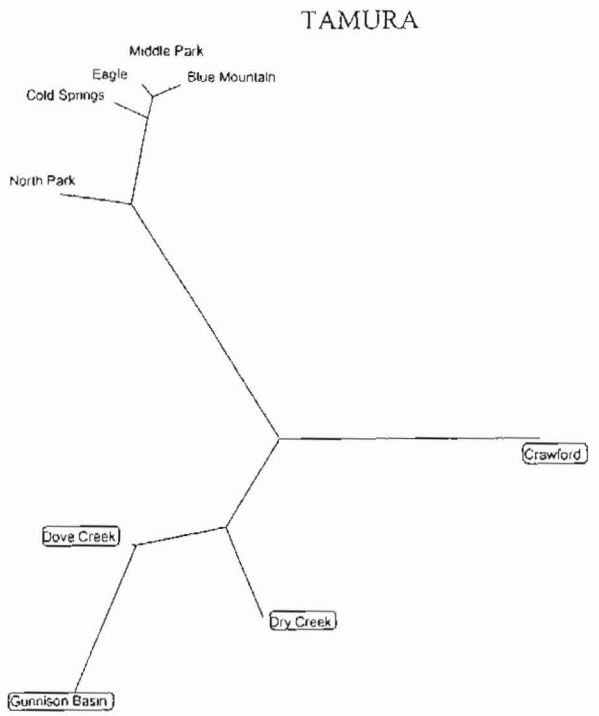

Figure 2.4. Neighbor joining tree of mtDNA genetic distances calculated using allele frequencies and haplotype distances (Tamura 1992). Small-bodied populations are identified by a box around the name.

Appendix 2A. Distribution of alleles (reported as fragment length in base pairs) for four microsatellite loci and mtDNA haplotypes among nine populations of sage grouse in Colorado. The first four populations are small-bodied sage grouse and the last five populations are large-bodied sage grouse.

Table 2A.1. Allele distributions for microsatellite LLST1 among nine populations of sage grouse in Colorado.

Gunnison			Crawford			Dry Creek			Dove Creek			Cold Springs			Blue Mountain			North Park			Middle Park			Eagle		
GB1	154	154	CR1	154	157	DYC1	154	154	DVC1	154	154	CS1			BM2	154	154	NP1	154	154	MP1	154	154	EG1	154	154
GB2	154	154	CR2	154	154	DYC2	154	154	DVC2	154	154	CS2	154	154	BM3			NP2	154	163	MP2	154	157	EG2	154	154
GB3	154	154	CR3	154	154	DYC3	154	154	DVC3	154	154	CS3	154	157	BM4	154	154	NP3	154	163	MP3	154	154	EG3	154	157
GB4	154	157	CR4	154	154	DYC4	154	154	DVC4	154	154	CS4	154	154	BM5	154	154	NP4	154	183	MP4	154	157	EG4	154	157
GB5	154	157	CR5	154	154	DYC5	154	154	DVC5	154	154	CS5	154	154	BM6	154	157	NP5			MP5	154	154	EG5	157	157
GB6	154	154	CR6	154	157	DYC6	154	154	DVC6	154	154	CS6	154	154	BM7	154	154	NP6	154	154	MP6	154	154	EG6	154	154
GB7	154	157	CR7	154	154	DYC7	154	154	DVC7	154	154	CS7	154	154	BM8	154	154	NP7	154	163	MP7	154	154	EG7	154	154
GB8	154	157	CR8	154	157	DYC8	154	154	DVC8	154	154	CS8	154	157	BM9	154	154	NP8	154	154	MP8	154	154	EG8	154	154
GB9	154	154	CR9	154	154	DYC9			DVC9	154	154	CS9	154	154	BM10	154	154	NP9	154	154	MP9	154	163	EG9	154	157
GG1	154	154	CR10	154	154	DYC10	154	154	DVC10	154	154	CS10	154	154	BM11	154	157	NP10	154	154	MP10			EG10		
GG2	154	154	CR11	154	157	DYC11	154	154	DVC11	154	154	CS11	154	154	BM12	154	154	NP11	154	157	MP11	154	154	EG11	154	154
GG3	154	157	CR12	154	157	DYC12	154	154	DVC12	154	154	CS12	154	157	BM13	154	157	NP12	154	154	MP12	151	157	EG12	154	157
GG4	157	157	CR13	154	154	DYCF1	154	154	DVCP1	154	154	CS13	154	154	BM14	154	157	NP13	154	157	MP13	154	157	EG13	154	154
GG5			CR14	154	154	DYCF2	154	154	DVCF2	154	154	CS14	154	157	BM15	154	157	NP14	154	157	MP14	154	154	EG14	154	157
GG6	154	154	CR15	154	157	DYCF3			DVCF3	154	154	CS15	154	157	BM16	154	157	NP15	154	154	MP15	154	154	EG16	154	154
GG7	154	157	CR16			DYCP4	154	154				CS16	154	154	BM17	154	154	NP16	154	154	MP16	151	157	EG18	154	154
GG8	154	157	CEF1	154	154	DYCF5						CS17			BM18	154	154	NP17	154	157	MP17	154	157	EG19	154	157
GG9	154	154	FM2			DYCF6	154	154				CS18	154	157	BM19	154	154	NP18	154	154	MP18	154	154	EG20	157	157
GG10	154	157	FM3	154	157	DYCF7	154	154				CS19	154	154	BM20	154	154	NP19	154	154	MP19	151	154	EG21	154	157
GG11	154	154	FM4			DYCF8	154	154				CS20	154	157	BM21	154	154	NP20	154	154	MP20	154	154	EG22	154	154
GG12	154	154	PM5			DYCF9	154	154				CS21	154	154	BM22	154	154	NP21	154	154	MP21	154	157	EG23	154	154
GG13	154	154				DYCF10	154	154				CS22			BM23	154	154	NP22	154	157				EG24	154	154
GG14												CS23			BM24	154	154	NP23								
GG15	154	154										CS24	154	157	BM25	154	154	NP24	154	157						
GG16	154	157										CS25	157	157				NP25	154	157						
GG17	154	157										CS26	154	157												
GG18	154	154																								
GG19	154	157																								
GG20	154	154																								

Table 2A.2. Allele distributions for microsatellite LLSD8 among nine populations of sage grouse in Colorado.

Gunnison			Crawford			Dry Creek			Dove Creek			Cold Springs			Blue Mountain			North Park			Middle Park			Eagle		
GB1	143	143	CR1	143	143	DYC1	143	143	DVC1	143	143	CS1	137	137	BM2	137	143	NP1	137	143	MP1	137	143	EG1	137	143
GB2	137	143	CR2	143	143	DYC2	143	143	DVC2	143	143	CS2	137	143	BM3			NP2	137	157	MP2	137	157	EG2	137	143
GB3	143	143	CR3	143	143	DYC3	143	143	DVC3	143	143	CS3	143	157	BM4			NP3	137	137	MP3	137	143	EG3	137	143
GB4	143	143	CR4	143	143	DYC4	143	143	DVC4	143	143	CS4	137	143	BM5	137	157	NP4	137	157	MP4	137	157	EG4	137	157
GB5	143	143	CR5	143	143	DYC5	143	143	DVC5	143	143	CS5	137	157	BM6	137	137	NP5	137	157	MP5	143	157	EG5	137	157
GB6	143	143	CR6	143	143	DYC6	143	143	DVC6	143	143	CS6	143	157	BM7	137	137	NP6	137	157	MP6	137	157	EG6	137	157
GB7	143	143	CR7	143	143	DYC7	143	143	DVC7	143	143	CS7	137	157	BM8	137	163	NP7	137	143	MP7	137	143	EG7	137	137
GB8	143	143	CR8	143	143	DYC8	143	143	DVC8	143	143	CS8	143	137	BM9	137	157	NP8	137	137	MP8	137	157	EG8	137	137
GB9	143	143	CR9	143	143	DYC9	143	143	DVC9	143	143	CS9	157	157	BM10	143	143	NP9	143	1557	MP9	137	137	EG9	137	157
GG1	143	143	CR10	143	143	DYC10	143	143	DVC10	143	143	CS10	137	143	BM11	137	137	NP10	137	137	MP10	137	143	EG10		
GG2	143	143	CR11	143	143	DYC11	143	143	DVC11	143	143	CS11	137	137	BM12	137	157	NP11	137	157	MP11	137	143	EG11	137	137
GG3	143	143	CR12	143	143	DYC12	143	143	DVC12	143	143	CS12	137	157	BM13	137	157	NP12	137	157	MP12	137	157	EG12	137	137
GG4	143	143	CR13	143	143	DYCF1	143	143	DVCF1	143	143	CS13	137	143	BM14	137	137	NP13	137	137	MP13	137	157	EG13	137	157
GG5	143	143	CR14	143	143	DYCF2	143	143	DVCF2	143	143	CS14	137	157	BM15	137	137	NP14	137	143	MP14	137	143	EG14	137	157
GG6	143	143	CR15	143	143	DYCF3			DVCF3	143	143	CS15	137	157	BM16	137	157	NP15	137	143	MP15	137	137	EG16	137	157
GG7	143	143	CR16	143	143	DYCF4	143	143				CS16	157	157	BM17	143	157	NP16	137	137	MP16	137	143	EG18	137	143
GG8	143	143	FM1			DYCF5						CS17			BM18	137	157	NP17	143	143	MP17	137	157	EG19	137	143
GG9	143	143	FM2			DYCF6						CS18	137	143	BM19	137	143	NP18	143	157	MP18	137	137	EG20	137	157
GG10	143	143	FM3			DYCF7	143	143				CS19	137	143	BM20	137	157	NP19	137	157	MP19	137	143	EG21	137	137
GG11	143	143	FM4			DYCF8	143	143				CS20	157	157	BM21	137	157	NP20	137	143	MP20	137	143	EG22	143	157
GG12	143	143	FM5			DYCF9	143	143				CS21	137	137	BM22	157	157	NP21	137	137	MP21			EG23	143	157
GG13	143	143	CRF1	143	143	DYCF10						CS22			BM23	137	157	NP22	137	157				EG24	137	143
GG14	143	143										CS23	143	143	BM24	137	143	NP23	137	143						
GG15	143	143										CS24			BM25	137	137	NP24	137	143						
GG16	143	143										CS25						NP25	143	143						
GG17	143	143										CS26														
GG18	143	143																								
GG19	143	143																								
GG20	143	143																								

Table 2A.3. Allele distributions for microsatellite LLSD3 among nine populations of sage grouse in Colorado.

Gunnison			Crawford			Dry Creek			Dove Creek			Cold Springs			Blue Mountain			North Park			Middle Park			Eagle		
GB1	135	137	CR1	133	133	DYC1	135	135	DVC1	133	135	CS1	133	137	BM2	133	141	NP1	133	133	MP1	133	153	EG1	133	141
GB2	133	135	CR2	133	133	DYC2	133	135	DVC2	135	135	CS2	133	141	BM3			NP2			MP2	141	153	EG2	133	141
GB3	133	133	CR3	133	133	DYC3	135	135	DVC3	135	135	CS3	133	133	BM4	141	153	NP3	133	143	MP3	133	141	EG3	133	137
GB4	133	135	CR4	133	133	DYC4	133	135	DVC4	135	135	CS4	133	133	BM5	133	133	NP4	133	133	MP4	133	153	EG4	133	137
GB5	133	133	CR5	133	135	DYC5	135	135	DVC5	133	135	CS5	133	133	BM6	133	137	NP5	133	133	MP5	133	133	EG5	141	153
GB6	133	133	CR6	133	135	DYC6	133	133	DVC6	135	135	CS6	133	133	BM7	141	141	NP6	133	153	MP6	133	137	EG6	133	137
GB7	133	135	CR7	133	133	DYC7	135	135	DVC7	135	135	CS7	133	137	BM8	141	141	NP7	133	141	MP7	141	153	EG7	133	141
GB8	135	135	CR8	133	133	DYC8	135	135	DVC8	135	135	CS8	133	141	BM9	133	133	NP8	133	133	MP8	133	141	EG8	133	141
GB9	133	135	CR9	133	133	DYC9	135	135	DVC9	135	135	CS9	133	135	BM10	133	141	NP9	133	133	MP9	135	141	EG9	133	137
GG1	133	135	CR10	133	133	DYC10	133	135	DVC10	133	133	CS10	133	137	BM11	133	137	NP10	141	141	MP10	133	141	EG10	133	141
GG2	133	135	CR11	133	133	DYC11	135	135	DVC11	133	135	CS11	133	133	BM12	133	141	NP11	133	133	MP11	133	133	EG11	133	137
GG3	133	133	CR12	133	133	DYC12	135	135	DVC12	135	135	CS12	133	133	BM13	133	141	NP12	141	153	MP12	133	141	EG12	133	133
GG4	133	135	CR13	133	135	DYCF1	135	135	DVCF1	135	135	CS13	133	133	BM14	133	141	NP13	141	153	MP13	133	137	EG13	133	137
GG5	133	135	CR14	133	133	DYCF2	135	135	DVCF2	135	135	CS14	133	133	BM15	133	133	NP14	133	141	MP14	133	153	EG14	133	137
GG6	135	135	CR15	133	133	DYCF3	133	133	DVCF3	135	135	CS15	133	133	BM16	133	133	NP15	133	141	MP15	133	133	EG16	133	137
GG7	135	135	CR18	133	133	DYCF4	135	135				CS16	135	141	BM17	133	141	NP16	133	153	MP16	133	141	EG18	133	153
GG8	133	133	FM1			DYCF5	135	135				CS17			BM18	133	153	NP17	153	153	MP17	133	153	EG19	133	137
GG9	133	135	FM2	133	133	DYCF6	135	135				CS18	135	141	BM19	133	141	NP18	133	141	MP18	133	135	EG20	133	137
GG10	133	135	FM3	133	133	DYCF7	133	133				CS19	133	141	BM20	133	141	NP19	133	133	MP19	133	137	EG21	133	141
GG11	133	135	FM4	133	133	DYCF8	133	133				CS20	133	133	BM21	133	137	NP20	133	141	MP20	133	133	EG22	133	137
GG12	133	133	FM5			DYCF9	135	135				CS21	133	141	BM22	153	153	NP21	133	133	MP21			EG23		
GG13	133	133	CRF1			DYCF10						CS22			BM23	133	153	NP22	133	153				EG24	133	141
GG14	133	133										CS23			BM24	133	141	NP23	133	141						
GG15	135	137										CS24			BM25	133	133	NP24	133	141						
GG16	133	135										CS25						NP25								
GG17	133	135										CS26														
GG18	133	137																								
GG19	133	135																								
GG20	135	135																								

Table 2A.4. Allele distributions for microsatellite LLSD4 among nine populations of sage grouse in Colorado.

Gunnison			Crawford			Dry Creek			Dove Creek			Cold Springs			Blue Mountain			North Park			Middle Park			Eagle		
GB1	191	191	CR1	191	203	DYC1	191	203	DVC1	199	201	CS1	195	197	BM2	193	193	NP1	187	207	MP1	207	207	EG1	185	329
GB2	191	201	CR2	203	203	DYC2			DVC2	191	201	CS2	195	243	BM3			NP2	195	215	MP2	193	193	EG2	185	329
GB3	191	191	CR3	191	191	DYC3	191	205	DVC3	191	201	CS3	189	189	BM4	187	197	NP3	189	215	MP3	189	189	EG3	189	215
GB4	191	225	CR4	193	193	DYC4	203	207	DVC4	201	201	CS4	193	225	BM5	191	289	NP4	191	205	MP4	193	215	EG4	189	245
GB5	191	191	CR5	191	225	DYC5	215	215	DVC5	191	199	CS5	195	243	BM6	185	329	NP5	203	215	MP5	183	209	EG5	215	267
GB6	191	191	CR6	193	203	DYC6	215	215	DVC6	201	201	CS6	189	195	BM7	281	329	NP6	205	245	MP6			EG6	187	267
GB7	203	225	CR7	191	203	DYC7	191	191	DVC7	201	201	CS7	193	203	BM8	189	357	NP7	203	245	MP7	193	205	EG7	219	297
GB8	191	203	CR8	191	203	DYC8	191	191	DVC8	201	201	CS8	195	215	BM9	267	321	NP8	193	205	MP8	183	189	EG8		
GB9	191	215	CR9	191	203	DYC9	191	215	DVC9	191	201	CS9	189	197	BM10	189	195	NP9	203	207	MP9			EG9	187	187
GG1	191	191	CR10	191	191	DYC10	191	217	DVC10	191	201	CS10	205	391	BM11	189	191	NP10	207	207	MP10	187	309	EG10		
GG2	191	191	CR11	191	225	DYC11			DVC11	191	201	CS11	195	243	BM12	195	195	NP11	205	215	MP11	189	189	EG11	215	267
GG3	191	203	CR12	193	203	DYC12			DVC12	191	191	CS12	197	391	BM13	169	195	NP12	205	205	MP12	183	207	EG12		
GG4	191	191	CR13	191	191	DYCF1	191	203	DVCF1	191	191	CS13	197	215	BM14	309	329	NP13	205	245	MP13	189	189	EG13	187	225
GG5	191	215	CR14	193	203	DYCF2	191	203	DVCF2	199	201	CS14	197	215	BM15	189	193	NP14	205	205	MP14	189	193	EG14		
GG6	215	225	CR15	193	203	DYCF3			DVCF3			CS15	197	215	BM16	223	267	NP15	191	245	MP15	189	193	EG16	195	267
GG7	191	191	CR16	203	203	DYCF4	191	217				CS16	183	197	BM17	185	323	NP18	195	205	MP16	183	195	EG18	195	215
GG8	191	205	FM1			DYCF5						CS17			BM18	187	289	NP17	205	239	MP17	189	205	EG19	187	267
GG9	191	191	FM2			DYCF6						CS18	187	193	BM19			NP18	191	205	MP18	189	193	EG20	187	193
GG10	191	205	FM3			DYCF7	217	217				CS19	197	255	BM20	187	187	NP19	187	189	MP19	187	189	EG21	189	287
GG11	193	219	FM4			DYCF8	191	191				CS20	189	255	BM21	189	329	NP20	209	245	MP20			EG22	195	267
GG12	191	191	FM5			DYCF9						CS21			BM22			NP21			MP21			EG23	187	205
GG13	191	203	CRF1			DYCF10						CS22			BM23			NP22						EG24	187	215
GG14	191	203										CS23			BM24			NP23								
GG15	191	191										CS24			BM25			NP24								
GG16	191	191										CS25						NP25								
GG17	193	215										CS26														
GG18	191	191																								
GG19	191	205																								
GG20	191	191																								

Table 2A.5. Distribution of mitochondrial DNA haplotypes among nine populations of sage grouse in Colorado.

Population	Haplotype																		
	A	B	C	D	E	G	H	L	S	X	Z	AA	AC	AD	AE	AF	AI	AL	AM
Gunnison Basin	38					2													
Crawford	2					15													
Dry Creek	4					6											8		
Dove Creek	11																2		
Cold Springs	3	7	10	1				2			1		1						
Blue Mountain	1	8	1	1					1		3	1	1	1	2	1			
Middle Park		7	9	2	1		1											1	
North Park	4	5	6	3	2		1			1									1
Eagle	2	2	15	4			3												

Table 2A.6. *P* values of pairwise population F_{ST} tests for microsatellites among all pairs of populations of sage grouse in Colorado. The first four populations are small-bodied and the last five populations are large-bodied. The average *P* value of comparisons among small-bodied bird is 0.0055, of comparisons between large vs. small-bodied birds is 0.0000, and of comparisons among large-bodied birds is 0.1171.

Population	Gunnison	Crawford	Dry Creek	Dove Creek	Cold Springs	Blue Mountain	North Park	Middle Park
Crawford	0.0009							
Dry Creek	0.0073	0.0000						
Dove Creek	0.0000	0.0000	0.0250					
Cold Springs	0.0000	0.0000	0.0000	0.0000				
Blue Mountain	0.0000	0.0000	0.0000	0.0000	0.1746			
North Park	0.0000	0.0000	0.0000	0.0000	0.0538	0.0903		
Middle Park	0.0000	0.0000	0.0000	0.0000	0.1666	0.2320	0.1058	
Eagle	0.0000	0.0000	0.0000	0.0000	0.0929	0.1672	0.0239	0.0641

Table 2A.7. P values of pairwise population F_{ST} tests for mtDNA among all pairs of populations of sage grouse in Colorado. The first four populations are small-bodied and the last five populations are large-bodied. The average P value of comparisons among small-bodied bird is 0.0124, of comparisons between large vs. small-bodied birds is 0.0000, and of comparisons among large-bodied birds is 0.3739.

Population	Gunnison	Crawford	Dry Creek	Dove Creek	Cold Springs	Blue Mountain	North Park	Middle Park
Crawford	0.0000							
Dry Creek	0.0000	0.0005						
Dove Creek	0.0020	0.0000	0.0717					
Cold Springs	0.0000	0.0000	0.0000	0.0000				
Blue Mountain	0.0000	0.0000	0.0000	0.0002	0.5890			
North Park	0.0000	0.0000	0.0000	0.0000	0.3888	0.1832		
Middle Park	0.0000	0.0000	0.0000	0.0000	0.4118	0.4737	0.0787	
Eagle	0.0000	0.0000	0.0000	0.0000	0.5838	0.3796	0.1481	0.5019

CHAPTER THREE

POPULATION GENETICS OF GUNNISON SAGE GROUSE: IMPLICATIONS FOR MANAGEMENT

INTRODUCTION

The distribution and abundance of sage grouse in Colorado have been greatly reduced primarily due to habitat loss and fragmentation (Braun 1995). Sage grouse have been extirpated from 12 of the 27 counties in Colorado in which they occurred in the 1900's and populations in nine of the remaining 15 counties are thought to number less than 500 breeding birds (Braun 1995). Sage grouse in southwestern Colorado have been the most severely impacted by destruction and fragmentation of habitat and, as a result, populations are small and isolated (Fig. 3.1).

Recently, sage grouse in southwestern Colorado and southeastern Utah have been described as a new species of sage grouse, i.e., the Gunnison sage grouse (*Centrocercus minimus*) (Braun and Young 1995). This new species distinction was based on morphological and behavioral data (Hupp and Braun 1991, Young 1994, Young et al. 1994), and later supported by genetic data (Kahn et al. 1999, Chapter Two). Because Gunnison sage grouse are 33% smaller than all other sage grouse, I refer to them as either Gunnison sage grouse or more generally as small-bodied sage grouse. In Chapter Two I

compared five large-bodied populations from northern Colorado with four small-bodied populations from southwestern Colorado using mitochondrial and nuclear markers. I found that small-bodied sage grouse have much less genetic diversity than large-bodied sage grouse and that there was markedly less gene flow among the four Gunnison sage grouse populations in southwestern Colorado than among the five populations of large-bodied sage grouse in northern Colorado. As a result, I argue that genetic data should be considered in management decisions for Gunnison sage grouse. This chapter uses the results from Chapter Two to address specific management implications for Gunnison sage grouse.

Microsatellites are thought to be among the most powerful markers in population genetic studies today because of their high rate of mutation (Goldstein and Pollock 1997), which makes them extremely useful in distinguishing differences among populations thought to have low genetic diversity. Mitochondrial DNA, while rapidly evolving, is maternally inherited and thus, masks any effect of male dispersal. Because I was interested in the implications of relative amounts of gene flow and isolation, I refer only to the microsatellite data presented in Chapter Two. The specific objectives of this chapter were to examine the genetic diversity of each Gunnison sage grouse population and gene flow among these populations and to make management recommendations based on this information.

STUDY AREA

Gunnison sage grouse have an extremely limited range as they are restricted to southwestern Colorado and southeastern Utah. In southwestern Colorado, five populations have been studied (Gunnison Basin, Dove Creek, Dry Creek, Crawford, and Glade Park). Only one other population near Poncha Pass has recently been documented consistently by lek surveys. Samples were obtained from all five studied populations, but the Glade Park population was omitted due to insufficient sample size.

The largest area of contiguous habitat and consequently the largest population occurs in the Gunnison Basin (Fig. 3.2) which supports approximately 2,600 birds in the breeding season and is thought to be a stable population (C. E. Braun, Colorado Division of Wildlife, unpublished data). The Crawford population (Fig. 3.2) underwent a severe decline until 1994 but as a result of a habitat manipulation, has rebounded (Commons 1997, Commons et al. 1999) to approximately 175 birds (C. E. Braun, Colorado Division of Wildlife, unpublished data). The Dry Creek population (Fig. 3.2) is stable or declining with approximately 300 birds and the Dove Creek population (Fig. 3.2) is declining with approximately 75 birds (C. E. Braun, Colorado Division of Wildlife, unpublished data).

The Gunnison Basin is an intermontane basin ranging in elevation from 2,300 to 2,900 m with several flat-topped mesas. The lower lands consist of broad, alluvial flood plains which abut major streams, and the uplands have moderate to steep slopes dissected by intermittent streams. Dominant vegetation includes mountain big sagebrush (*Artemisia tridentata vaseyana*), and black sagebrush (*A. nova*), intermixed with antelope bitterbrush

(*Purshia tridentata*), and mountain snowberry (*Symphoricarpos oreophilus*) (Hupp and Braun 1989).

The Crawford population in Montrose County is northwest of the Gunnison Basin separated by the Black Canyon of the Gunnison River and Black Mesa with elevations ranging from 1,968 to 2,952 m. Large mesas dominate the landscape around the town of Crawford and the area is bisected north to south by deep canyons. The dominant vegetation consists of a mix of mountain big sagebrush, black sagebrush, pinon pine (*Pinus edulis*), and juniper (*Juniperus* spp.). At higher elevations, gambel oak (*Quercus gambelii*) and serviceberry (*Amelanchier* spp.) intermix with mountain big sagebrush (Commons 1997).

The Dry Creek population in San Miguel County is southwest of Naturita and Norwood. This area is semi-arid high desert and ranges in elevation from 1,936 m in Dry Creek Basin to 2,385 m at Miramonte Resevoir. Dry Creek Basin is an old glacial bed which consists of flats, gently rolling hills, and deep drainages surrounded by mesas to the north, south, and east. This area is dominated by basin big sagebrush, low sagebrush (*A. arbuscula*), and winterfat (*Eurotia lanata*). The sagebrush dominated area north of Miramonte Reservoir is characterized by gently rolling hills and shallow drainages. The dominant vegetation surrounding the reservoir is black sagebrush and mountain big sagebrush with pinon pine and juniper invading (Commons 1997).

The Dove Creek population is approximately 58 km southwest of Dry Creek isolated by the Dolores Canyon and Disappointment Valley. Dove Creek, in Dolores County, is semi-arid desert ranging in elevation from 2,020 to 2,303 m. The town of

Dove Creek bisects the population with the northern area dominated by pinto bean, alfalfa, and wheat production, and the southern half used mainly for wheat production. Portions of the agricultural areas were enrolled in the Conservation Reserve Program (CRP) in the late 1980's. The area consists mostly of rolling hills and deep drainages bounded to the south by Squaw Canyon and to the northeast by Dolores Canyon. The northern part of the Dove Creek area consists of farmland and areas dominated by mountain big sagebrush, black sagebrush, gambel oak, pinon pine, juniper, ponderosa pine (*P. ponderosa*), mountain snowberry, serviceberry, antelope bitterbrush, and chokecherry (*Prunus* spp.). The southern Dove Creek area is dominated by farmland with small areas of basin and mountain big sagebrush, rabbitbrush (*Chrysothamnus* spp.), and broom snakeweed (*Gutierrezia sarothrae*).

METHODS

Complete methods for blood and feather sample collection, DNA extraction, PCR, and visualization are presented in Chapter Two.

Data Analysis

Two measures of genetic distance were calculated for all pairs of small-bodied populations, the proportion of shared alleles (Bowcock et al. 1994), and chord distance (Cavalli-Sforza and Edwards 1967). Each genetic distance metric was calculated by determining the genetic distance for each locus and averaging across loci. I used a Mantel test to determine whether there was a relationship between genetic difference

(F_{ST}) and geographic distance. To compare the amount of population subdivision within the small-bodied birds to the amount of population subdivision within the large-bodied birds, I calculated Wright's (1951) F_{ST} statistic for both groups of populations. Pairwise population F_{ST} significance tests were also conducted among all populations to test whether pairs of populations were statistically different. I documented the amount of genetic diversity per population by calculating mean heterozygosity and mean number of alleles per locus for each population, and by counting the number of unique alleles for each population.

RESULTS

The two measures of genetic distance show somewhat similar patterns (Table 3.1). Using the proportion of shared alleles distance, the smallest genetic distance was between Dove Creek and Dry Creek and the largest was between Crawford and Dove Creek. The chord distance metric showed a similar ranking, yet found the pairs of Gunnison Basin/Crawford and Gunnison Basin/Dry Creek to be closer than the Dove Creek/Dry Creek pair that the other metric ranked as closest. Both metrics agreed that Gunnison Basin/Dove Creek, Crawford/Dry Creek, and Crawford/Dove Creek should be ranked at four, five, and six. The relationship among these four populations was represented using a neighbor joining tree (Fig. 3.3). Both genetic distance measures produced similar trees with Gunnison Basin and Crawford clustering together and Dove Creek and Dry Creek clustering together. Separate Mantel tests for the large and small-

bodied birds both revealed no significant isolation by distance for the small (P = 0.3127) or large-bodied bird (P = 0.4356) populations.

A comparison of F_{ST} values between large and small-bodied birds revealed that the small-bodied birds were significantly more subdivided (F_{ST} = 0.2178, 95% CI 0.1230 - 0.3339) than the large-bodied birds (F_{ST} = 0.0266, 95% CI -0.0016 - 0.0528). Pairwise population F_{ST} significance tests also indicated significant population subdivision (Table 3.2). A P value of 0.005 was used to indicate statistical significance because of the multiple comparisons nature of the analysis. As would be expected for comparisons of populations from different species, all small vs. large-bodied populations were significantly different. There were no differences between pairs of large-bodied populations suggesting substantial gene flow among the five large-bodied populations. Only two pairs of populations within the small-bodied birds were not significantly different at P = 0.005 (Dry Creek and Gunnison Basin, P = 0.0073; Dry Creek and Dove Creek, P = 0.025) suggesting isolation and reduced gene flow among the small-bodied birds.

In comparing the genetic diversity of large and small-bodied sage grouse, the small-bodied sage grouse populations had less genetic diversity, reduced heterozygosity and fewer polymorphic loci (Table 3.3). The amount of diversity of Gunnison sage grouse populations varies substantially with the Gunnison Basin having the highest number of unique alleles, the highest mean number of alleles per locus and the highest mean heterozygosity. The Dove Creek population had the fewest number of unique

alleles, the lowest mean number of alleles per locus, and the second lowest heterozygosity.

DISCUSSION

My comparison of large and small-bodied sage grouse in Colorado has shown that small-bodied Gunnison sage grouse populations are isolated with relatively little gene flow among populations and much less genetic diversity than the large-bodied sage grouse. Furthermore, three of the four Gunnison sage grouse populations are small, and at risk of extinction. Together these factors provide evidence suggesting that the viability of at least three of the Gunnison sage grouse populations should be addressed.

There has been much concern about the viability of small populations and how it might be affected by demographic, environmental, and genetic stochasticity, as well as catastrophes (Shaffer 1981, Soule 1987). Although minimum viable population sizes vary enormously among different species, it is generally thought that populations smaller than a few hundred individuals warrant at least investigation into possible negative effects that accompany small populations (Shaffer 1987). The persistence of wild populations is usually influenced more by ecological effects (such as the direct effects of catastrophes and environmental and demographic stochasticity) than by genetic effects. Yet when wild populations are reduced to small populations by artificial means such as habitat destruction, genetic factors and their interaction with ecological factors become increasingly important (Lande 1995).

Historically, Dove Creek, Dry Creek, and Crawford all had much larger populations which were somewhat connected through more contiguous areas of sagebrush habitat (Fig. 3.1). It is Braun's (1995) assertion that clearing of sagebrush for cultivated crops, highway construction, ranch development, powerline placement, reservoir construction, and other facets of human settlement have resulted in fragmentation and loss of sagebrush habitats in southwestern Colorado leading to the current isolation of these populations which is consistent with the relatively low amounts of gene flow documented in this dissertation. This human-induced reduction in population sizes of Gunnison sage grouse leads me to believe that the Dove Creek, Dry Creek, and Crawford populations are at risk from the direct effects of catastrophes, environmental and demographic stochasticity, and, potentially, to the effects of inbreeding.

Being a lek breeding species, sage grouse have less genetic diversity than other non-lekking grouse (Leberg 1991, Young 1994). Because only approximately 10 - 20 % of males on leks actually breed and up to 80% of all matings on a lek each year are by one or two males (Wiley 1973, Vehrencamp et al. 1989, J. R. Young, Western State College, unpublished data) the effective size of sage grouse populations is likely much lower than the actual population size. Many equations exist to quantify effective population size which take into account different scenarios. A simple equation for effective population size which takes into account unequal sex ratios is described by Hartl and Clark (1989) as

$$Ne = \frac{4(N_{males})(N_{females})}{N_{males} + N_{females}}.$$

This is a simplified model for calculating effective population size which assumes discrete, non overlapping generations, and accounts only for differences in sex ratio. However, it can be used to provide an idea of the reduction in effective population size of sage grouse due to the lek breeding system. If a breeding population has 300 birds, 100 males and 200 females (the sex ratio for sage grouse is generally two females to one male), the effective population size can be calculated to be between 38 and 73 birds depending on whether it is assumed that 10 or 20% of the males breed. Either way, this is a substantial reduction from the natural population size of 300.

It is highly debated whether reduced genetic variation reduces the viability of a population. Avise (1994) warns that caution should be used in interpreting low variation in populations for a variety of reasons including knowledge that at least a few successful, widespread species have low genetic variation; in some endangered species like the elephant seal, (*Mirounga angustirostris*) (Bonnel and Selander 1974), lack of genetic variation has not seemed to seriously inhibit population recovery, and the effect of inbreeding on fitness differs widely among species with some being highly affected and some seemingly unaffected (Price and Waser 1979, Ralls and Ballou 1983, Ralls et al. 1988, Laikre and Ryman 1991). Lande (1988) argues that low reproductive output in small populations may be due to non-genetic factors such as the Allee effect (Andrewartha and Birch 1954). Furthermore, small populations, (regardless of the

amount of genetic variation) are at risk of extinction because of demographic fluctuations (Gilpin and Soule 1986). Because of such factors, Lande (1988) argued that, for conservation plans, demographic and behavioral concerns should be a higher priority than genetic concerns.

Other authors, however, are adamant that genetic variation is extremely relevant to the health and viability of populations and that it must be addressed and monitored in management plans (O'Brien and Evermann 1988, Quattro and Vrijenhoek 1989). Examples of how inbreeding have affected some characters of fitness include the survival, growth, early fecundity, and developmental stability of the Sonoran topminnow (*Poeciliopsis occidentalis sonorensis*) (Quattro and Vrijenhoek 1989), fertility and hatching success of greater prairie-chickens *(Tympanuchus cupido)* (Westemeier, et al. 1998), pair bonding behavior in wolves *(Canis lupus)* (Wayne et al. 1991), and high instances of abnormal sperm in cheetahs *(Acinonyx jubatus)* (O'Brien and Evermann 1988). Further, O'Brien and Evermann (1988) found low variation in the major histocompatibility complex (MHC) in cheetahs and documented a 50 - 60% mortality in cheetahs over a three year period due to a corona virus. They advocate that genetically depauperate populations face enhanced susceptibility to infectious disease or parasitic agents.

MANAGEMENT IMPLICATIONS

Acknowledging Lande's (1988) assertion not to emphasize genetic concerns over other concerns and O'Brien and Evermann's (1988) belief that genetic considerations are

vital to conservation, I advocate addressing both genetic concerns and other ecological concerns (habitat loss, fragmentation) in management of Gunnison sage grouse. This echos Soule and Mills' (1998) idea of not isolating genetics from other factors affecting small populations, but rather addressing all factors (demographic, genetic, habitat-based) together in the preservation of small populations. I believe every attempt should be made to prevent further loss of habitat in any area inhabited by Gunnison sage grouse. Further, I believe the habitat of Gunnison sage grouse should be managed according to established general procedures (Braun et al. 1977), with case by case specifications to address specific problems in different areas (Commons 1997). Conservation plans have been developed for the Gunnison Basin, Dove Creek, Dry Creek, and Crawford populations, and working groups are developing plans for Glade Park and Poncha Springs. The purpose of these community-based conservation working groups is to provide coordinated management across jurisdictional/ownership boundaries and to develop community-wide support necessary to assure the survival of Gunnison sage grouse. With the support of the conservation working groups, population sizes in Dove Creek, Dry Creek, and Crawford should stabilize and/or increase, lessening the extinction risk from demographic and environmental stochasticity.

Because Gunnison sage grouse overall have much lower genetic diversity tban other sage grouse, I feel that a management plan for Gunnison sage grouse should address genetic concerns. Within the Gunnison sage grouse, the Gunnison Basin population has the most diversity followed by Dry Creek, Crawford, and Dove Creek. Because these populations are isolated and because there is little gene flow (relative to the amount of

gene flow among large-bodied birds), I suggest translocating birds from the Gunnison Basin into at least the Dove Creek population, and potentially into the Crawford and Dry Creek populations. The Dove Creek population is of most concern with the lowest genetic diversity (only seven unique alleles and an average of 1.8 alleles per locus) and the smallest actual population size (approximately 75 birds) and effective population size (11 birds, as calculated from equation one, with 50 females, 25 males, and 10% of the males breeding). I advocate translocating four to six females from the Gunnison Basin population into the Dove Creek population every few years to increase the genetic variability in Dove Creek. My recommendation to translocate females rather than males is because only a few males actually breed in each population whereas most females breed. The number of birds to translocate is based on the idea that loss of genetic variability can be countered by immigration from an outside population, assuming it is large enough to maintain genetic variability. Immigration from other populations also has the effect of slowing genetic drift and fixation of alleles (Lande 1995). Wright (1931, 1951, 1969) has shown that analysis of the 'island model' indicated that immigration of a few individuals per generation will prevent loss of genetic variability. I believe that if four to six birds are translocated, then two or three will likely survive the translocation and successfully reproduce. Similar translocations from the Gunnison Basin to Crawford or Dry Creek are advised, yet are not as high of a priority. Natural gene flow is more likely to occur between Gunnison Basin and Crawford since they are geographically closer and there is evidence that gene flow between the Gunnison Basin and Crawford is

higher than between the Gunnison Basin and either Dry Creek or Dove Creek (Table 3.1, Fig. 3.2).

Some may argue that the translocation of birds from the Gunnison Basin to Dove Creek may have a negative impact on the Dove Creek population because of potential outbreeding effects (Dove Creek birds may be highly adapted to the Dove Creek area and birds from the Gunnison Basin would lower the overall fitness of the population). Such effects have been well documented in fisheries management. For example, Chum salmon (*Onochynchus keta*) eggs introduced from a foreign stock resulted in a total decline in stock size to 5% of the original number (Altukhov and Salmenlova 1987). I do not believe this will be the case for sage grouse for several reasons. First, habitat destruction has caused Gunnison sage grouse populations to be isolated. Historically, populations were much larger and well connected (Fig. 3.1). It is conceivable even today, however, that interchange among these four populations could occur naturally as sage grouse have been documented to move up to 114 km between autumn and spring (Berry and Eng 1985). My data show some population differentiation (Table 3.2) and less gene flow relative to large-bodied sage grouse, yet movements between Dry Creek and Dove Creek and between Gunnison Basin and Crawford are not impossible. I do not believe these populations have been truly isolated long enough to develop real genetic differences that might be associated with outbreeding depression. Second, the birds that I advocate translocating are from wild populations, not birds in captivity that have been manipulated by humans. Third, if birds in Dove Creek were extremely well adapted to their habitat and birds from the Gunnison Basin were not adapted to that environment, the birds from

the Gunnison Basin would be at a selective disadvantage and would be outcompeted by the Dove Creek birds. Because I only advocate moving a few birds into an area (contrasted with the huge numbers of eggs moved in fishery studies), I do not believe this would have a negative impact on the population.

The decision whether to translocate birds to increase the genetic diversity in a population like Dove Creek is difficult. There are several scenarios to consider in this situation. First, genetic diversity may not be associated with fitness at all, in which case translocating birds has no effect on the fitness of the population. Second, birds in an area like Dove Creek might be highly adapted to the environment and better suited to succeed than the few birds moved from an area like the Gunnison Basin. Although I believe this scenario is least likely, even if it were true, the few birds translocated would quickly be selected against and their genes eliminated from the population. This should have little or no negative impact on the population. Finally, if genetic diversity is associated with fitness or with the ability for birds to adapt to future environmental changes, then translocating birds into the population will have a positive impact on the population.

The low genetic diversity among all Gunnison sage grouse and the extremely low effective population sizes in Dove Creek, Dry Creek, and Crawford are causes for concern. Maintaining/improving habitats and translocating birds may be insufficient to assure viability of this species. Characteristics of fitness as they relate to genetic diversity must be more closely examined. Research on reproductive features (sperm function, egg normality), parasite load, and disease resistance (e.g., MHC) should be

conducted, comparing both within the small-bodied Gunnison sage grouse and between the large and small-bodied sage grouse.

While genetic concerns may not be the highest priority for Gunnison sage grouse conservation and management, I believe that along with other issues (habitat loss and quality) they should at least be considered. An overall management plan including monitoring and maintaining genetic diversity, preventing future habitat loss and fragmentation, and sound management of current populations and habitat must be implemented to assure the viability of Gunnison sage grouse.

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Table 3.1. Two different genetic distance measures calculated from four microsatellite loci for all pairs of small-bodied sage grouse populations in Colorado. The number in parentheses represents the rank among all populations, one being the pair of populations with the smallest genetic distance and six being the pair of populations with the largest genetic distance.

Population Pairs	Proportion of Shared Alleles	Chord Distance
Gunnison Basin and Crawford	0.252 (2)	0.250 (1)
Gunnison Basin and Dry Creek	0.264 (3)	0.280 (2)
Gunnison Basin and Dove Creek	0.344 (4)	0.367 (4)
Crawford and Dry Creek	0.411 (5)	0.384 (5)
Crawford and Dove Creek	0.456 (6)	0.471 (6)
Dry Creek and Dove Creek	0.188 (1)	0.349 (3)

Table 3.2. Significance ($P < 0.005$) of pairwise population F_{ST} tests for the microsatellite data for sage grouse populations in Colorado. Pairs of populations significantly different are shown by + and those not significantly different are shown by -.

	Small-bodied				Large-bodied			
	Gunnison Basin	Crawford	Dry Creek	Dove Creek	Cold Springs	Blue Mountain	North Park	Eagle
Crawford	+							
Dry Creek	-	+						
Dove Creek	+	+	-					
Cold Springs	+	+	+	+				
Blue Mountain	+	+	+	+	-			
North Park	-	+	+	+	-	-		
Eagle	+	+	+	+	-	-	-	
Middle Park	+	+	+	+	-	-	-	-

Table 3.3. Genetic diversity measures for each sampled population of sage grouse in Colorado.

Population	% Loci Polymorphic	Mean # of alleles per locus		Heterozygosity		Unique Alleles (N)
		$\bar{x}$	SD	$\bar{x}$	SD	
Large-bodied						
Cold Springs	100	5.5	2.5	0.631	0.118	22
Blue Mountain	100	6.5	3.2	0.596	0.120	26
Middle Park	100	5.5	2.2	0.701	0.089	22
North Park	100	5.5	1.6	0.643	0.080	22
Eagle	100	5.5	2.5	0.748	0.145	22
Small-bodied						
Gunnison Basin	75	3.8	1.4	0.386	0.123	15
Crawford	75	2.3	0.6	0.299	0.138	9
Dry Creek	50	2.5	0.6	0.179	0.135	10
Dove Creek	50	1.8	0.5	0.193	0.135	7

Figure 3.1. Historic (top) and current (bottom) distribution of sage grouse and Gunnison sage grouse (lower left cut out) in Colorado.

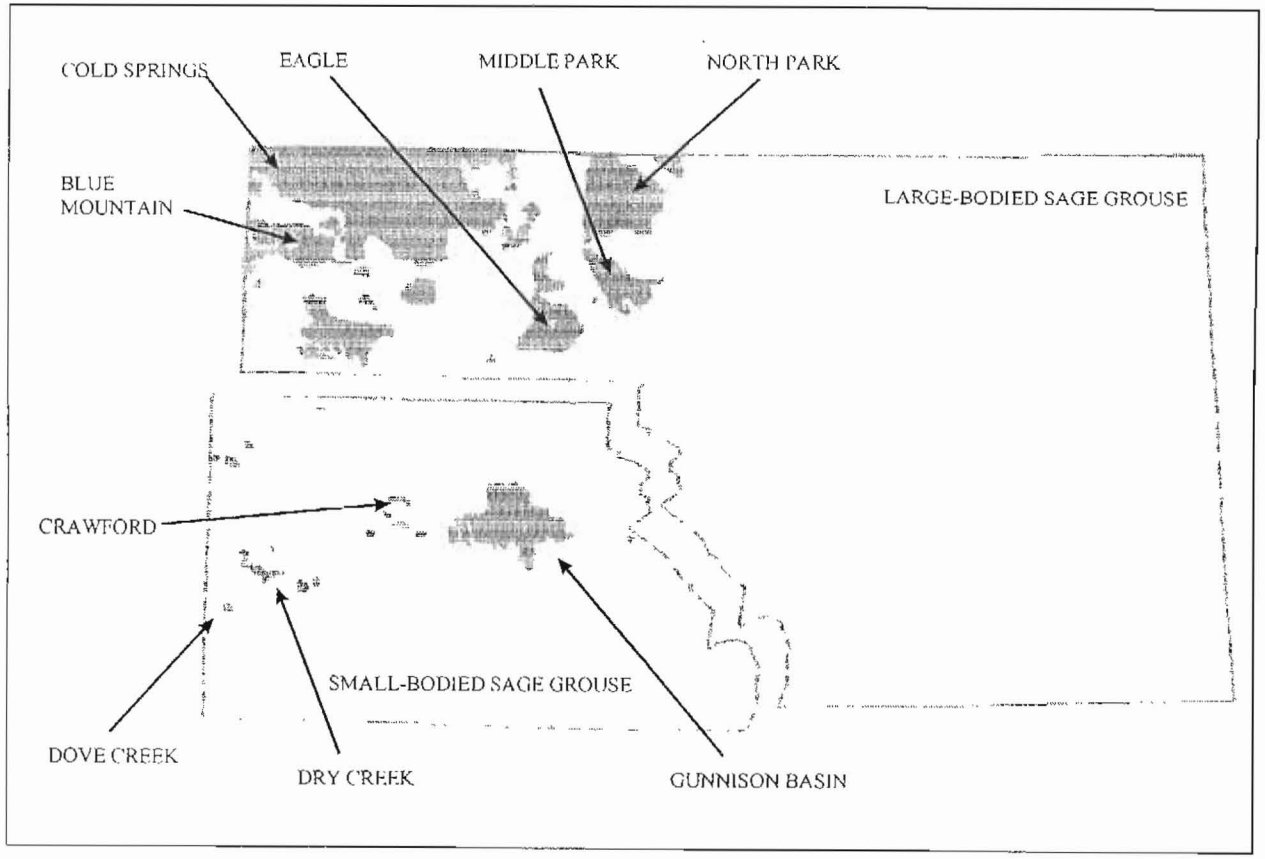

Figure 3.2. Populations of sage grouse sampled in Colorado.

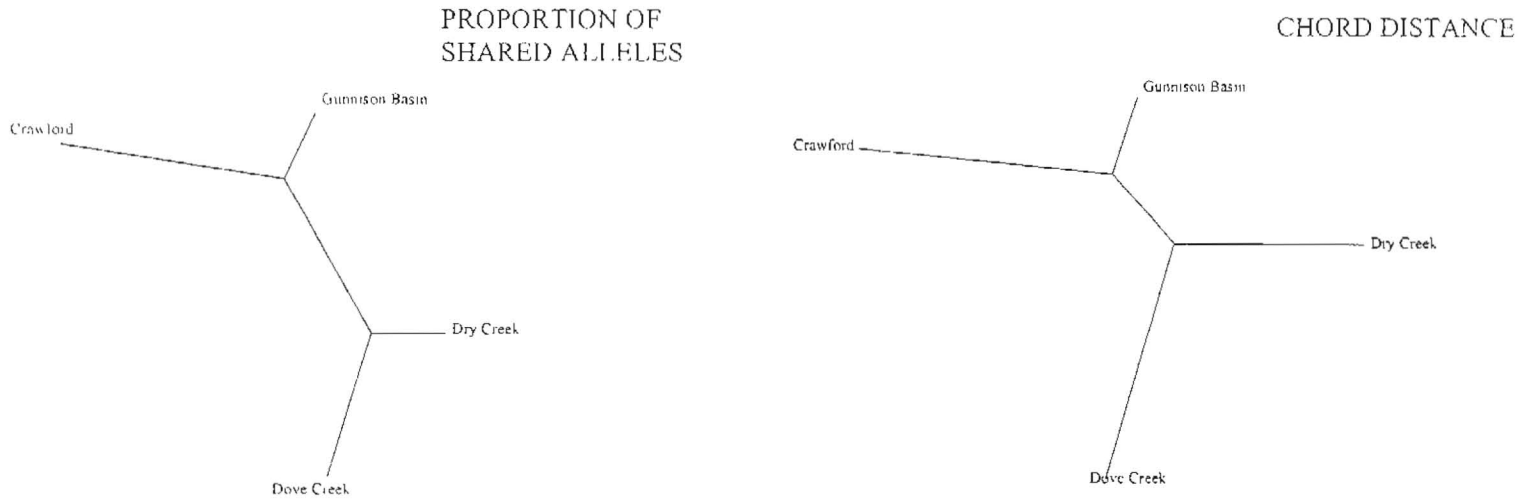

Figure 3.3. Neighbor joining trees of microsatellite data of Gunnison sage grouse populations using two different distance measures.

CHAPTER FOUR

QUANTIFYING CHANGES IN SAGEBRUSH HABITAT IN SOUTHWESTERN COLORADO FROM THE MID-50'S TO THE MID-90'S

INTRODUCTION

Sage grouse, *Centrocercus urophasianus,* historically occurred in at least 15 states and three provinces (Aldrich 1963, Johnsgard 1973), they currently occupy only 11 states and two provinces (Braun 1998). In Colorado, the distribution and abundance of sage grouse has been dramatically reduced (Braun 1995). Sage grouse have been extirpated from 12 of the 27 counties in Colorado in which they occurred in the 1900's and populations in nine of the remaining 15 counties are thought to number less than 500 breeding birds (Braun 1995). Population declines appear to be related to habitat loss (conversion of big sagebrush, *Artemisia tridentata,* into farmland or housing developments), habitat degradation (heavy grazing, sagebrush removal, road and powerline development through sagebrush, and human disturbance), and habitat fragmentation (Braun 1995). Sage grouse habitat in southwestern Colorado, the range of the newly described Gunnison sage grouse, *Centrocercus minimus* (Braun and Young 1995), has been most severely impacted by these processes (Fig. 4.1).

In winter, sage grouse are dependent solely on sagebrush leaves (primarily big sagebrush) for food (Patterson 1952, Wallestad et al. 1975). Due to lack of a grinding gizzard, sage grouse cannot digest plant fiber well (Remington 1989) and, as a result, are dependent upon sagebrush because it retains nutritious leaves all winter. Thus, the loss of sagebrush habitat is likely linked to the decline of Gunnison sage grouse in southwestern Colorado.

Historical records document the occurrence of six species of sagebrush in Colorado (James 1823). Cary (1911:246) described sagebrush to be "omnipresent on the higher plains of western Colorado and also in most of the higher mountain parks up to 10,000 feet". In southwestern Colorado, sagebrush areas included by Cary (1911) were: Debeque to Glenwood and Dotsero, Wolcott, Roaring Fork Valley to Aspen, Uncompahgre Plateau, Lone Cone, Lone Mesa, Naturita, Cerro Summit, Somerset, Sapinero, Gunnison, Creede, Poncha Pass, Buena Vista, Leadville, Hotchkiss, Saguache, Bayfield, Arboles, and McElmo Canyon. Rogers (1964) reported that all sagebrush areas listed by Cary (1911) still contained some amount of sagebrush in the early 1960's, yet due to human activities, many no longer were dominated by sagebrush. Human activities mentioned by Rogers (1964) included overgrazing, irrigation projects, and dry-farming. The distribution of sagebrush and sage grouse in the early 1960's (Fig. 4.2) was documented by Rogers (1964). Braun (1995) compared the distribution of sage grouse in 1993-94 to the range of sage grouse described by Rogers in 1964. Braun (1995) reported extirpation of sage grouse from 12 of 17 counties in southwestern Colorado which once supported them. This led me to believe that sagebrush habitats in southwestern Colorado

had been lost to other land uses.

Changes in vegetation types and land uses have often been documented successfully using aerial photography. For example, the National Wetlands Inventory mapped large scale changes in wetland distributions (Tiner 1990, Dahl and Johnson 1991) using this technology. Other examples include documenting tree invasion into grasslands (Mast et al. 1997), monitoring land cover change of a heathland region (Csaplovics 1992), quantifying temporal changes in seagrass areal coverage (Robbins 1997), and inventorying and monitoring arid rangeland vegetation (Knapp et al. 1990). I used aerial photographic analysis, to document and quantify changes in sagebrush-dominated habitats in southwestern Colorado which may be affecting the persistence of Gunnison sage grouse.

METHODS

Plot Selection

I identified 10 areas in southwestern Colorado which in the early 1960's (Rogers 1964) contained sagebrush-dominated habitat. Rough polygons (Fig. 4.3) were digitized around the 10 sagebrush areas in a geographic information system (GIS). I constructed a grid of sampling plots (sampling frame) covering each of the 10 polygons, with each sampling plot being a square, 4 km on a side (16 km^2/plot).

Although I did not have prior data suggesting what the change in habitat might be, I did expect habitat loss. Thus, I chose to compute a total sample size based on a worst case scenario that the proportion of sagebrush habitat in the first time period was 0.5

(worst case because if it was higher or lower the precision would be greater). I decided that a coefficient of variation (CV) of less than 10% on my estimate of habitat change would be acceptable for this study. I then calculated the appropriate sample sizes to achieve a CV of 4 or 10% of the estimate of the proportion of sagebrush habitat. The sample sizes calculated were 625 for a CV of 4% and 100 for a CV of 10%. I chose a target sample size of 200 since it was between 100 and 625 and the CV would be around 7%. Thus, my sampling fraction was approximately 9% (200 plots sampled from 2274 total plots). The number of plots to be sampled per stratum were calculated (rounding this number to the nearest integer) such that the sampling fraction was approximately 9% in each stratum. I then randomly chose the appropriate number of plots within each stratum to achieve a stratified random sampling design (Table 4.1). Because I rounded the number of plots to the nearest integer, the total number of plots sampled increased to 202.

Aerial Photography Acquisition and Interpretation

I attempted to obtain low level (between 1:20,000 and 1:30,000) black and white aerial photographs of each plot in the 1950's, 1970's, and 1990's. I chose my sampled plot size such that an entire plot could fit on one low level photo (occasionally a plot was covered by a group of photos from the same flight). Aerial photographs (either black and white film positive or color infrared) for all plots in the 1990's time period were obtained. Color infrared photos were used only when black and white photos were not available. Aerial photos from the early time periods were more difficult to obtain. For each plot, I developed a list of available photos (from different time periods) covering that plot and

chose the earliest available photos for each plot. If there were photos approximately mid way between the earliest date and the 1990's date, I chose those photos as well. In most cases, photos for only two time periods could be obtained. I did obtain photos from three time periods for 37 plots which allowed me to examine rates of habitat change over time. I omitted eight plots because there was insufficient photography covering those plots. Aerial photos were obtained from the U.S. Geological Survey (USGS) Eros Data Center.

Each plot boundary was identified and traced onto a 1:24,000 7.5-minute USGS topographic quad map (or groups of maps if needed). From features on the quad map, the plot was identified on the corresponding photo (or group of photos). A photo adjacent (along the same flight line) to the one containing the plot was identified for use on a stereoscope to visualize the plot in three dimensions. Acetate was then overlaid and taped to the appropriate photo (or groups of photos). The plot was then photo-interpreted to identify sagebrush-dominated (big sagebrush > than 50%) areas. These areas were traced onto the acetate using a Koh-i-noor Rapidograph pen with a tip to draw lines no thicker than 0.25 m, using Rapidograph Rapidraw 3084-F ink in the drawing pen. Forty-three of the 194 plots were ground truthed by first interpreting the photo, then going to the area on the ground and confirming its classification as sagebrush-dominated habitat.

A zoom transfer scope was used to standardize the scale and georeference the data because the photos from different time periods were taken at different scales (photos taken at different elevations). A mylar sheet was taped to each quad map and the appropriate plot was traced onto the mylar correctly overlaying the plot traced onto the quad map. Each photo with interpretation was placed on the zoom transfer scope and

focused to the appropriate scale so that features in the photo were lined up with features on the quad map. The interpreted sagebrush areas were traced onto the mylar sheet, removed from the quad map, scanned into a computer, and converted into a bitmap image using Adobe Photoshop. Each bitmap image was edited to correct anomalies such as closing polygons, deleting stray marks picked up by the scanner, and thinning polygon edges. The bitmap images were then imported into the GIS software ArcView (ESRI 1996) where total area of sagebrush, number of sagebrush polygons, and area and perimeter of each sagebrush polygon were calculated.

Data Analysis

I calculated the total amount of sagebrush-dominated habitat in each stratum and overall using standard methods for a stratified, simple random sampling design (Thompson 1992, Thompson 1997). The total estimated amount of sagebrush, $\hat{T}$ was calculated as

$$\hat{T} = \sum_{j=1}^{L} N_j \bar{x}_j$$

where L is the number of strata (10), N_j is the total number of plots in stratum j, and $\bar{x}_j$ is the mean amount of sagebrush habitat in sampled plots from stratum j. The sampling variance of $\hat{T}$ was calculated as

$$\hat{\text{var}}(\hat{T}) = \sum_{j=1}^{L} \left[N_j \right]^2 \left[\frac{s_j^2}{n_j} \left(1 - \frac{n_j}{N_j} \right) \right]$$

where n_j is the number of plots sampled in stratum j, and s_j^2 is the sampling variance estimate for stratum j. From the estimated total area of sagebrush I estimated the proportion of area that represented sagebrush-dominated habitat in each stratum and also overall.

I considered the most recent time period for each plot to be the late photo and the earliest time period for each plot to be the early photo. To determine the average time span between early and late photos, I calculated the average difference between the years of the early and late photos. I calculated the annual change in proportion of habitat between the early and middle photos, and between middle and late photos for the 37 plots with three time periods. Annual change r_{annual} was calculated as

$$r_{annual} = 1 - (1 - R_{period})^{\frac{1}{\Delta}}$$

where Δ is the time period in years and R_{period} is the estimated rate of change over a given time period. I then subtracted the two annual rates of change and tested whether this difference was different from zero. Because I did not find a significant difference between the annual rates of change from the two time periods (early to middle and middle

to late), I assumed that annual rates of change were reasonably constant over the 35 year time period.

Photos representing each time period (early and late) were taken in different years across plots (e.g., the early time period could be represented by a photo from mid-40's to late-60's). This made it difficult to compare changes in habitat across plots. Thus, I chose a model-based approach to standardize the data to a common early and late year for comparisons across plots. I used the average early year (1958) as my standard early year and the average late year (1993) as my standard late year. Using the assumption of montonicity of change between time periods, I calculated the proportion of habitat available per plot in the standard early and standard late years using a logistic function

$$\log\left(\frac{p_{early}}{1-p_{early}}\right) = \alpha_{early}$$

$$\log\left(\frac{p_{late}}{1-p_{late}}\right) = \alpha_{late}$$

where p_{early} was the proportion of area on a plot which was sagebrush-dominated habitat in the early time period and p_{late} was the proportion of area per plot which was sagebrush-dominated habitat in the late time period. Computing α_{early} and α_{late}, I then used the following equations

$$\alpha_{early} = a + bt_{early}$$
$$\alpha_{late} = a + bt_{late}$$

to solve for $\hat{a}$ and $\hat{b}$. I then set a specific year (e.g., $t = 58$), computed $\hat{\alpha}_{58}$ using

$$\hat{\alpha}_{58} = \hat{a} + \hat{b}(58)$$

and then computed an estimated proportion of sagebrush in the given plot in year 58, $\hat{p}_{58}$, using the following equation

$$\hat{p}_{58} = \frac{1}{1 + e^{(-\hat{\alpha}_{58})}}.$$

Thus, for every plot I used the logistic function and estimated the proportion of sagebrush-dominated habitat in 1958 and in 1993. Similar standardization and projection to a given year are explained in Terrazas-Gonzalez (1997).

To obtain better estimates of within stratum variance and confidence intervals on the amount of sagebrush for strata with small sample sizes (strata 3, 4, 5, 6, 8, 9, and 10) I calculated the CV of the amount of sagebrush habitat in 1958 and 1993 for each stratum. Because CVs tend to be stable (Eberhart 1978) I calculated the average CV and used it as an estimate of CV for strata with small sample sizes. This methodology, while not commonly used, is valid and documented in Carroll and Ruppert (1988) and Buckland et al. (1993). This allowed me to calculate the $z_{\alpha/2}$ multiplier for a confidence interval using 175 degrees of freedom, instead of much smaller degrees of freedom if this procedure is not used (Tukey 1977).

The actual confidence intervals around the estimates of the amount of sagebrush in 1958 and 1993 for each stratum were calculated using a log transform approach (Burnham et al. 1987). This is primarily to assure that the lower bound of the confidence interval cannot be less than the actual amount of sagebrush seen per stratum, a_j, calculated as

$$a_j = \sum_{i=1}^{n_j} p_{i,j} * 1600$$

where n_j is the number of plots in strata *j*, and $p_{i,j}$ is the proportion of sagebrush habitat in plot *i*, stratum *j* (the quantity is multiplied by 1600 to give the area in ha). This quantity, a_j, was then subtracted from the estimated total amount of sagebrush for a given stratum, $\hat{T}_j$ to give a normalized lower limit $\tilde{T}_j$ for that stratum ($\tilde{T}_j = \hat{T}_j - a_j$). For each stratum, this lower limit was then used to calculate upper and lower confidence intervals, $\hat{T}_U$ and $\hat{T}_L$ using the following equations

$$\hat{T}_L = (\tilde{T}/C) + a$$

and

$$\hat{T}_U = (\tilde{T}C) + a$$

where

$$C = \exp(z_{\alpha/2}\sqrt{\ln(1+\left[cv(\hat{T})^2\right])})$$

and

$$cv(\hat{T}) = \frac{SE(\hat{T})}{\hat{T}\text{-}a}.$$

Because it is logical that confidence intervals around the estimate of the amount of habitat lost could be negative, confidence intervals for this parameter were calculated in a traditional way, i.e., $\pm$ 1.96 (SE [loss]).

RESULTS

Habitat Loss

The years of the early photos ranged from 1944 to 1976 and the late photos from 1988 to 1995. The average date for the early photos was 1958 (SD = 6.7) and 1993 (SD = 1.3) for the late photos. The average number of years between the early and late photos was 35.2 (SD = 6.7). A difference of approximately 35 years should reflect changes in sagebrush habitat. The difference in annual rate of change in habitat between the early to mid time periods for the 37 plots from three time periods and the mid to late time periods was not significantly different from zero ($T = 0.83$, $P = 0.4124$).

Thirty-one of the 194 plots had no sagebrush-dominated habitat in either early or late photos. Of those plots with some amount of sagebrush in the early date, 10 plots had an increase in the amount of sagebrush and 153 had a decrease. Without standardizing to

a given early and late year, the mean proportion of sagebrush habitat in the early years was 0.212 (SE = 0.016) and the mean proportion in the late years was 0.173 (SE = 0.015). This corresponds to 772,358 ha (SE = 59,307) in the early years and 630,274 (SE = 55,944) in the late years. This represents an 18% loss in sagebrush habitat between early and late years.

After adjusting the data based on the logistic method, the mean proportion of sagebrush habitat available in 1958 was 0.2161 (SE = 0.0166) and in 1993 was 0.1734 (SE = 0.0154) (Table 4.2) which converts to 786,411 ha in 1958 and 630,725 ha in 1993 with a loss of 155,673 ha (95% CI 124,819 - 186,527) (Table 4.3). Overall, this represents a 20% loss of sagebrush-dominated habitat in the 35 years measured or a 0.64% annual loss rate (95% CI 0.49% - 0.77%). Habitat loss per stratum varied (Tables 4.2, 4.3), yet only some strata gave reliable estimates because of small sample size. Of those strata with greater than 10 plots sampled, the rate of habitat loss was variable with rates as high as 50% in stratum two and as low as 11% in strata seven and eight (Table 4.2).

A comparison of the historic and current distributions of sage grouse reveals that only one area in southwestern Colorado seems not to have changed much (Fig. 4.1). This area is the Gunnison Basin which, in this study, is represented by stratum seven. Because of this *a priori* knowledge, I combined data from all strata except stratum seven and compared the rates of habitat loss from the Gunnison Basin to all other areas. The proportion of sagebrush habitat available was much higher in the Gunnison Basin than in the rest of the areas (Table 4.4). In 1958, the estimated proportion of sagebrush habitat in

the Gunnison Basin was over twice the proportion in all other areas combined (0.3673 in Gunnison vs. 0.1552 in all other areas), whereas, in 1993 the proportion of habitat in the Gunnison Basin was almost three times higher than in all other areas (0.3267 in Gunnison vs. 0.1116 in all other areas). The Gunnison Basin experienced a loss rate of only 11% compared to the combined loss rate of 28% elsewhere.

Habitat Fragmentation

My results clearly document habitat loss, yet habitat fragmentation was more difficult to document. In each plot I recorded the number of sagebrush polygons, the total area of sagebrush and the total amount of edge (total perimeter). Holding the area of sagebrush constant, it is intuitive that as fragmentation occurs, the number of polygons should increase and the ratio of the square root of total area to total perimeter (A/P ratio) should decrease. When area is not held constant, however, it is not clear what these variables will do (Groom and Schumaker 1993). With decreasing area, for example, the number of polygons could either increase or decrease (Fig. 4.4) as could the A/P ratio. In general terms, example A (Fig. 4.4) seems to be affected mostly by fragmentation. This is the case when the number of polygons increases (large areas broken into smaller areas) and A/P ratio decreases (more perimeter per unit area). Example C, however, is affected by habitat loss rather than fragmentation (Fig 4.4). Here, the number of polygons decreases and the A/P ratio increases. Thus, with habitat loss and fragmentation both occurring, merely reporting trends in the A/P ratio and the number of polygons would be misleading.

I looked at the relationship between the change in number of polygons for each plot and the change in A/P ratio (Fig. 4.5) and found that most of my data fell into one of two categories. Sixty-six plots (37%) had an increase in the number of polygons and a decrease in A/P ratio. This represents cases where fragmentation tends to be the dominant process. Eighty-one plots (50%) had increases in the A/P ratio and decreases in the number of polygons. In these plots, habitat loss was presumably the dominating process.

DISCUSSION

I found little difference in estimates of the proportion of habitat lost between the analysis with the raw and the standardized data (0.039, SE = 0.0034 for raw data and 0.0428, SE = 0.0043 for standardized data). This gave me confidence that the model-based standardization using a logistic function represented the data in a reasonable way.

Although I did not find a difference in the annual rate of change between the early and middle time period and between the middle and late period for 37 plots in this analysis, it seems reasonable that rates might differ and be higher in more recent years due to tremendous increases in human population growth in Colorado. It has been estimated that human population growth in western Colorado was 3.1% per year between 1990 and 1996, much higher than the national average of 0.9% (Theobald *in press*).

I documented substantial amounts of sagebrush-dominated habitat loss throughout southwestern Colorado. It is important to note that much of what was once sagebrush habitat was already lost to other land uses before the oldest photos in this study were

taken. While not quantified, the loss of sagebrush habitat before the 1950's appeared substantial. This is in agreement with Rogers (1964) statement that much of the area once abundant with sagebrush, had been converted to other land uses by 1964. I could only document habitat loss since 1958. Overall, the change in the proportion of sagebrush-dominated habitat between 1958 and 1993 was 0.0428 (SE = 0.0043). This translates to a loss rate of 20% with 155,673 ha lost over 35 years. Average loss of habitat per year was 0.64% or 5,033 ha (95% CI 3,853 - 6,055).

Certain areas had much higher loss rates, especially stratum two (an area south of Durango and Pagosa Springs) which had an estimated loss rate of almost 50% (SE = 13.54). Sage grouse have been extirpated from this area. The Gunnison Basin had the highest proportion of existing sagebrush habitat and had one of the lowest rates of habitat loss. My comparison of Gunnison Basin with all other data combined showed that the loss rate of 11% (SE = 1.14) in the Gunnison Basin was much lower than the loss rate of 28% (SE = 3.64) elsewhere. This is not surprising in light of Braun's (1995) comparison of historic and current sage grouse distributions (Fig. 4.1) in which the Gunnison Basin seems to be the only population which has not been severely reduced.

Habitat fragmentation also was considerable in this study. I found 66 plots in which habitat fragmentation was a dominating process in that there were more polygons in the late time period (evidence of fragmentation into smaller polygons) than in the early time period and lower A/P ratios (evidence of more perimeter per unit area). Fragmentation typically results in a few remnant sagebrush patches surrounded by a matrix of land that is unsuitable for sage grouse use due to development and land-use

changes. This makes movement among patches potentially dangerous as sage grouse are more vulnerable to predators in these instances. In this study, fragmentation was often the result of road development which is known to have a negative impact on Gunnison sage grouse (Braun 1995, Oyler et al. 1997). Powerlines often line roads and provide perches for avian predators. Sage grouse have also been known to fly into and be killed by powerlines.

While this study documented the amount of habitat loss and the prevalence of habitat fragmentation, it did not measure habitat quality (with respect to sage grouse). Certainly fragmenting once continuous sagebrush habitat can influence the quality of that habitat for sage grouse by allowing the invasion of non native plants, and creating perches and travel corridors for predators. Road development also affects the quality of sage grouse habitat because it is associated with increased human activity within or near sagebrush patches. Paved roads specifically, and all human activities associated with them, have been negatively associated with Gunnison sage grouse (Oyler et al. 1997, Chapter One). Habitat requirements for sage grouse are well documented (Klebenow 1969, Wallestad 1971, Eng and Schladweiler 1972, Wallestad and Pyrah 1974, Beck 1977, and others). Such habitat characteristics (e.g., % cover of sagebrush, height of sagebrush, % cover of forbs) were not measured in this study. Thus, a portion of the remaining sagebrush habitat is likely not suitable for Gunnison sage grouse, making estimates of the total amount of 'suitable' sagebrush less than the amount of sagebrush documented here.

The decline in the distribution and abundance of Gunnison sage grouse is alarming (Braun 1995). I believe this decline to be the direct result of habitat loss and fragmentation, and to a decline in the quality of the remaining habitat. While this study could not address habitat quality, I have been able to document a steady loss of sagebrush habitat since 1958 and habitat fragmentation in a substantial number of areas. If current trends of habitat loss and fragmentation continue, Gunnison sage grouse will undoubtedly become extinct. Protecting the remaining habitat from further loss and fragmentation is paramount to the survival of this species (Chapter Five).

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Table 4.1. Characteristics of strata sampled for sagebrush in southwestern Colorado.

Stratum	Area (ha)	Plots per stratum	Plots sampled per stratum
1	1,364,800	853	74
2	476,800	298	25
3	44,800	28	3
4	238,400	149	12
5	102,400	64	5
6	49,600	31	3
7	1,044,800	653	54
8	222,400	139	13
9	52,800	33	3
10	41,600	26	2

Table 4.2. Differences in the proportion of sagebrush habitat in southwestern Colorado between 1958 and 1993. Mean difference in available habitat is the proportion of habitat available in 1958 minus the mean proportion of habitat available in 1993.

Stratum	Sampling fraction (sampled/total)	Mean proportion of habitat available (1958)	SE	Mean proportion of habitat available (1993)	SE	Mean difference in available habitat (1958-1993)	SE	Rate of habitat loss (%)	SE
1	74/853	0.1640	0.0237	0.1289	0.0221	0.0351	0.0052	21 40	3 19
2	25/298	0.1777	0 0385	0 0895	0.0265	0.0882	0 0241	49 63	13.54
3	3/28	0 0485	0.0458	0.0207	0.0196	0.0278	0.0263	57.32	54.11
4	12/149	0 2366	0 0737	0.1650	0.0578	0.0716	0.0221	30 26	9 33
5	5/64	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0	0
6	3/31	0.0041	0 0039	0.0013	0.0013	0.0028	0.0027	68.29	64.70
7	54/653	0.3673	0.0414	0.3267	0.0407	0.0406	0.0053	11.05	1.44
8	13/139	0.0867	0.0401	0.0773	0.0353	0.0095	0.0052	10.84	5.99
9	3/33	0.1099	0.0447	0.0968	0.0528	0.0131	0.0109	11.92	9.95
10	2/26	0.2448	0.0065	0.1957	0.0286	0 0490	0.0220	20.06	8 99
Overall	**194/2274**	**0.2161**	**0.0166**	**0.1734**	**0.0154**	**0.0428**	**0.0043**	**19.80**	1.99

Table 4.3. Differences in the amount of sagebrush habitat in southwestern Colorado between 1958 and 1993. Habitat lost is the amount of habitat available (ha) in 1958 minus the amount of habitat available (ha) in 1993.

Stratum	Area (ha)	Habitat available in 1958 (ha)	95% Confidence interval	Habitat available in 1993 (ha)	95% Confidence interval	Habitat lost (ha)	95% Confidence interval
1	1,364,800	223,827	168,988 - 296,588	175,923	126,051 - 245,632	47,896	33,658 - 62,134
2	476,800	84,732	55,735 - 128,974	42,669	24,193 - 75,352	42,065	18,441 - 65,688
3	44,800	2,174	701 - 6,920	929	258 - 3,451	1,245	-516 - 3,006
4	238,400	56,415	30,581 - 104,342	39,338	19,397 - 79,999	17,076	4,997 - 29,156
5	102,400	0	0 - 0	0	0 - 0	0	0 - 0
6	49,600	205	66 - 651	65	18 - 241	139	-58 - 337
7	1,044,800	383,786	308,079 - 478,346	341,368	267,748 - 435,457	42,414	31,335 - 53,492
8	222,400	19,289	10,703 - 34,872	17,180	8,699 - 34,043	2,109	676 - 3,542
9	52,800	5,801	1,861 - 18,436	5,111	1,409 - 18,952	690	-286 - 1,667
10	41,600	10,182	2,664 - 39,868	8,142	1,806 - 37,762	2,039	-1,494 - 5,573
Overall	3,638,400	786,411	667,337 - 905,484	630,725	520,220 - 741,230	155,673	124,819 - 186,527

Table 4.4. Differences in the proportion of sagebrush habitat in southwestern Colorado between 1958 and 1993, when data from the Gunnison Basin (stratum seven) was compared to all other strata combined. Data were standardized to 1958 and 1993. Mean difference in available habitat is the proportion of habitat available in 1958 minus the mean proportion of habitat available in 1993.

Stratum	Sampling fraction (sampled/total)	Mean proportion of habitat available (1958)	SE	Mean proportion of habitat available (1993)	SE	Mean difference in available habitat (1958-1993)	SE	Rate of habitat loss (%)	SE
Gunnison Basin (7)	54/653	0.3673	0.0414	0.3267	0.0407	0.0406	0.0053	11.05	1.44
All others	140/1621	0 1552	0.0163	0.1116	0.0141	0.0437	0.0056	28.09	3.64
Overall	**194/2274**	**0.2161**	**0.0166**	**0.1734**	**0.0154**	**0.0428**	**0.0043**	**19.80**	**1.99**

Table 4.5. Differences in the amount of sagebrush habitat in southwestern Colorado between 1958 and 1993, when data from the Gunnison Basin (stratum seven) was compared to all other strata combined. Data were standardized to 1958 and 1993. Habitat lost is the amount of habitat available (ha) in 1958 minus the amount of habitat available (ha) in 1993.

Stratum	Area (ha)	Habitat available in 1958 (ha)	95 % Confidence interval	Habitat available in 1993 (ha)	95 % Confidence interval	Habitat lost (ha)	95 % Confidence interval
Gunnison Basin (7)	1,044,800	383,786	297,137 - 470,436	341,368	256,111 - 426,624	42,414	31,337 - 53,491
All others	2,593,600	402,624	318,434 - 486,814	289,358	216,497 - 362,218	113,260	84,032 - 142,488
Overall	**3,638,400**	**786,411**	**667,337 - 905,484**	**630,725**	**520,220 - 741,230**	**155,673**	**124,819 - 186,527**

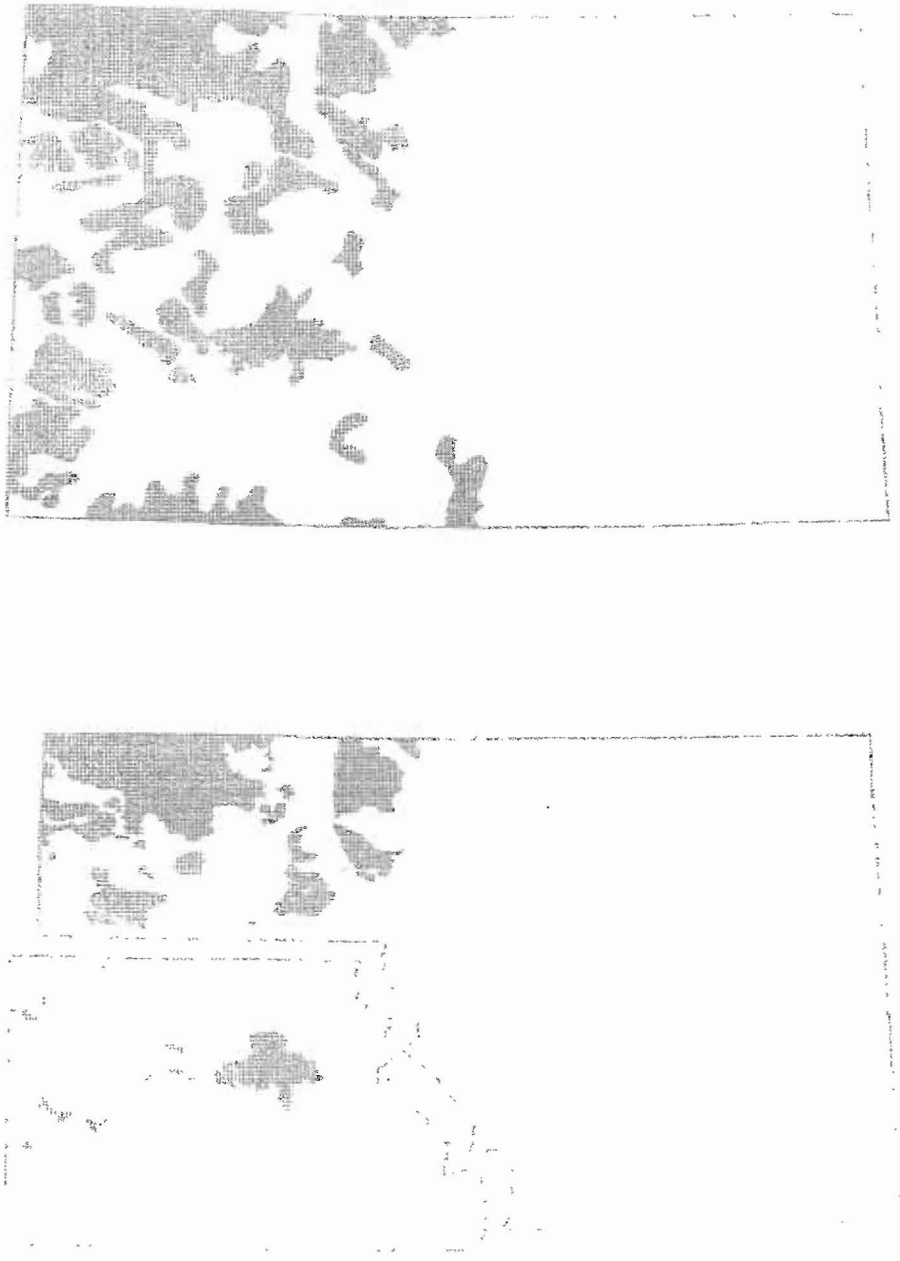


Figure 4.1. Historic (top) and current (bottom) distribution of sage grouse and Gunnison sage grouse (lower left cut out) in Colorado.

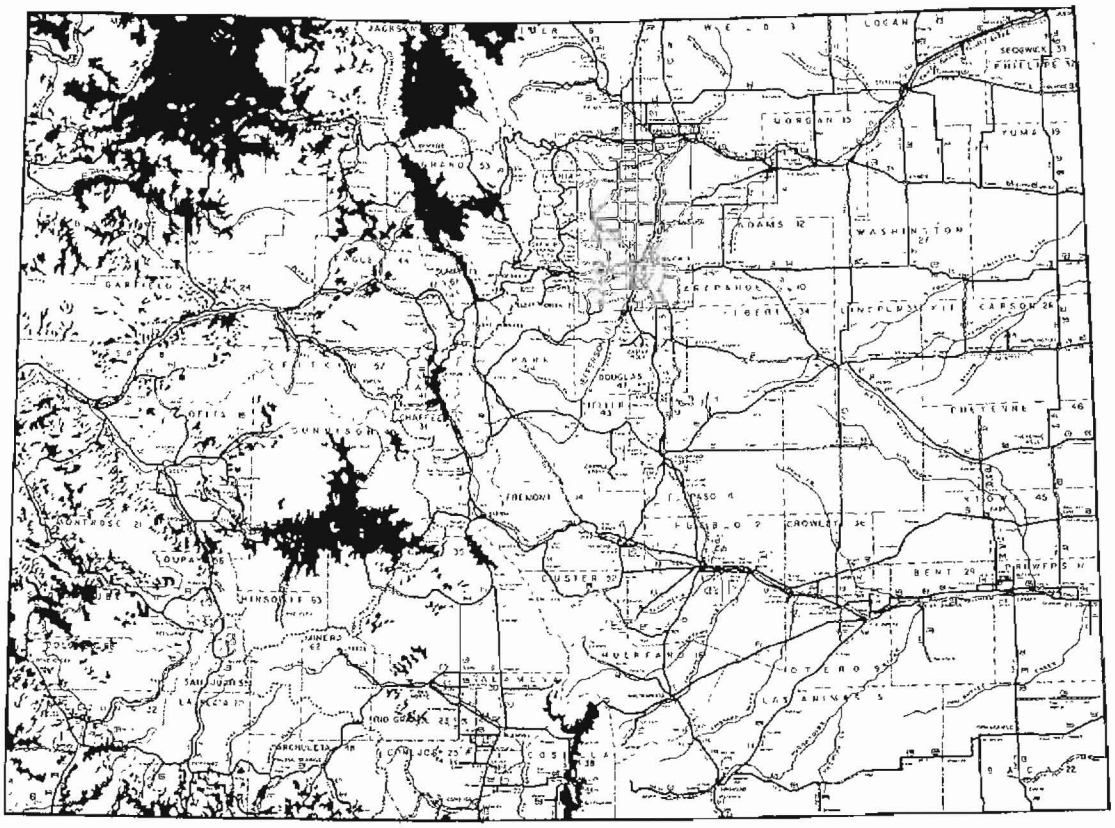


Figure 4.2. Distribution of sagebrush-dominated habitat in Colorado in the early 1960's (From Rogers 1964).

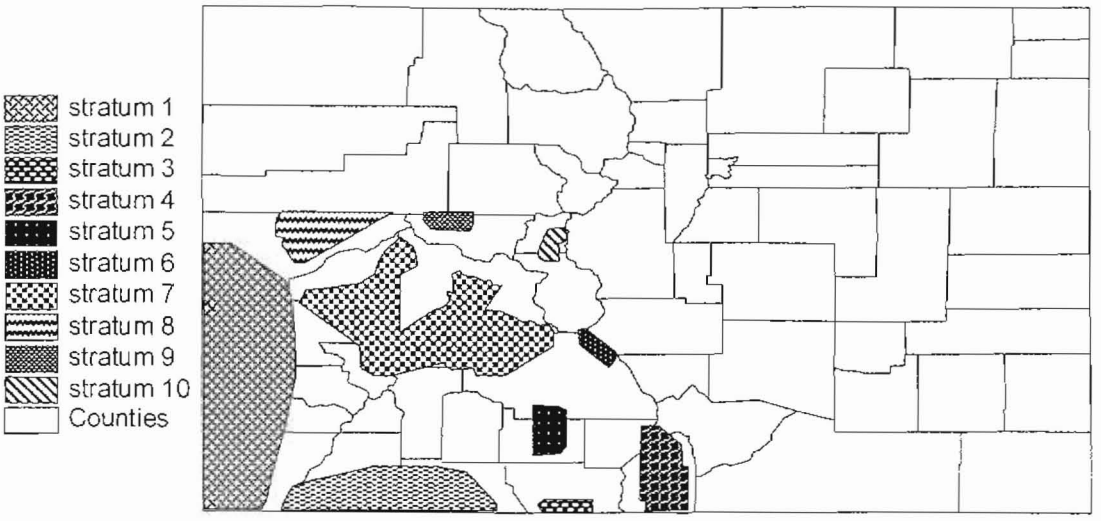
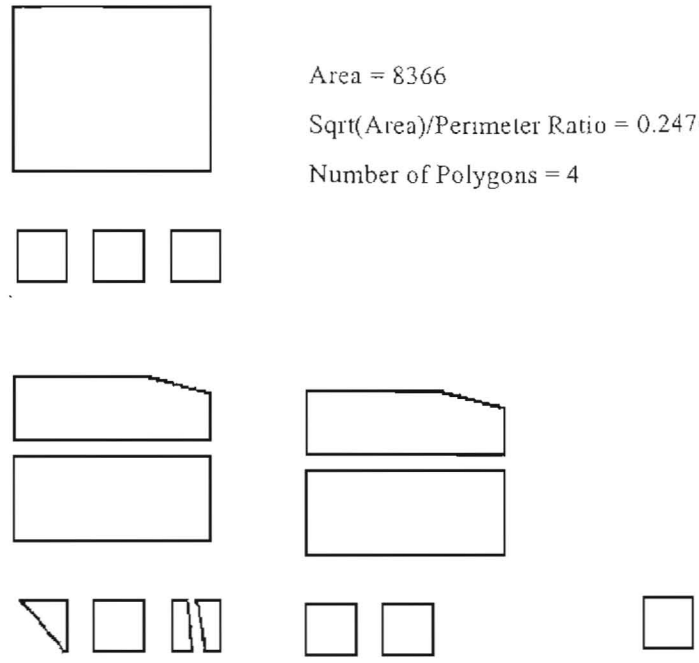


Figure 4.3. Strata used as a sampling frame for the distribution of sagebrush habitat in the early 1960's (From Rogers 1964).



Area = 8366

Sqrt(Area)/Perimeter Ratio = 0.2476

Number of Polygons = 4

A.	B.	C.
Area = 7571	Area = 7333	Area = 600
Sqrt(A)/P Ratio = 0.221	Sqrt(A)/P Ratio = 0.225	Sqrt(A)/P Ratio = 0.250
# Polygons = 6	# Polygons = 4	# Polygons = 1

Figure 4.4. Misleading relationship between habitat loss and fragmentation. In this example the top scenario is one large patch and three small patches. As habitat is lost and fragmented there are many different scenarios. In example A, there is some amount of fragmentation and loss. The ratio of square root of area to perimeter decreases and the number of polygons increases. In example B, the ratio decreases and the number of polygons stays the same. In example C the ratio increases and the number of polygons decreases.

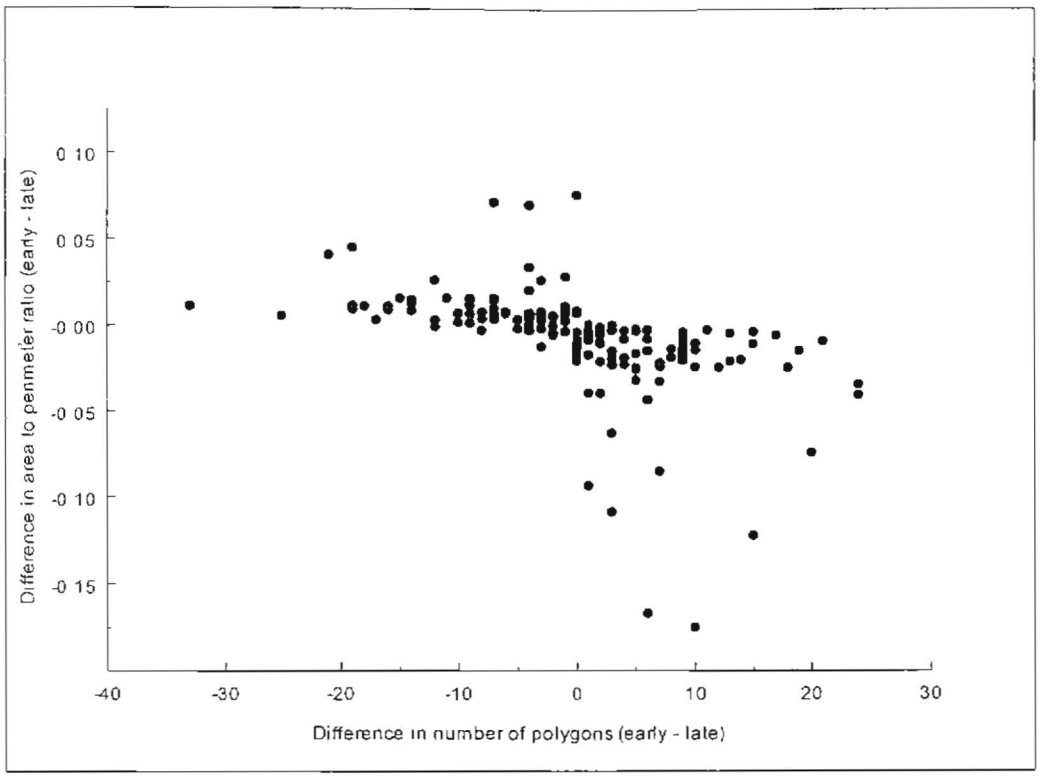


Figure 4.5. Relationship between the difference in number of polygons and the area/ratio perimeter. Data in upper left portion of the graph represents plots affected primarily by fragmentation and data in the lower right portion of the graph represent plots affected primarily by habitat loss.

CHAPTER FIVE

DEVELOPMENT OF A MODEL TO ASSESS MANAGEMENT AND CONSERVATION STRATEGIES FOR GUNNISON SAGE GROUSE IN COLORADO

INTRODUCTION

The distribution and abundance of sage grouse (*Centrocercus urophasianus*) have markedly declined throughout its entire range. The historic distribution, which included at least 15 states and three provinces (Aldrich 1963, Johnsgard 1973) has been reduced, with extirpation from four states and one province (Braun 1998). In those areas where sage grouse still exist, their range has declined markedly (Braun et al. 1994, Braun 1998). Populations in Colorado have also been greatly reduced as sage grouse have been extirpated from 12 of the 27 counties in which they occurred in the 1900's and breeding populations in nine of the remaining 15 counties are thought to number less than 500 breeding birds (Braun 1995).

Declines appear to be related to habitat loss (conversion of big sagebrush [*Artemisia tridentata*] into farmland or housing developments), habitat degradation (heavy grazing, sagebrush removal, road and powerline development through sagebrush, and human disturbance), and habitat fragmentation (Braun 1995, Braun 1998).

Populations in southwestern Colorado, the range of almost all the newly described Gunnison sage grouse (*C. minimus*) (Braun and Young 1995), have been most severely impacted by these processes (Fig. 5.1). Populations that remain are small, widely scattered, and exist in degraded, fragmented habitats isolated from a larger population in the Gunnison Basin. For this reason, Gunnison sage grouse have become a focus of management and conservation concerns.

Management options for Gunnison sage grouse include actions such as habitat protection, land mitigation, translocation among existing populations, and reintroduction into unoccupied areas. Habitat improvement is another management option, but could not be used in my model due to the coarse-scale nature of the data available. Often managers have to make decisions about specific issues using only a limited amount of information (usually that which is specific to the local population). My goal was to consolidate what is known about Gunnison sage grouse, represent it spatially, and make this information accessible to managers so they can assess how their decisions might affect not only a specific population, but the entire group of populations. Thus, I developed a Geographic Information System (GIS)-based computer model using the GIS software ArcView (ESRI 1996) which utilizes a variety of different layers of information to assess potential management strategies.

The use of GIS technology is rapidly becoming an integral part of conservation and wildlife studies. GIS models have been used to identify areas of highest biodiversity (Scott et al. 1993), assess existing conservation units in nature reserves (Peres and Terbough 1995), and address effects of forest management on species (Liu et al. 1995,

Rempel et al. 1997). Homer et al. (1995) used GIS to model structural and compositional attributes of sage grouse winter habitat in Utah. Their GIS model was on a much smaller scale and described the structural components of an area 2,548 km^2 in size. My model operates on a much larger scale (covering an area roughly 100,000 km^2) and addresses broad management questions of Gunnison sage grouse across all of southwestern Colorado.

METHODS

The model included information on all Gunnison sage grouse populations, information on all sagebrush patches in southwestern Colorado, and information on current and future human housing densities. The data on specific Gunnison sage grouse populations included population size (C.E. Braun, Colorado Division of Wildlife, unpublished data), distance to the centroid of the nearest population, and genetic diversity (Chapters Two and Three). Information on sagebrush patches included patch area, perimeter, distance to the nearest paved road from the centroid of each patch, distance to the nearest occupied patch, rate of habitat loss (Chapter Four), and the probability of occupancy (calculated using methods from Chapter One). Data on current and future human housing densities were classified into one of six groups based on the number of housing units per ac (Theobald in press). I represented all available information spatially by obtaining or developing GIS coverages.

I obtained a GIS coverage of the present distribution of Gunnison sage grouse from the Colorado Division of Wildlife. This coverage was edited to reflect recent

extirpation of populations so that it represented the most current distribution information available. Data on population size (C.E. Braun, Colorado Division of Wildlife, unpublished data), and genetic diversity (Chapters Two and Three) were entered so the information could be accessed by selecting a given population. The distance to the centroid of the nearest population from each population's centroid was calculated in ArcView (ESRI 1996).

A coverage of paved roads in southwestern Colorado was developed using 1:100,000-scale digital line graphs obtained from the U. S. Geological Survey (USGS). These files were derived from cartographic source materials using manual and automated digitizing methods from USGS topographic maps published as 30 x 60-minute quadrangles. I downloaded transportation data corresponding to the following USGS 1:100,000 scale maps: Grand Junction, Delta, Nucla, Dove Creek, Cortez, Durango, Silverton, Montrose, Paonia, Carbondale, Glenwood Springs, Vail, Leadville, Gunnison, Saguache, Del Norte, Antonito, Alamosa, Blanca Peak, Canon City, and Pikes Peak. I then merged all of the data in Arc/Info (ESRI 1987) into one coverage and selected only paved roads (attribute codes 170201 - 170208).

To represent sagebrush patches in southwestern Colorado, I obtained a GIS coverage of vegetation types in southwestern Colorado from the Colorado Gap Analysis project. This coverage was developed for relatively coarse scale (1:100,000) projects with each mapping unit in non-riparian areas equal to 100 ha. This coverage (currently being tested and validated) maps only generalized distributions of vegetation types based on the USGS 1:100,000 mapping scale. These data seemed appropriate because my goal

was to assess management strategies of Gunnison sage grouse across southwestern Colorado . From this coverage, I selected only those polygons described as sagebrush habitat. Two different classifications were found in this coverage, Wyoming or Mountain big sagebrush (*A. t. wyomingensis* or *A. t. vaseyana*) and Basin Big sagebrush (*A. t. tridentata*). The habitat was classified as Wyoming or Mountain big sagebrush if it comprised more than 25% of the total vegetative cover. This classification was variable and included dense, homogenous sagebrush stands as well as more sparsely vegetated areas. Often, patches of sagebrush were found with patches of mixed grasses. In these cases, the area was classified as sagebrush if sagebrush patches occupied more than 50% of the total ground cover.

The area and perimeter of each sagebrush patch were given in the coverage. Using the sagebrush and sage grouse coverages, I calculated the distance from each sagebrush polygon centroid to the centroid of the nearest population. To report the average annual rate of habitat loss for each sagebrush polygon, I determined the stratum (Chapter Four) to which each polygon belonged. Because the sample size in some strata were small, (strata 3, 4, 5, 6, 9, and 10) estimates of habitat loss from those strata were poor, yet when I pooled data across strata with small sample sizes, the estimates of habitat loss were much better. Thus, I calculated a weighted average (0.98%) across those six strata and assigned each polygon that value. Polygons in strata with adequate estimates of loss rates were assigned the appropriate annual loss rate (stratum 1 = 0.68%, 2 = 1.9%, 7 = 0.33%, and 8 = 0.33%). I also calculated the probability of occupancy for each patch using the model averaging approach and the three models discussed in Chapter One.

Two coverages of human housing density (one from 1990 and one projected to 2020) were obtained from the Natural Resource Ecology Laboratory (NREL) at Colorado State University. These raster maps were based on 1990 U.S. Census Bureau block-group level data (a block-group is a subdivision of census tract containing between 250 and 500 housing units). Theobald (in press) mapped housing density at quarter-section (65 ha) resolution into one of six categories: no data, rural (< 1 unit per 80 ac), ranchette (< 1 unit per 40 ac), exurban (< 1 unit per 10 ac), suburban (< 1 unit per 2 ac), and urban (> 1 unit per 2 ac) for the 1990 map. The 2020 map was classified the same way and is a projection from the 1990 data (Theobald and Hobbs 1998).

To address the issue of land protection and mitigation I decided to prioritize patches of sagebrush for protection which were currently occupied by Gunnison sage grouse. My criterion for ranking patches was a combination of the size of the sage grouse population in that patch, the distance to the nearest population, and the probability of occupancy of that patch. There are many other ways in which these areas could be prioritized; this is just one example of an application of this model. To prioritize patches for this example, I converted the vector sage grouse distribution coverage into two raster coverages, one with population size and one with the distance to the nearest population. I then standardized the distances so that they ranged from zero to one (with zero being the farthest and one being the closest) by dividing each value by the largest distance and subtracting this value from one. Population sizes were standardized by dividing each value by the largest value such that large populations had values close to one. I also converted my vector sagebrush coverage into a raster coverage using the probability of

occupancy attribute as a cell value. Using ArcView's Spatial Analyst (ESRI 1996), I averaged the values of probability of occupancy, standardized population size, and standardized distance to the nearest population to create a coverage which prioritized areas for land mitigation and protection. This, in effect, gives equal weight to the three factors considered. Unequal weighting could be used if there was a reasonable basis for its use.

I was also interested in determining which unoccupied sagebrush patches might be good reintroduction sites. Sites could be prioritized in different ways, but for this example I defined good sites as patches with a high probability of occupancy which were close to existing populations so that a network of somewhat connected populations could be established. I calculated the distance between the centroid of each sagebrush patch and the centroid of the nearest sage grouse population to determine which patches would make the best reintroduction sites. I then converted the vector sagebrush coverage to two raster coverages, one with each cell representing the probability of occupancy and one with each cell representing the distance to the nearest population. I then standardized the distances so that they ranged from zero to one (with zero being the farthest and one being the closest) by dividing each value by the largest distance and subtracting this value from one. In ArcView's Spatial Analyst (ESRI 1996), the two raster coverages were then combined by averaging the probability of occupancy and the standardized distance to the nearest population. This produced a coverage which represented the suitability of each patch with values near one being the most suitable and those near zero being the least suitable.

An issue which might also be important for land mitigation and reintroduction is to determine which patches of sagebrush and which populations of sage grouse would potentially be impacted most by human population densities. Thus, I created four new coverages; two represented overlays of the sagebrush coverage and two (one each) of the human housing densities (1990 and 2020) coverages. Two additional coverages represented overlays of the sage grouse distribution coverage and each of the human housing densities. These coverages were developed by converting the sagebrush and sage grouse vector coverages into raster coverages and combining them with human housing densities in 1990 and 2020.

To investigate the possibility of translocating birds among populations to increase genetic diversity, I decided that areas with the lowest genetic diversity, furthest from the area with the highest genetic diversity would be considered a priority for translocation. Many other criterion could also be used to prioritize populations for translocation. From the sage grouse vector coverage, I made two raster grid coverages, one with the attribute called number of unique alleles as the grid value (populations with no genetic data received a zero), and one with the distance from the centroid of the Gunnison Basin attribute as a grid value. I then standardized both values so they ranged from zero to one (zero being the lowest diversity for the genetic coverage and the furthest distance from Gunnison Basin for the distance coverage). These coverages were then combined in ArcView's Spatial Analyst (ESRI 1996) by averaging the two values.

RESULTS

I classified the distribution of Gunnison sage grouse into eight populations based on knowledge of movements (Commons 1997, C. Woods and C. E. Braun. 1995. Sage grouse investigations, Colorado Division of Wildlife, Fort Collins, CO, USA) and expert judgement (Fig. 5.2). Polygons shown in the same color belong to the same population. For ease of discussion I developed a map showing key towns in southwestern Colorado (Fig. 5.3).

I found similar patterns in size and genetic diversity of populations (i.e., large populations had high genetic diversity and small populations had low diversity). The largest population in the Gunnison Basin (Fig. 5.4) had the most genetic diversity (Fig. 5.5) with approximately 2000 breeding birds and an average of 3.75 alleles per locus. The Dry Creek Basin population and the Crawford population are intermediate in size and in genetic diversity. The Dove Creek population, with approximately 100 birds had the lowest genetic diversity with an average of 1.75 alleles per locus.

Sagebrush patches with a high probability of occupancy (Fig. 5.6) generally occurred in areas where sage grouse currently exist (Gunnison Basin, Dry Creek Basin, Glade Park/Pinon Mesa and Crawford). An exception is the sagebrush patch near Poncha Pass which has a relatively low probability of occupancy. This population is small and was a transplant from the Gunnison Basin. Unoccupied areas with a high probability of occupancy include areas east of Dry Creek Basin near the town of Norwood, areas west of Montrose, areas southeast of Pinon Mesa, areas north of Crawford, an area south of interstate 70 near Glenwood Springs and Gypsum, a few patches near the New Mexico

border west of Durango, and areas near Del Norte. Areas of low probability of occupancy generally occurred in areas near towns and cities such as Grand Junction, Paonia, Aspen, Alamosa, and Cortez.

Areas with the lowest annual rate of habitat loss occur in the Gunnison Basin and north of it (Fig. 5.7). Highest loss rates occur southeast of Cortez along the border of New Mexico. Areas east of the Gunnison Basin also have high annual rates of habitat loss.

I identified those sagebrush areas within the existing sage grouse distribution which had a high priority for protection and land mitigation (Fig. 5.8). Areas with a high priority for mitigation and protection included most of the Gunnison Basin, most of Dry Creek Basin, Glade Park/Pinon Mesa, Crawford, and Sim's Mesa. The area with the lowest priority was the Poncha Pass population. The Dove Creek population was classified as "no data" because the sagebrush coverage did not identify the area as sagebrush because the sagebrush in Dove Creek occurs in a patchy distribution intermixed with agricultural fields.

Potential reintroduction sites with a high probability of occupancy and close to an existing population included areas mostly west of the Gunnison Basin (Fig. 5.9). Patches west of Montrose and southeast of Pinon Mesa had a high suitability as did sites east of Dry Creek Basin near Norwood. Other potential reintroduction sites include patches east of Dove Creek and patches north of Crawford.

Areas within the current distribution of Gunnison sage grouse with the highest human housing density (urban with > 2 unit per 2 ac, suburban with < 2 units per ac) in

1990 occurred exclusively in the Gunnison Basin in the town of Gunnison (Fig. 5.10). The Gunnison Basin was mostly categorized as rural (< 1 unit per 80 ac) and ranchette (< 1 unit per 40 ac) and rural. All other areas inhabited by Gunnison sage grouse were classified as rural. The 2020 projection of human housing densities (Fig. 5.11) reveals that the Gunnison Basin will likely experience great increases in human development and disturbance particularly along highway 50 which bisects the Gunnison Basin. North of the town of Gunnison toward Crested Butte is also projected to have a substantial increases in human density. The only other area within the current distribution of Gunnison sage grouse which will likely experience such increases is on Sim's Mesa south of Montrose which is projected to receive a ranchette classification by 2020. All other areas are projected to remain in the rural classification. Overall, changes in human density within areas currently occupied by sage grouse will be greatest in the the categories of rural and exurban (Table 5.1) with the amount of area classified as urban decreasing by 2059 km^2 and the amount of area classified as exurban increasing by 1694 km^2.

Of all the sagebrush areas in southwestern Colorado, areas with the highest human housing density (urban and suburban) in 1990 occurred near Aspen and Glenwood Springs (Fig. 5.12). The Gunnison Basin had a few areas classified as ranchette and small area around the town of Gunnison classified as exurban, suburban, and urban. A few other areas were classified higher than rural including areas around Cortez and Durango. For the most part all other areas were classified as rural. The 2020 projection is for substantial increases in human density in the Gunnison Basin (particularly between

Gunnison and Crested Butte), Aspen and Glenwood Springs, Cortez, Durango, areas east of Dry Creek Basin near Norwood, Paonia, and areas east of Grand Junction (Fig. 5.13). Changes between 1990 and 2020 (Table 5.2) will be greatest in the categories of rural (decrease of 6528 km^2), ranchette (increase of 2635 km^2), and exurban (increase of 3791 km^2).

The Dove Creek population was determined to have the most potential for translocating birds from the Gunnison Basin to increase genetic diversity (Fig. 5.14). Four populations, however, were omitted from consideration due to a lack of genetic data.

DISCUSSION

I found the highest number of birds with the highest genetic diversity in the largest sagebrush areas (Figs. 5.4, 5.5). Of the populations for which I had genetic data, the lowest diversity was found in the smallest population, Dove Creek. This is not surprising in light of the theories of inbreeding and genetic drift (Hartl and Clark 1989). I advocate translocating four to six females from the Gunnison Basin to the Dove Creek population every few years to increase its genetic diversity (Chapter Three). The Crawford and Dry Creek Basin populations are also candidates for translocation (Fig. 5.14). Further, genetic data (Chapters Two, Three) suggest that Gunnison sage grouse populations are isolated with relatively low gene flow among populations. Thus, protecting existing habitat and preventing further loss and fragmentation is paramount. Potential reintroduction sites should be large enough to sustain a considerable number of birds and be close enough to an existing population so that natural dispersal is possible.

Large sites that are far from an existing population could still be considered, yet dispersal would need to be facilitated by humans which is a less favorable option.

I found that the areas with the highest probability of occupancy occurred mostly in areas already occupied by Gunnison sage grouse. The Poncha Pass population is an exception, yet the population size is less than 50 birds (C. E. Braun, Colorado Division of Wildlife, unpublished data) and that population was a transplant from the Gunnison Basin. Unoccupied areas with a high probability of occupancy include areas between Dry Creek Basin, the Gunnison Basin, and Glade Park/Pinon Mesa, and areas north of Crawford, areas south of Glenwood Springs, areas between Cortez and Durango, and areas near Del Norte. These probabilities are based on the area of the patch and the distance to the nearest road from the centroid of the patch (Chapter One). This represents a coarse scale approach to finding potentially suitable sites which should be supplemented by ground measurements to assure that the habitat characteristics of the habitat meet known requirements of sage grouse (Klebenow 1969, Wallestad 1971, Eng and Schladweiler 1972, Wallestad and Pyrah 1974, Beck 1977, and others).

Historically, the highest rates of habitat loss occurred in areas removed from the Gunnison Basin including areas near Durango, and areas east of Alamosa and north to Poncha Pass. Interestingly, these areas of high annual rates of habitat loss are not areas which are predicted to have high human densities by 2020 and those areas with the highest predicted human densities occur in areas where the rate of habitat loss is low (Figs. 5.7, 5.13). This is likely because historic habitat loss occurred from the conversion of sagebrush habitat into farmland (Rogers 1964), not housing developments. Future

human impacts, however, appear to be concentrated in the Gunnison Basin (Fig. 5.13) which is the one area with a currently stable population. This is cause for concern and efforts should be made to minimize housing developments in areas crucial to Gunnison sage grouse survival.

Land protection and mitigation is an important management option. The areas within the current distribution of Gunnison sage grouse which I found to have a high priority for protection and mitigation were Glade Park/Pinon Mesa, Dry Creek Basin, Crawford, and Sim's Mesa, and much of the Gunnison Basin (Fig. 5.8). Of those areas, the only area that is projected to have a substantial increase in human population density is in the Gunnison Basin (Fig. 5.11). The other areas, however, may be subject to habitat loss from changing land use patterns other than human development such as conversion into farmland or destruction of sagebrush to promote growth of grass for grazing.

I identified potential reintroduction sites by determining sites with a high probability of occupancy that were close to existing populations (Fig. 5.9). On the ground measurements need to be collected in these sites, however, to assure that the characteristics of the habitat meet the known requirements of sage grouse (Klebenow 1969, Wallestad 1971, Eng and Schladweiler 1972, Wallestad and Pyrah 1974, Beck 1977, and others). Further, there may be other factors not measured here which could make these sites less suitable such as powerlines or oil and gas development. The ownership of these sites might also make reintroduction unlikely. My recommendations of potential sites here should be considered as a first step for considering reintroduction. Many other issues need to be considered before a reintroduction should be implemented.

The best sites I found included areas between Dry Creek Basin, the Gunnison Basin, and Glade Park/Pinon Mesa, areas east of Dove Creek, and areas north of Crawford. The areas east of Dry Creek Basin near Norwood seem to be a logical place to reintroduce birds because a population in this area would bridge the gap between the Gunnison Basin and Dry Creek populations and hopefully facilitate gene flow among the populations. The human population projection (Fig. 5.13), however, shows considerable increases in human densities in these areas. The areas north of Crawford and east of Dove Creek, however, are not predicted to have substantial increases in human densities.

This model contains current information about Gunnison sage grouse populations and sagebrush patches in southwestern Colorado. It was designed so that information could be updated or added as it becomes available. Various scenarios can be addressed with this model and I have shown examples of some questions that can be addressed including prioritizing areas for land protection and mitigation, prioritizing areas for reintroduction, and prioritizing areas for translocation to increase genetic diversity. These examples are not exhaustive and are used to illustrate different aspects of the model. Other applications of the model include determining how the probability of occupancy changes with changes in patch size or distance to the nearest paved road, and investigating specific scenarios such as identifying all sagebrush patches within a given distance from a population that satisfy certain size and probability of occupancy criterion. Additional data (e.g., data on land ownership, fine scale habitat quality measured on the ground, and population data) can be easily added to strengthen the utility of the model.

This model, when used by managers with specific questions, will help prioritize strategies for the conservation and management of Gunnisou sage grouse.

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Table 5.1. Amount of area within the current distribution of Gunnison sage grouse in southwestern Colorado classified into each of five human housing densities in 1990 and projected to the year 2020.

Housing density	Amount (km^2) in 1990	Amount (km^2) in 2020
Rural (< 1 unit per 80 ac)	6,950	4,891
Ranchette (< 1 unit per 40 ac)	1,750	2,058
Exurban (< 1 unit per 10 ac)	273	1,967
Suburban (<1 unit per 2 ac)	74	130
Urban (> 1 unit per 2 ac)	16	16

Table 5.2. Amount of area within the current distribution of sagebrush in southwestern Colorado classified into each of five human housing densities in 1990 and projected to the year 2020.

Housing density	Amount (km^2) in 1990	Amount (km^2) in 2020
Rural (< 1 unit per 80 ac)	30,797	24,269
Ranchette (< 1 unit per 40 ac)	3,712	6,347
Exurban (< 1 unit per 10 ac)	2,580	6,371
Suburban (<1 unit per 2 ac)	320	347
Urban (> 1 unit per 2 ac)	38	113

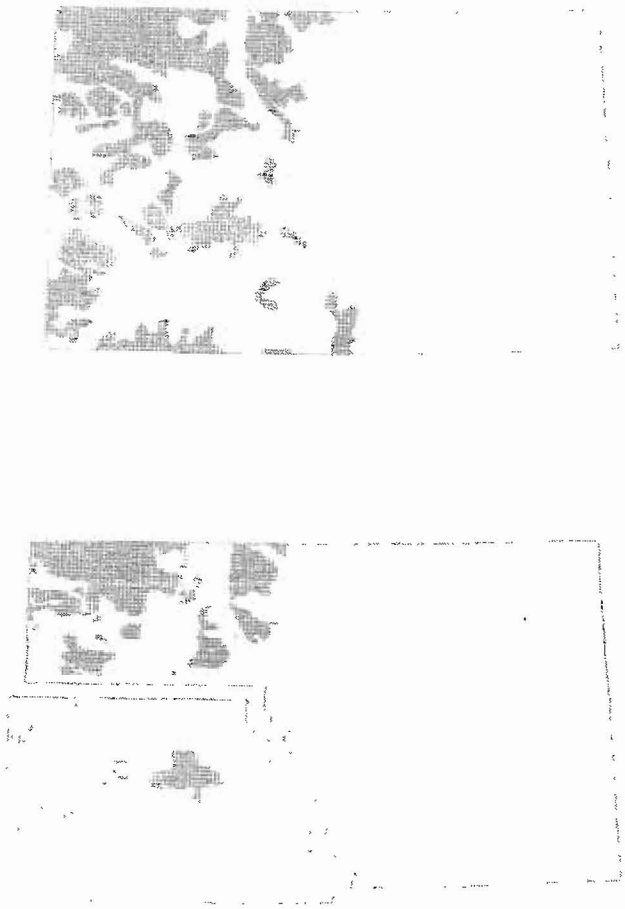

Figure 5.1. Historic (top) and current (bottom) distribution of sage grouse and Gunnison sage grouse (lower left) in Colorado.

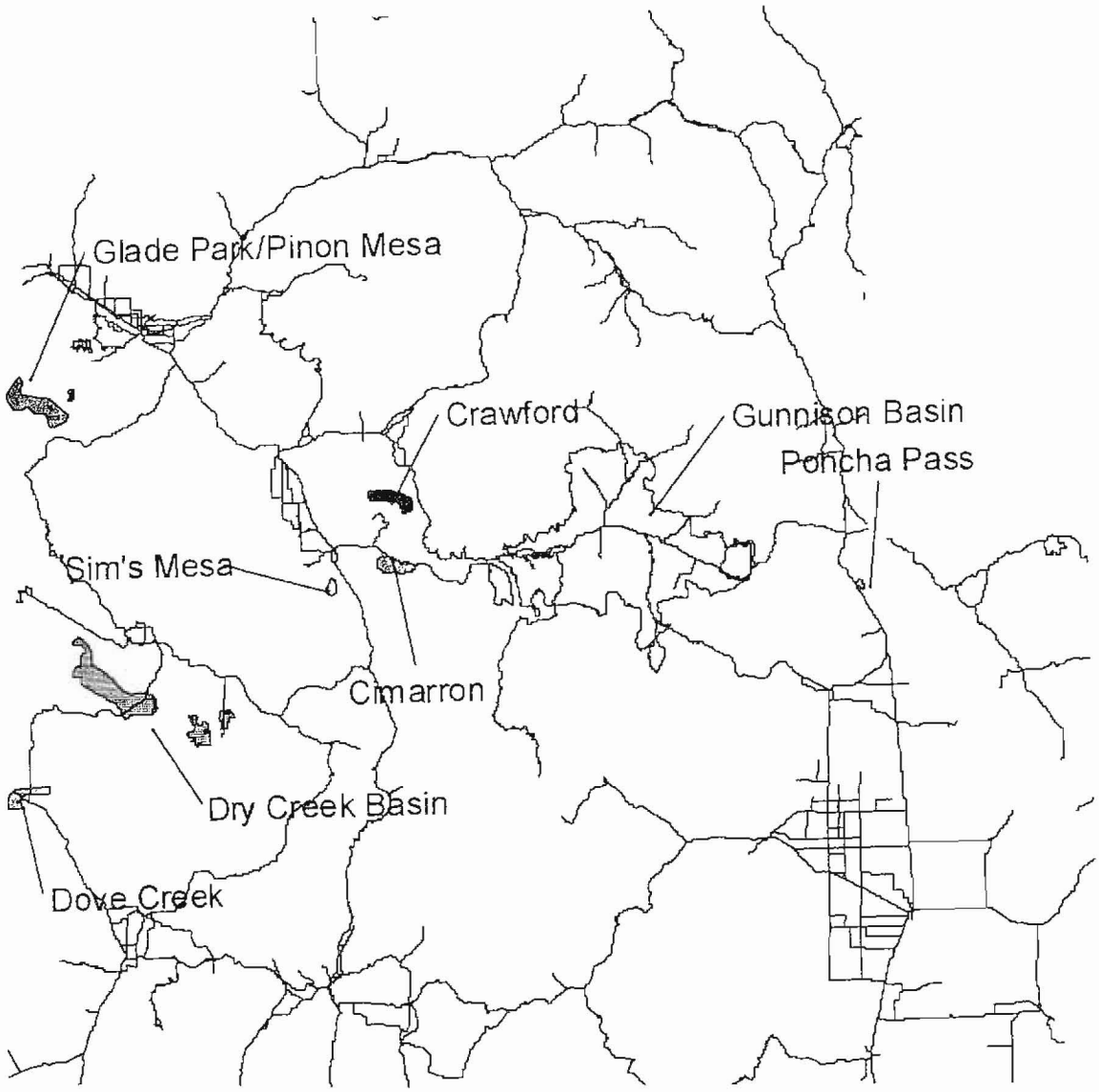

Figure 5.2. Distribution of 8 populations of Gunnison sage grouse in southwestern Colorado. Polygons with the same color represent the same population.

Figure 5.3. Location of cities and towns in southwestern Colorado.

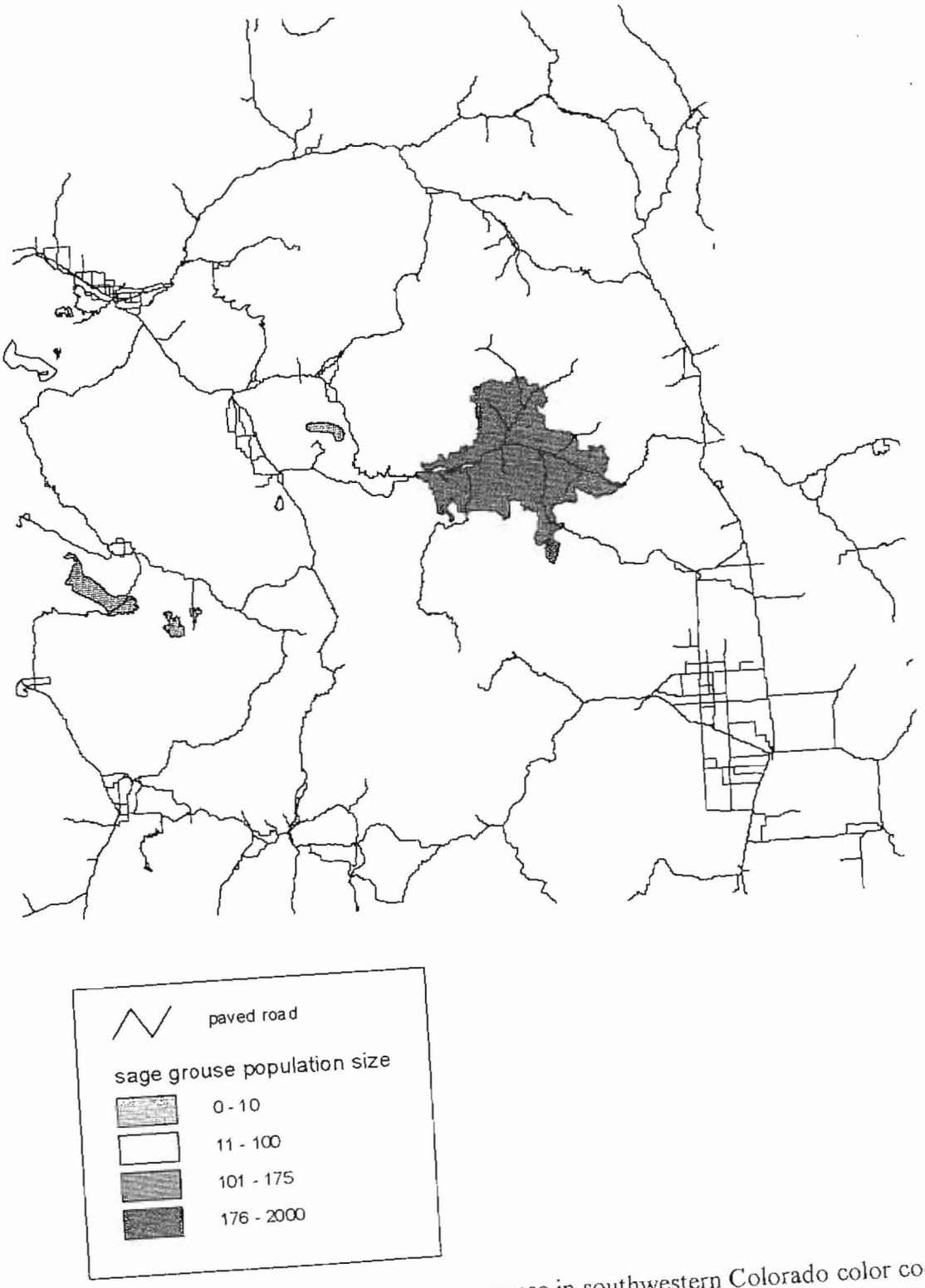

Figure 5.4. Distribution of Gunnison sage grouse in southwestern Colorado color coded to show differences in population size.

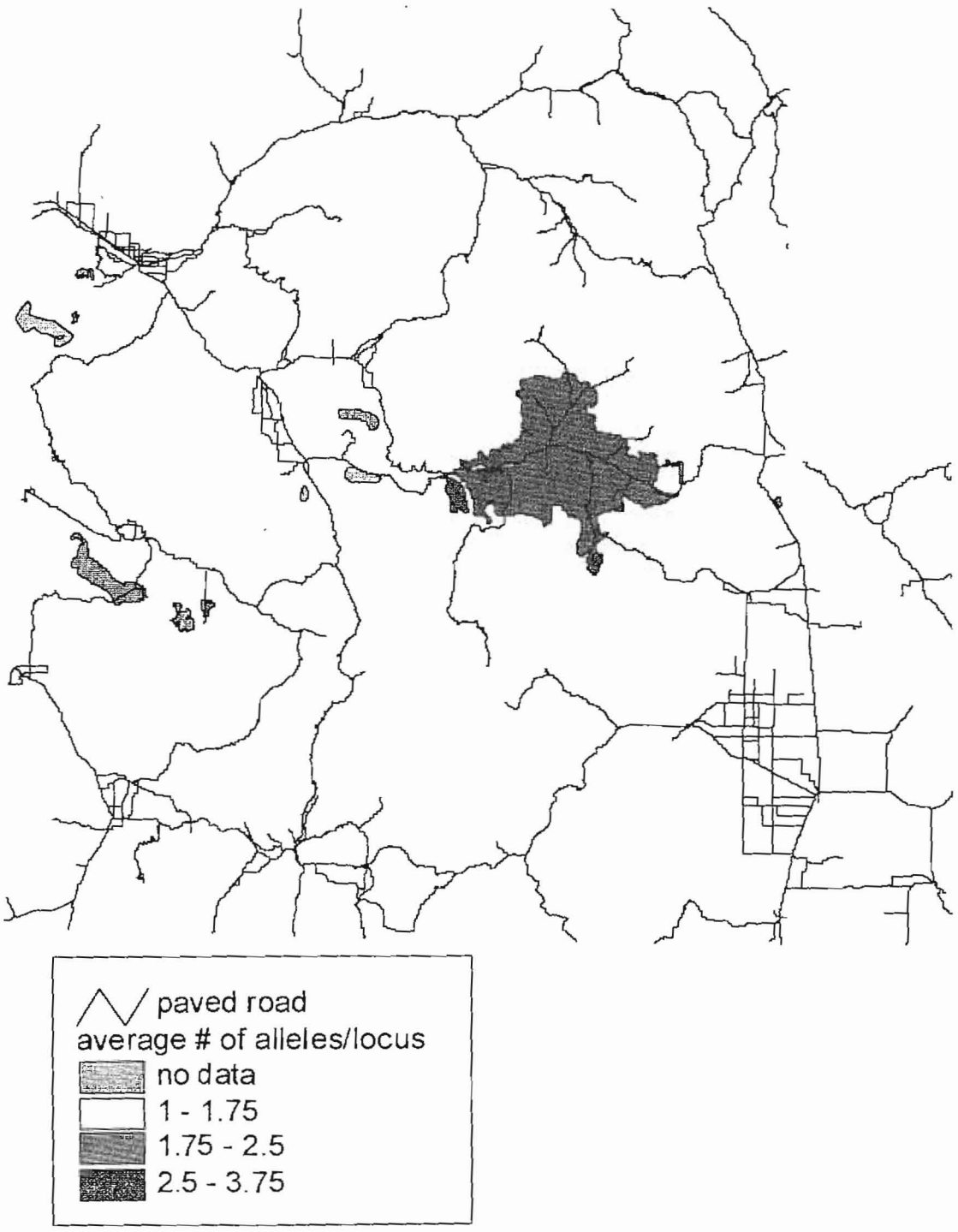

Figure 5.5. Genetic diversity (average number of alleles per locus) of four populations of Gunnison sage grouse in southwestern Colorado (Chapters Two, Three).

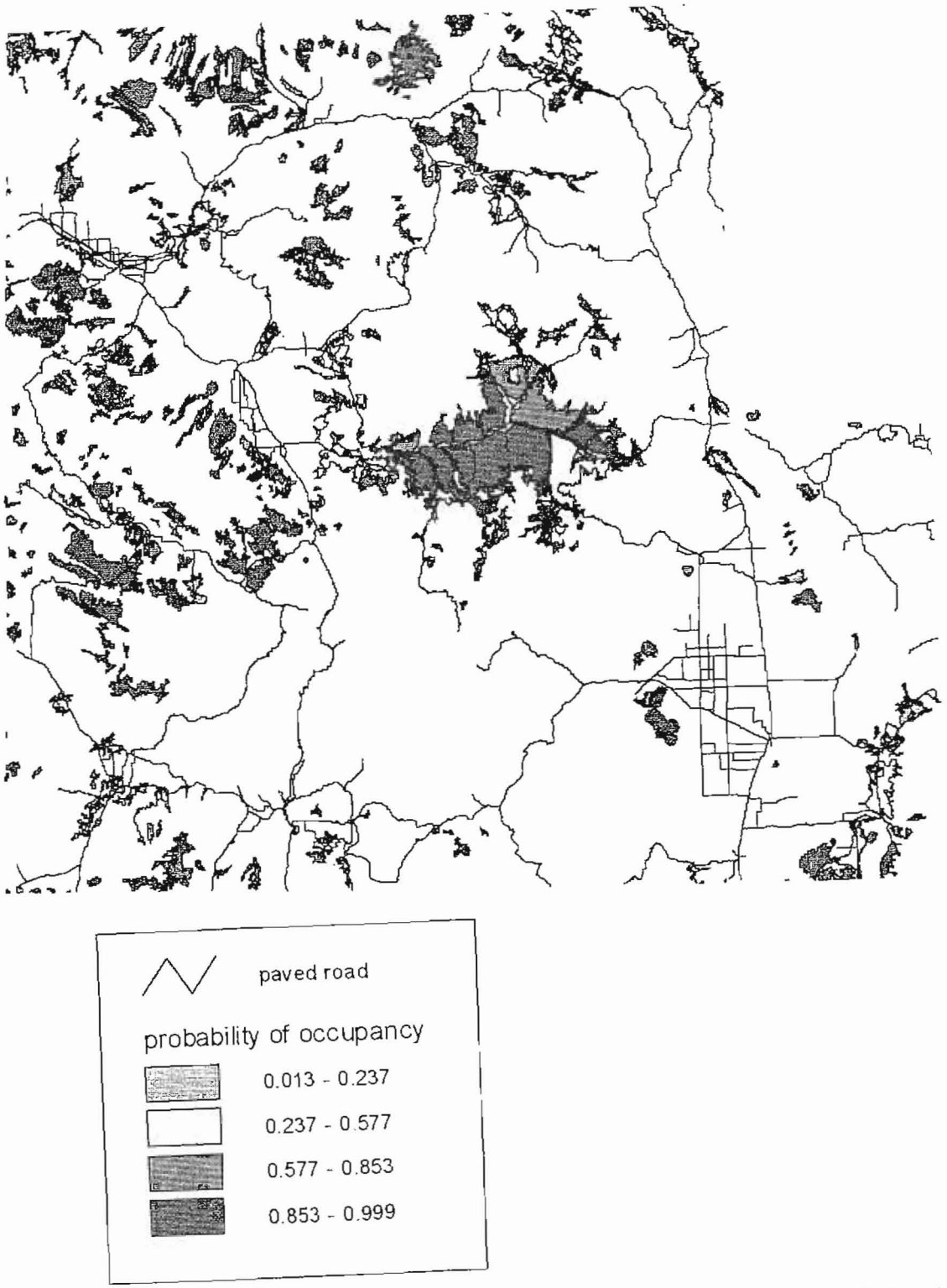

Figure 5.6. Probability of occupancy of all sagebrush patches in southwestern Colorado (calculated using model averaging procedure in Chapter One).

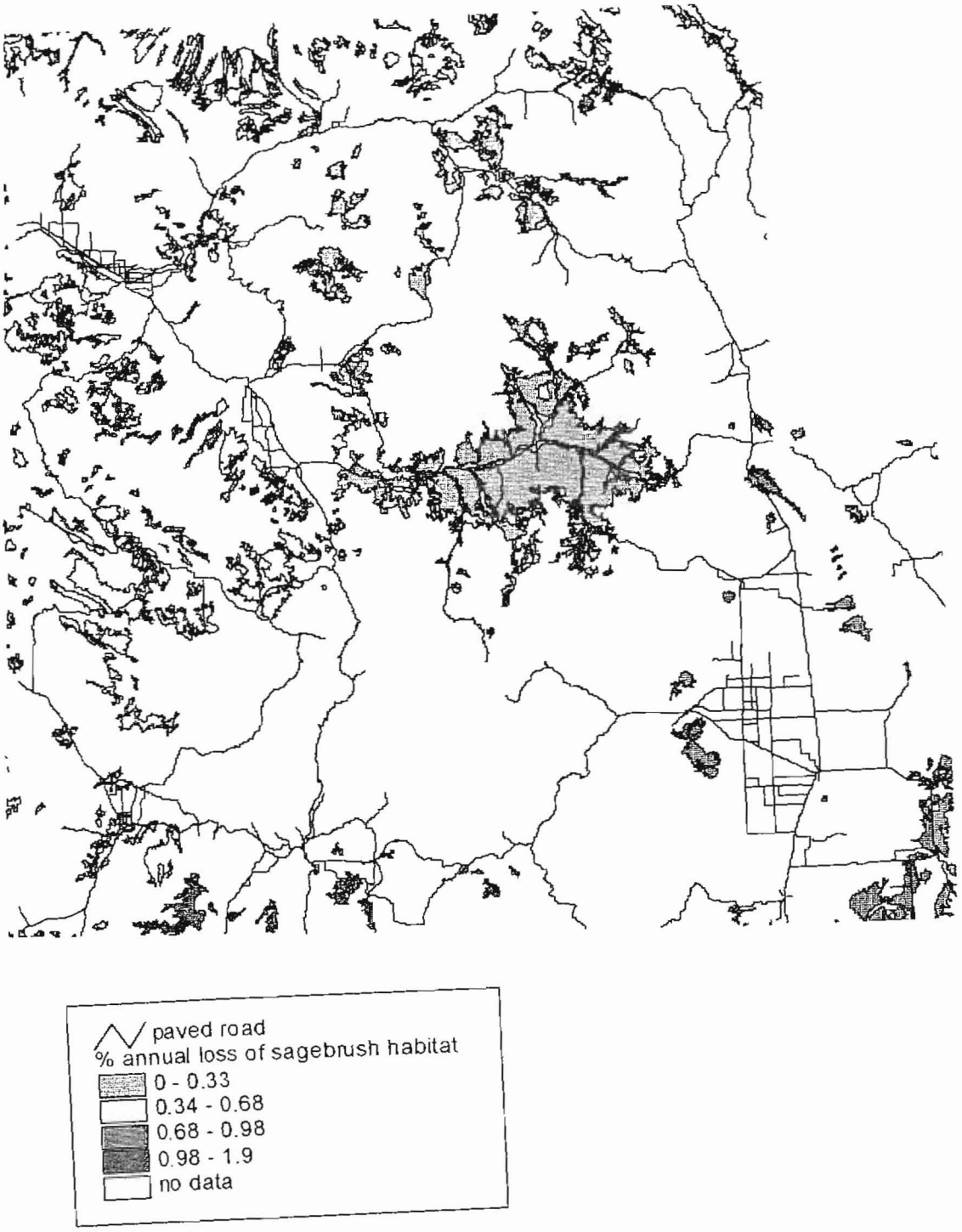

Figure 5.7. Annual loss (%) of sagebrush habitat (as measured in Chapter Four) in southwestern Colorado.

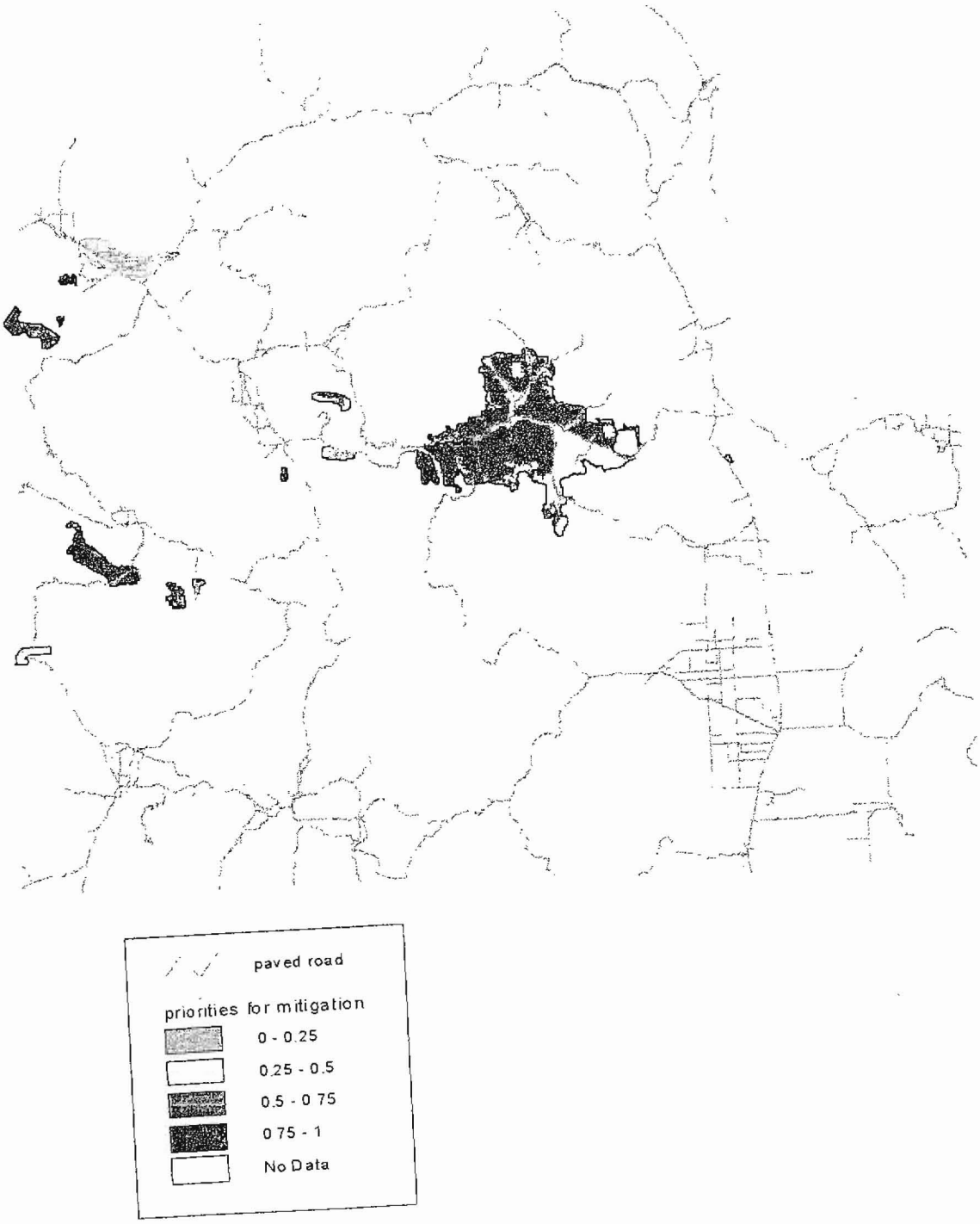

Figure 5.8. Priorities for land protection and mitigation in southwestern Colorado (based on size of the existing sage grouse population, distance to the nearest population, and probability of occupancy).

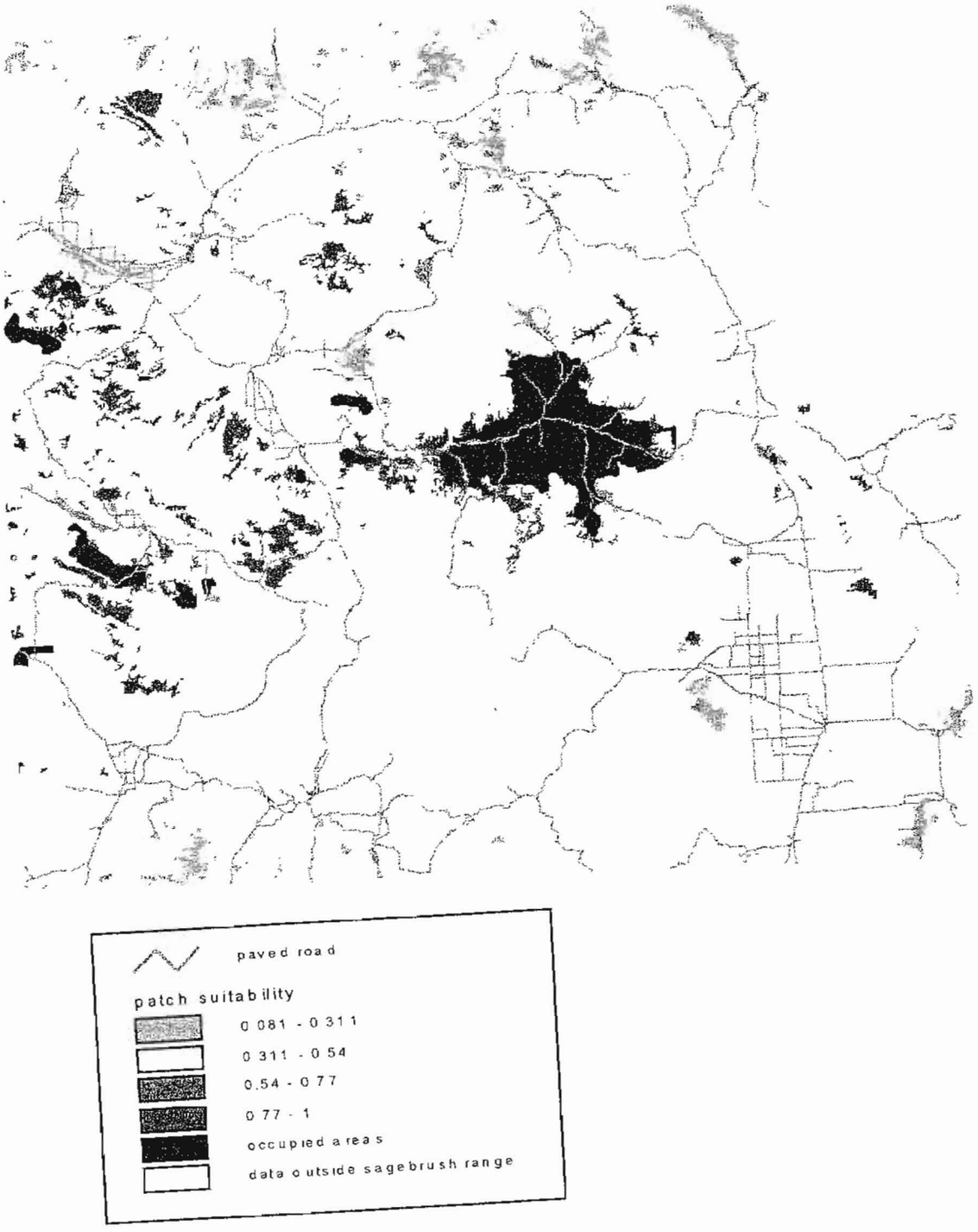

Figure 5.9. Priorities for sage grouse reintroduction into unoccupied areas of southwestern Colorado. Priorities are based on a combination of distance to the nearest population and probability of occupancy.

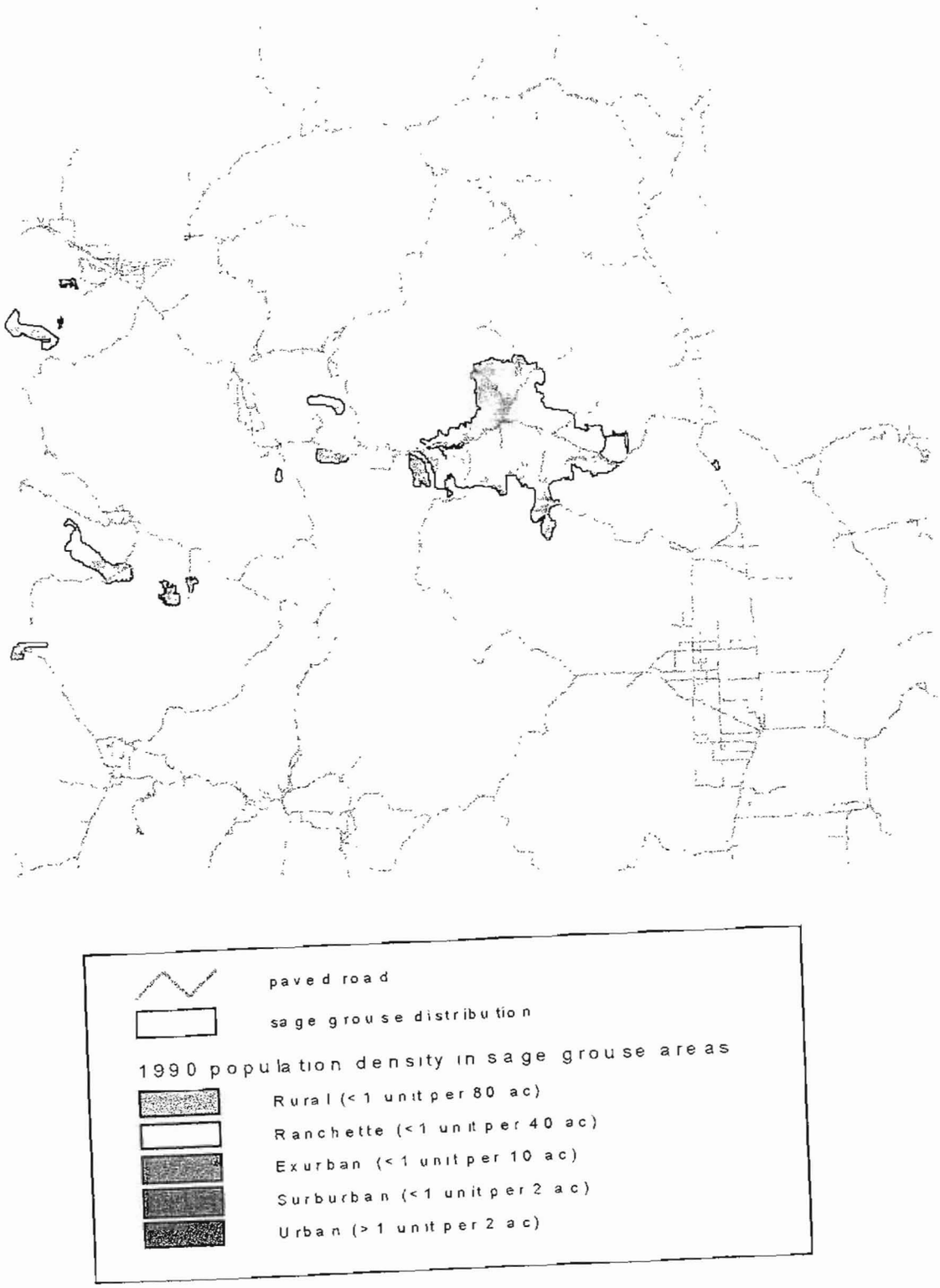

Figure 5.10. Human housing density (1990) in areas currently occupied by Gunnison sage grouse in southwestern Colorado.

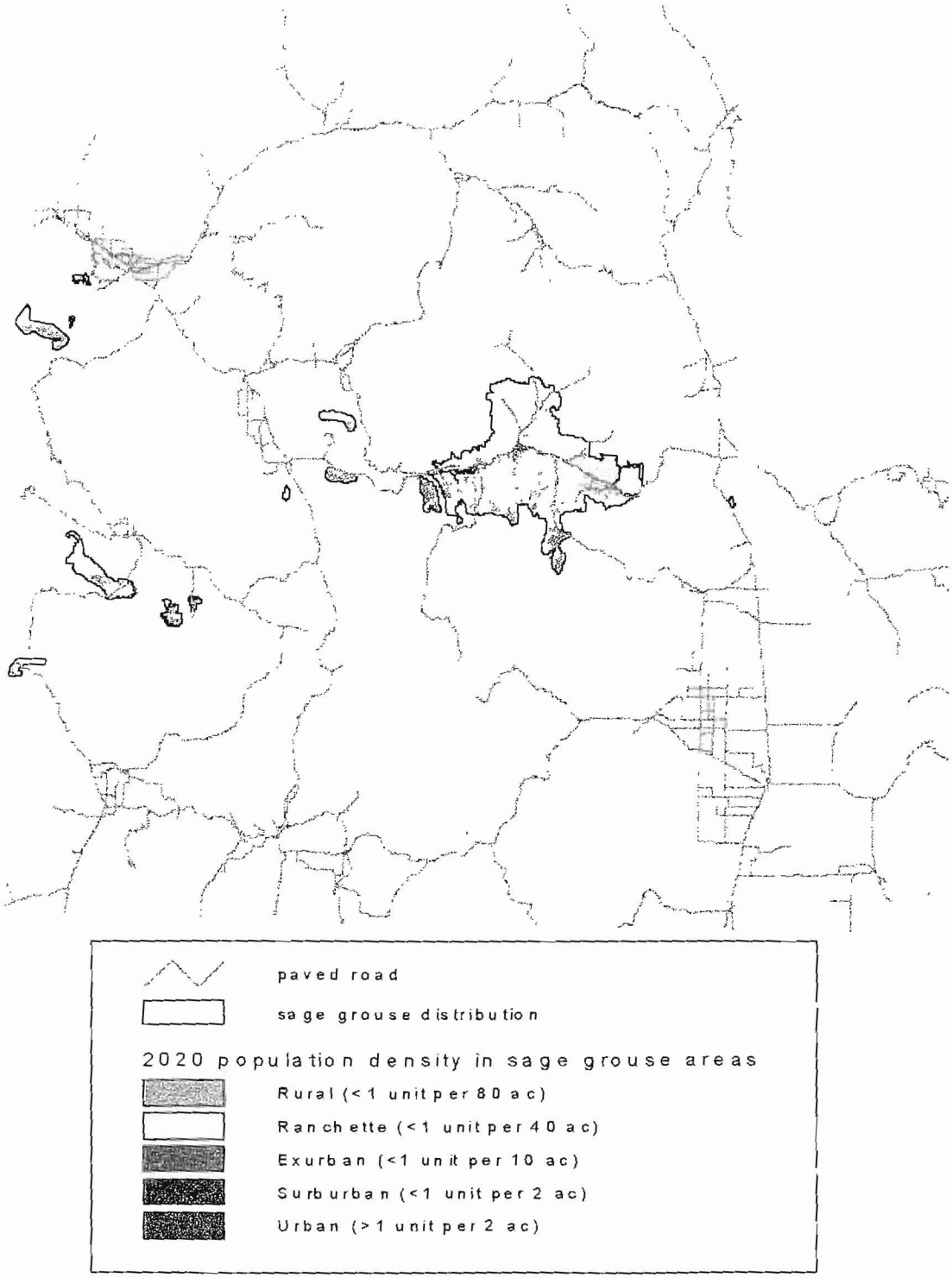

Figure 5.11. Human housing density projected to 2020 in areas currently occupied by Gunnison sage grouse.

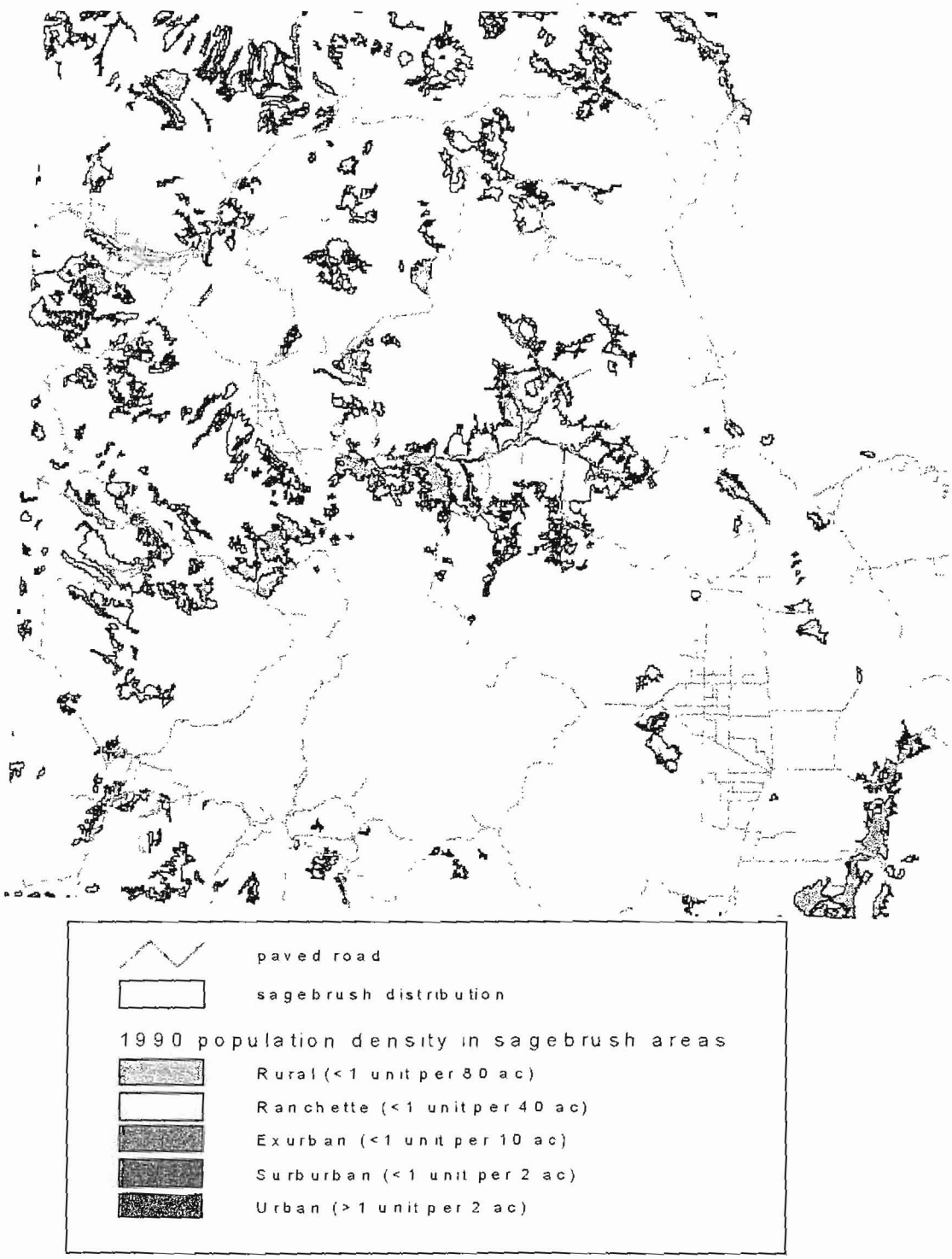

Figure 5.12. Human housing density (1990) in sagebrush areas in southwestern Colorado.

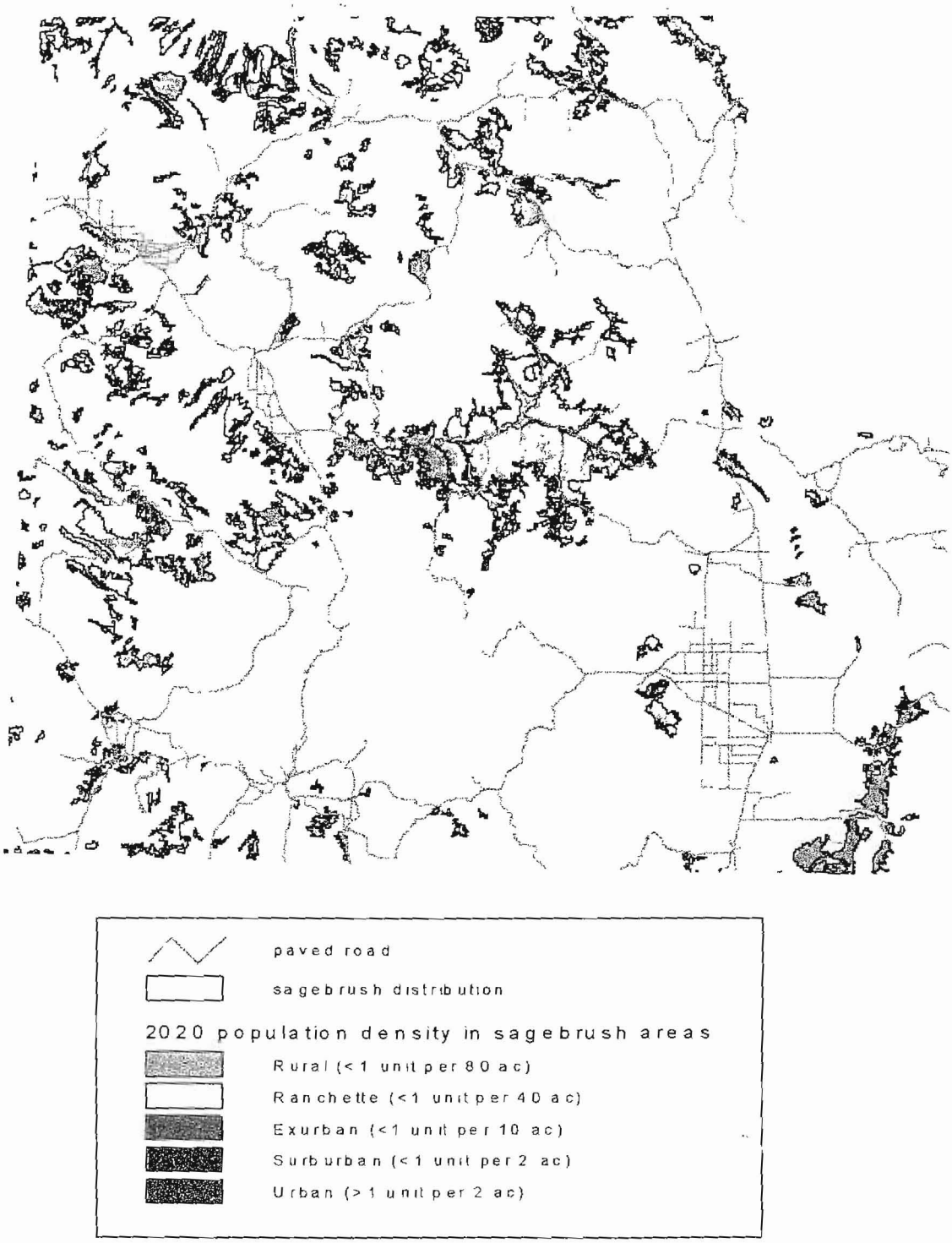

Figure 5.13. Human housing density projected to 2020 in sagebrush areas in southwestern Colorado.

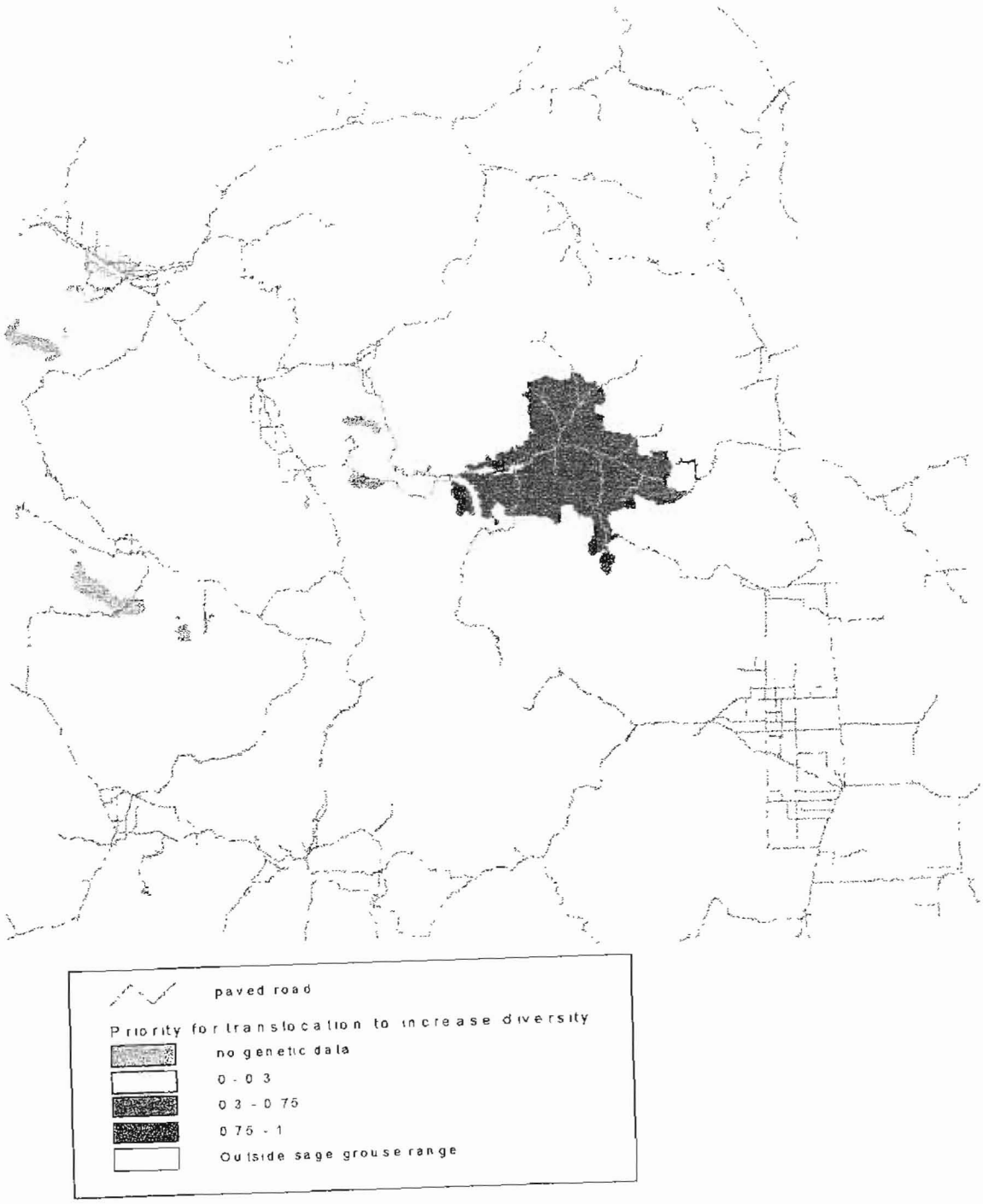

Figure 5.14. Priority of populations into which sage grouse from the Gunnison Basin should be translocated. Low values (yellow) receive the highest priority.

DISCUSSION

Through the course of this dissertation research, much was learned about conservation and management of Gunnison sage grouse (*Centrocercus minimus*), research and methodology in general, and limitations associated with research. In these final pages, I comment on what was learned, how my work could have been improved, and what future research might follow.

From the habitat-based model that I developed in Chapter One, I learned that the model which best described the data included the variables distance to the nearest paved road from the patch centroid, and patch area. The major limitation of this model was small sample size (25 patches sampled). With a much larger sample size, many more candidate models with more variables could have been considered. Sample size was small because of the criterion I used for choosing patches to sample (had to have supported populations in the past 20 years) and the time constraint of only one field season. If this criterion was relaxed (although the number of historically occupied patches is limited) and more time was allotted for sampling, more patches (from the sagebrush coverage in Chapter Five) could be visited and sample size could be raised somewhat.

In Chapter Two I found that the small-bodied Gunnison sage grouse were distinct from the large-bodied sage grouse (*C. urophasianus*) and that the small-bodied birds had

less genetic diversity and gene flow relative to the large-bodied birds. The main limitation in this study was the small number of microsatellite loci (four) used. With only four loci examined, it becomes more difficult to characterize the uncertainty of the relationship among populations as bootstrap analyses on the neighbor-joining trees are not reliable with such few loci. Further, the data analyses associated with microsatellite markers are not well developed. It has been suggested that microsatellites follow a stepwise mutation model rather than the typical infinite alleles model of mutation. Published genetic distances based on the stepwise mutation model produced spurious results with my data and thus were not used in this dissertation. More research on these models of mutation need to be conducted and better genetic distance measures based on the stepwise mutation model need to be developed to improve the analysis of microsatellite data. Future additions to my genetic study might include developing primers specifically for sage grouse to increase the number of loci and also to obtain samples from areas without adequate representation (such as Pinon Mesa and Poncha Pass). Genetic samples from the Poncha Pass population would be particularly interesting because it is an extremely small population which was a transplant from the Gunnison Basin a known number of years ago. This would allow us to look at isolation and genetic drift in this population.

The management implications of my genetic study are discussed in Chapter Three. I noted the low genetic diversity in the small-bodied birds, particularly within the population near Dove Creek. I suggested translocating females from the Gunnison Basin population to at least the Dove Creek population to increase its genetic diversity. Again,

data used in this study would be improved by obtaining more microsatellite loci which would allow for a better understanding of the relationship among the small-bodied populations. Additionally, future research should focus on monitoring survival and reproductive success of Dove Creek birds before and after any transplant to assess any effects (positive or negative). Genes associated with immunity to disease such as the Major Histocompatibility Complex should be examined in these birds to see if the genetic diversity of this gene is low which might have severe implications should a disease outbreak occur.

Analysis of aerial photography in Chapter Four showed a 20% loss and substantial fragmentation of sagebrush-dominated habitat between the mid-50's and the mid-90's. The Gunnison Basin had a lower loss rate than all other strata examined. The design and analysis of this study were sound and likely do not need to be improved. I ground truthed approximately 20 % of the photos and am confident that classification errors were minimal. Errors associated with the zoom transfer scope, scanning, and importing the data into Photoshop were not quantified. To quantify these errors, multiple people would have to be used to assess variability among people and associated errors. I did not have this option for this study, but future studies could incorporate multiple people repeating the same techniques to quantify this error. This has been done by the National Wetlands Institute and was found to be a minimal source of error in their study. The analysis of fragmentation could likely have been documented better by examining all plots and reporting the fate of each sagebrush polygon. However, this would be extremely time intensive and would not likely add much to the analysis. It would be

interesting to look at photos from the same plots in another 10 - 20 years. I believe that much of the loss in the past has been the result of conversion of sagebrush into farmland, but that this trend might change given current human influx into Colorado. I predict that future loss and fragmentation will be due to human development rather than farming and ranching.

In Chapter Five, I developed a GIS-based model to assess potential conservation strategies for Gunnison sage grouse. It is a course scale model based on data from the Colorado GAP Project. Finer scale data could be used, yet it is prohibitive now due to the enormous size of the computer files containing this type of data. Currently, this model contains information on sage grouse populations (size and genetic diversity), information on sagebrush patches in southwestern Colorado, and information on human population densities. Additional information which could be incorporated into this model include data on whether land is public or private, more complete genetic data, population data on Gunnison sage grouse (such as survival and reproduction), and habitat quality data on sagebrush patches. Predications from this model could be made and field tested (e.g., patch suitability).

Overall, I believe that the information in this dissertation has improved the knowledge base of certain aspects of Gunnison sage grouse and that it can be incorporated into conservation plans for this species. This species will likely be petitioned for listing as a threatened or endangered species. My habitat-based model (Chapter One) and GIS-based final model (Chapter Five) can be used to address habitat issues and to determine areas to protect and areas for reintroduction. Data on habitat loss

(Chapter Four) will help assess past causes of extirpation and may be used to assess how human population growth may affect Gunnison sage grouse in the future. The genetic data (Chapters Two, Three) support the distinction of Gunnison sage grouse as a species and hopefully can be used to manage against the loss of genetic diversity. A conservation plan that integrates the information from this dissertation and from other studies, should be completed and implemented to assure the persistence of this species.