GENETIC VARIATION OF PRONGHORN POPULATIONS IN TEXAS

A Thesis

By

Renee C. Keleher

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Approved as to style and content by:

Louis A. Harveson, Ph.D.	Randall DeYoung, Ph.D.
(Committee Chair)	(Member)
Patricia M. Harveson, Ph.D.	Billy Tarrant
(Member)	(TPWD Representative)

Robert J. Kinucan, Ph.D.

(Dean of Agricultural and Natural Resource Sciences)

ABSTRACT

Texas Parks and Wildlife Department (TPWD) established approximately 100 pronghorn herd units in the 1970s using large land holdings, historic habitat conditions, survey data, and suggested movement barriers. Today, these same herd units are the basis of Texas' pronghorn survey and harvest management program. An updated assessment of pronghorn population structure is needed. I sampled 351 pronghorn throughout their distribution in Texas during the 2007-2008 harvest seasons and genotyped 344 pronghorn at 8 microsatellite loci. My goals were to assess geographic patterns of genetic similarity and to investigate the spatial scale of population structure in Texas. I detected moderate levels of genetic diversity within sampled pronghorn populations, and a small but significant level of genetic structure among populations (F_{ST} = 0.034). Bayesian analyses of population structure revealed that sampled populations could be clustered into 2 groups and a weak correlation (r^2 = 0.024) between genetic distance and geographic distance among populations. I concluded that population structure in Texas is not strongly differentiated. This may suggest that either gene flow is occurring among and within populations, historical genetic structure is still being detected, or previous pronghorn translocations has affected the genetic structure of pronghorn populations in Texas. Future research should involve more molecular markers, and increased sample sizes from the Panhandle and Rocker b populations. Overall, information from this project can aid TPWD in delineating pronghorn metaherd units and may assist in future trap, transport, and translocation projects in Texas.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LITERATURE REVIEW

Origin and Early History

Pronghorn (*Antilocapra americana*) belong to the order Artiodactyla and are the only extant members of the North American family Antilocapridae (Frick 1937). Yet, controversy over the taxonomic position of Antilocapridae among the Bovidae and Cervidae families exists (O'Gara and Matson 1975, Janis 1982, Gentry and Hooker 1988). Pronghorn are traditionally placed in the family Antilocapridae, allied with the Bovidae in the suprafamily Bovidoidae (Simpson 1945). However, O'Gara and Matson (1975) placed pronghorn in the subfamily Antilocaprinae within the family Bovidae due to similar horn and teeth characteristics. Bovidae and Antilocapridae are characterized by hypsodont cheek teeth and permanent bony horn cores covered by keratinous sheaths. In contrast, other investigators have allied the family Antilocapridae with the Cervidae in the suprafamily Cervidoidae because horns and horn-like structures could have evolved independently a number of times in ruminant lineages (Leinders and Heintz 1980). Therefore, antler and horn core development was used for placement of pronghorn in the Cervidoidae suprafamily (Byers 1997). For example, antler development in cervids and horn development in pronghorn is apophyseal, in which antler and horn core develop as elongations of the periosteum of the frontal bone; horn development in all Bovidae is epiphyseal, in which the horn core develops from fusion of the embryonic ossa cornua to the frontal bone (Solounias 1988). Further, 2 lacrimal orifices and closed grooves above the distal ends of the cannon bones have led some taxonomists to consider pronghorn to be related more closely to cervids (Koopman 1967). It was the presence of annually

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deciduous horn sheaths that placed pronghorn in a family separate from Bovidae and Cervidae (O'Gara and Yoakum 2004). Kraus and Miyamoto (1991) were unable to resolve phylogenetic relationships among Antilocapridae, Bovidae, Cervidae, and Giraffidae using mitochondrial DNA sequence data due to rapid divergence of the families occurring 23-28 million years ago. Limited fossil evidence and conflicting genetic analyses continues to complicate the taxonomic classification of pronghorn.

The Antilocapridae family is divided into 2 subfamilies, Merycodontinae and Antilocaprinae (Frick 1937, Ahearn 1988). The Merycodontinae first appeared in the mid-Miocene epoch and went extinct by the end of the Miocene; the Antilocaprinae appeared in the late Miocene but have persisted to the present as the single species *Antilocapra americana* (Janis 1982). Antilocaprinae were generally larger (30-80 kg: Janis 1982) than Merycodontinae (7-30 kg; Janis 1982), but the most noticeable difference between the two groups was in horn structure. Many species of Merycodontinae bore palmate antler like structures with burred pedicels resembling cervid antler; horns of Antilocaprinae were smooth and covered with the deciduous sheath characteristic of living pronghorn (Frick 1937, Webb 1973).

Historically, over 30 million pronghorn occurred in North America, equal to or exceeding the American bison (*Bos bison*) population (Nelson 1925, Yoakum 1978). Pronghorn were nearly extirpated during the 19th century because of human exploitation. Westward expansion during the 1800s resulted in habitat degradation, excessive hunting, agricultural cultivation, and uncontrolled grazing of pronghorn habitat which decimated the population (Schmidly 2002). Pronghorn numbers were at their lowest around 1915, with a gross continent-wide population estimate of 13,000 animals (Hoover et al. 1959).

By then, most states realized the imminent danger of pronghorn extirpation and passed laws ceasing hunting. Conservation-minded organizations and concerned individuals supported those state and federal agencies or programs offering protection for the remaining pronghorn population. These refuge and management initiatives lead to the dramatic recovery of the pronghorn. Today, there are roughly 850,000 pronghorn in the United States, most of them occurring in Wyoming (O'Gara and Yoakum 2004).

Classification and Distribution

Pronghorn occur in western North America from southern Canada to northern Mexico and in the United States from Washington, Oregon and California east to Texas and the Great Plains states. Approximately two-thirds of the North American pronghorn population lives on the short, mixed, and tall grasslands of the central Great Plains, one-third occupies the shrub-steppe biome, and <1% live in desert ecosystems (Yoakum 1994). Pronghorn are adapted to low, open vegetation that allows broad visibility and does not inhibit mobility so their defense mechanisms of keen eyesight and speed can be used as protection against predators (Kitchen 1974, Hailey 1991, Ockenfels and Wennerlund 1994). Although the total area of suitable habitat has been greatly restricted by human settlement, pronghorn still inhabit much of their historic range (Hall 1981, Autenrieth et al. 2006).

There are 5 subspecies of pronghorn: American pronghorn (*A. a. americana*, Ord 1815), Oregon pronghorn (*A. a. oregona*, Bailey 1932), Mexican pronghorn (*A. a. mexicana*, Merriam 1901), Sonoran pronghorn (*A. a. sonorensis*, Goldman 1945), and Peninsular pronghorn (*A. a. peninsularis*, Nelson 1912). Morphological characteristics

(e.g. color, size and form) are the primary reason for the naming of separate subspecies. Mitochondrial DNA and protein electrophoresis analyses have indicated no divergence between *A. americana* and *A. oregona*, and suggest intergrade zones between *A. mexicana* and *A. americana* as well as *A. mexicana* and *A. sonorensis* (Lee et al. 1994). The subspecific integrity of pronghorn populations was further complicated by restocking efforts throughout much of their historic range earlier this century (Kitchen and O'Gara 1982). Thirty thousand pronghorn were translocated into 17 states within their historic distribution from 1940-1997 (O'Gara et al. 2004). Translocations of *A. americana* into ranges of other supposed subspecies may have altered the genetic integrity of remnant herds (Lee et al. 1994). Taxonomic uncertainties and distributional ranges of some subspecies are still poorly known (Hall 1981, O'Gara and Yoakum 2004).

Physical Description

Pronghorn are medium sized (1.3-1.4 m, 43-55 kg) ungulates with tan coloration on the upper portions of their bodies with white on the face, chest, stomach, and rump (Kays and Wilson 2002). Males are distinguished from females by large pronged horns and a dark triangular-shaped cheek patch at the corner of the jaw. Male horn length averages 25-38 cm and female horn length averages 2.5-10 cm and both sexes shed their sheaths annually (Yoakum and O'Gara 2000). Pronghorn have several morphological and physiological adaptations that allow them to maintain speeds of 48.3-64.4 km/hr for several kilometers (Bullock 1974). Specifically, pronghorn have lightweight bones, long slender limbs, and lack dewclaws (O'Gara and Yoakum 2004). In addition, pronghorn have an enlarged respiratory system and heart to maximize oxygen intake, countercurrent

hear exchange to keep cool, and a comparatively small digestive tract (Sundstrom et al. 1973, O'Gara and Yoakum 2004).

Diet

Pronghorn are opportunistic herbivores and select their food based on availability, nutritional value, palatability, season, and succulence. Adult pronghorn consume 1.1-1.3 kg (dry weight) quality forage per day to meet their nutritional needs (Zarn 1981). Studies indicate a diet composition ranging from 65-70% forbs, 25-30% browse, and 5-8% grasses making them concentrate selectors due to their small rumen volume to body weight ratio (Buechner 1950, Hailey 1979, Roebuck 1982). Therefore, pronghorn require a higher quality diet compared to domestic ruminants (Schwartz et al. 1977). Pronghorn diets vary due to land management practices, geographic location, climate, soils, and habitat types. For example, in the Trans-Pecos region of Texas common plants in the pronghorn diet are black dalea (*Dalea frutescens*), tall buckwheat (*Eriogonum tenellum*), scarlet beeblossom (*Gaura coccinea*), stemmed bitterweed (*Hymenoxys scaposa*), cutleaf daisy (*Erigeron composites*), sideoats grama (*Bouteloua curtipendula*), blue grama (*Bouteloua gracillis)*, deer vetch (*Aeschynomene americana)*, coneflower (*Echinacea purpurea*), and paper flower (*Psilostrophe cooperi*) (Buechner 1950). Pronghorn drink water from free-standing sources, although water obtained from forage and metabolic production provides enough moisture for body functions during limited rainfall (Fox 1997, Autenrieth et al. 2006).

Biology

Pronghorn are gregarious and form herds for seasonal movements and protection against predators, with herd composition varying seasonally (Buechner 1950, Yoakum 1978, Yoakum and O'Gara 2000). During the rut mature bucks separate from herds to defend a territory or to form a harem, after the rut pronghorn group together in mixed gender herds (Buechner 1950).

Pronghorn are nomadic and move within their home range in response to unsuitable habitat conditions such as drought, blizzards, disturbance, and limited forage or water availability (Ockenfels and Wennerlund 1994). Most pronghorn exhibit seasonal movements and few populations participate in traditional migrations. In the northern parts of their range, pronghorn can move ≤320 km in response to deep snow or to reach available winter forage. During dry seasons, southern populations may increase mobility in search of forage and water (Yoakum 1975, Riddle 1990). Size of pronghorn home ranges and daily movements are highly variable due to differing habitat quality, population density, season, and land use practices (O'Gara and Yoakum 2004, Canon and Bryant 2006, Barnowe-Meyer 2009). Natural and artificial barriers can impede pronghorn movements and exclude the occupancy of otherwise suitable habitats in their home range (Autenrieth et al. 2006, Gavin and Komers 2006). Pronghorn have a disinclination to jump over fences or other objects. Ordinarily they crawl under barbed-wire fences. Goat and sheep net-wire fencing can be detrimental to herds of pronghorn threatened with starvation because of their impermeability (van Riper and Ockenfels 1998, O'Gara and Yoakum 2004).

Pronghorn begin reproducing when they are 16 months of age and continue to breed annually until they are 8-10 years. The gestation period averages 252 days and is relatively long compared to similar sized ruminants (Asdell 1964). Twinning is common with an average of 1.9 fawns/doe (Byers 1997). During gestation, female pronghorn are more susceptible to malnutrition and will reabsorb fetuses if nutrition is not adequate (Yoakum and O'Gara 2000). The length of the breeding season varies considerably across their distribution. Most pronghorn in northern ranges breed during a short period from mid September to early October, while southern counterparts may breed from July through October (Buechner 1950, O'Gara 1968). Pronghorn are polygynous and their reproductive strategy consists of forming harems and a territorial defense system. However, switching between 2 mating systems in 1 reproductive season is not uncommon (Byers 1997). When forage quality varies between areas and the best resources are clumped, pronghorn tend to be territorial and males on the best territory do most of the breeding. As resource distribution becomes more uniform, the system shifts towards dominions (Byers and Kitchen 1988). Males exhibit different degrees of defense against exclusive areas which appears to be related to their food source, population density, and sex ratio (Maher 1994).

Currently, coyotes (*Canis latrans*) are the pronghorn's primary predator while bobcats (*Lynx rufus*), golden eagles (*Aquila chrysaetos*), and mountain lions (*Puma concolor*) are of less concern (Canon and Bryant 2006, Jacques et al. 2007, Barnowe-Meyer 2009). Predation claims a high percentage of fawns, especially in areas where predators are numerous and pronghorn numbers are scarce. Pronghorn mortality rates are greatest for fawns 11-20 days of age, beyond 20 days most fawns can outrun a coyote

(Byers 1997). The most common threat to pronghorn is malnutrition. This problem becomes acute when net-wire fences restrict pronghorn from access to areas with higher quality of habitat. Population crashes resulting from malnutrition are not uncommon and usually the blame falls on poor rangeland conditions, severe weather conditions and the inability to access adequate habitat (Compton 1970, McKenzie 1970, Simpson and Leftwich 1978, Hailey 1979, O'Gara and Yoakum 2004).

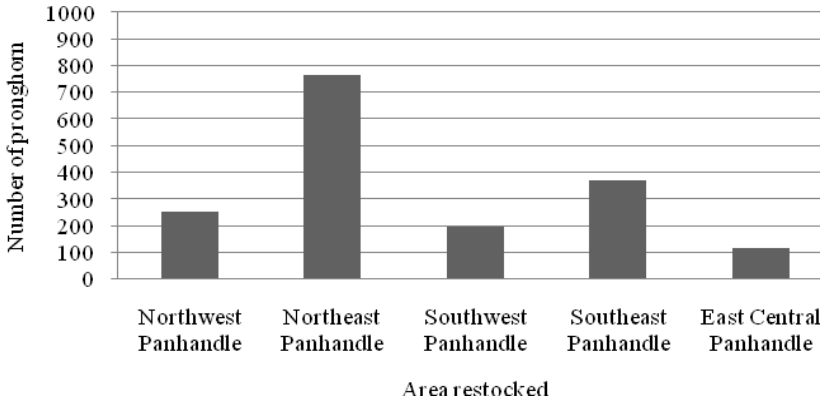
History and Management of Pronghorn in Texas

Historically, 1,000,000 pronghorn were estimated to have occurred throughout two-thirds of Texas (Yoakum 1978, DelMonte and Kothmann 1984). Today, the geographic range of pronghorn in Texas can be described as discontinuous in comparison to pronghorn ranges in more northern latitudes (Lee et al. 1989). The settling of west Texas during the late 1800's resulted in a significant decline in the pronghorn population (DelMonte and Kothmann 1984). Long periods of uncontrolled hunting, overgrazing of grasslands by domestic livestock, and extensive cultivation of prairie habitat decimated the distribution and numbers of pronghorn. In fact, concerns of extirpation lead to a closed hunting season in Texas in 1903 (Buechner 1950, Swepston and Hailey 1991). By 1924, Texas' pronghorn population estimate was only 2,407, of which 692 occurred in small isolated herds in the Trans-Pecos region (Swepston and Hailey 1991, Schmidly 2002). Further conservation efforts of the state legislature and increased protection from law enforcement, landowners, and ranchers led to pronghorn population recovery. Hence the population in the Trans-Pecos area reached 3,888 in 1938 and increased to 4,742 in 1941 (DelMonte and Kothmann 1984).

For more than a century, translocation of wildlife species for the purpose of reintroduction, introduction, or supplementation has been one of the most commonly used techniques in wildlife management (Latch et al. 2006). Trapping and transplanting pronghorn in Texas was initiated in Texas in 1939 with an objective of statewide restoration of the species to suitable habitat within its historic range (Hailey 1979). Initial trapping efforts were aimed at removing pronghorn from overstocked sheep and goat ranges and from enclosed areas where population surpluses existed (DelMonte and Kothmann 1984). Transplants were made to previously inspected and approved sites within the historic range as established by Bailey (1905). The majority of pronghorn broodstock came from within Texas but a small number (~230 animals) of sources came from Colorado, Utah, and Wyoming (Texas Parks and Wildlife Department, unpublished data).

From 1939-1982 approximately 5,700 pronghorn were trapped and transplanted into areas of perceived suitable habitat in Texas; most of the restocking occurred in the Trans-Pecos and Panhandle regions (DelMonte and Kothmann 1984, Figures 1A, 2A). The early restocking efforts of 1939-1956 transplanted roughly 4,000 pronghorn in Texas (Figures 1B, 2B). Several factors such as predation, movement barriers, competition with livestock, oil field development, illegal hunting, drought, agricultural development, and brush encroachment limited the success of the transplants (Jones 1949, DelMonte and Kothmann 1984). By 1953, the pronghorn population had increased enough to allow a closely regulated hunting season.

In 1972, another intensive restocking effort began when surplus pronghorn numbers became available on a large ranch (Rocker b, Irion Co., Texas) located on the



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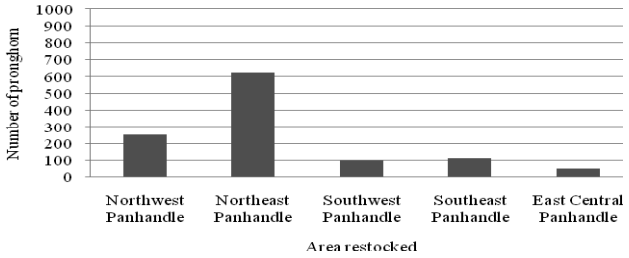

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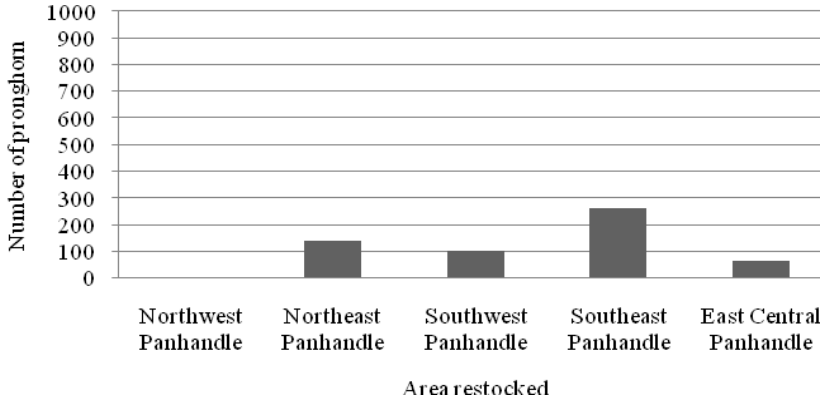
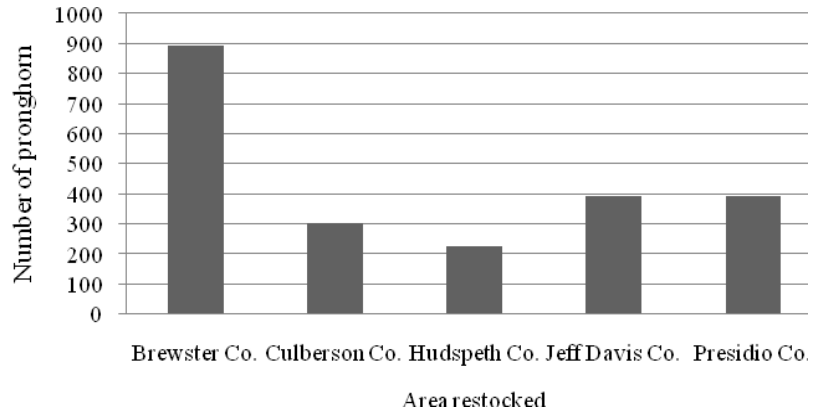
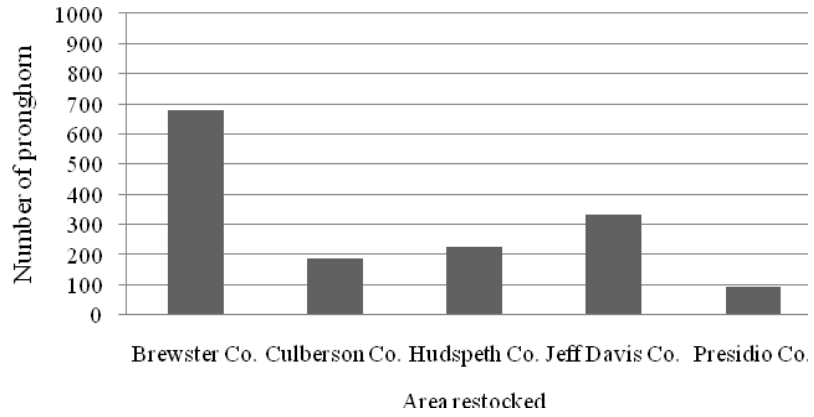


(C)

Figure 1. Total number of pronghorn transplanted in the Panhandle, Texas from 1939-1991 (A), from 1939-1956 (B), and from 1972-1991 (C).



(A)



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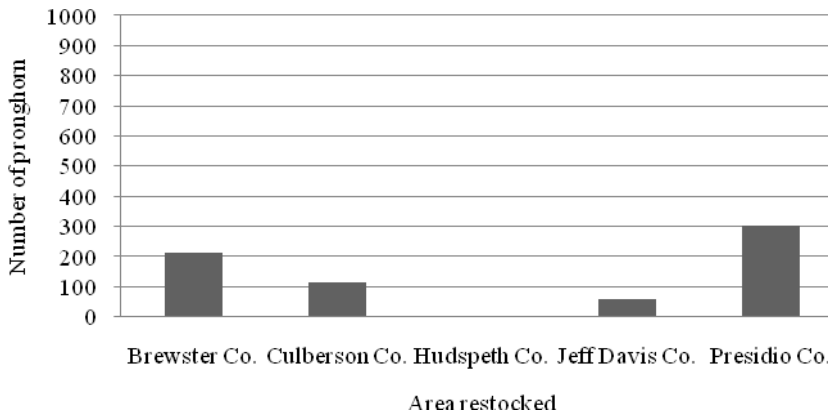

(C)

Figure 2. Total number of pronghorn transplanted in the Trans-Pecos, Texas from 1939-1991 (A), from 1939-1956 (B), and from 1972-1991 (C).

border of the lower Rolling Plains and Edwards Plateau region of Texas. In 2 years (1972 – 1974), TPWD trapped 1,100 pronghorn on the Rocker b ranch and transplanted them to release sites throughout the Trans-Pecos and Panhandle regions (Figures 1C, 2C). From 1979-1982, an estimated 230 pronghorn were released in Texas that utilized sources from Colorado, Wyoming, and Utah. One hundred forty of those numbers were transplanted to the northeast Panhandle (Texas Parks and Wildlife Department, unpublished data, Figure 1C).

In summary, from 1972 – 1982, 1,658 pronghorn (including those from the Rocker b) were released on 78 sites in 29 counties of Texas. A total of 714 bucks (43%) and 944 does (57%) was stocked for a sex ratio of 1.3 does per buck (DelMonte and Kothmann 1984). From 1982 to 1991, small numbers of pronghorn were released primarily in the Texas panhandle (Texas Parks and Wildlife Department, unpublished data). The status of the restocked herds was evaluated 5 years after the restocking event. By 1984, 66 of the 78 release sites were evaluated; 12 (18.2%) of the transplanted herds were rated successful, 23 (34.8%) of the transplanted herds were rated undetermined with decreased numbers of broodstock surviving, and failures accounted for 31 (47%) of the remaining herds with loss of broodstock. Coyote predation, illegal hunting, prolonged drought, and the small numbers of animals per translocation were thought to contribute to the failures at many of the unsuccessful release sites.

Today, pronghorn populations are managed in approximately 100 herd units throughout the Trans-Pecos, Panhandle, and southern Rolling Plains regions of Texas (Figure 3). TPWD established pronghorn herd units in the 1970s. They used large land

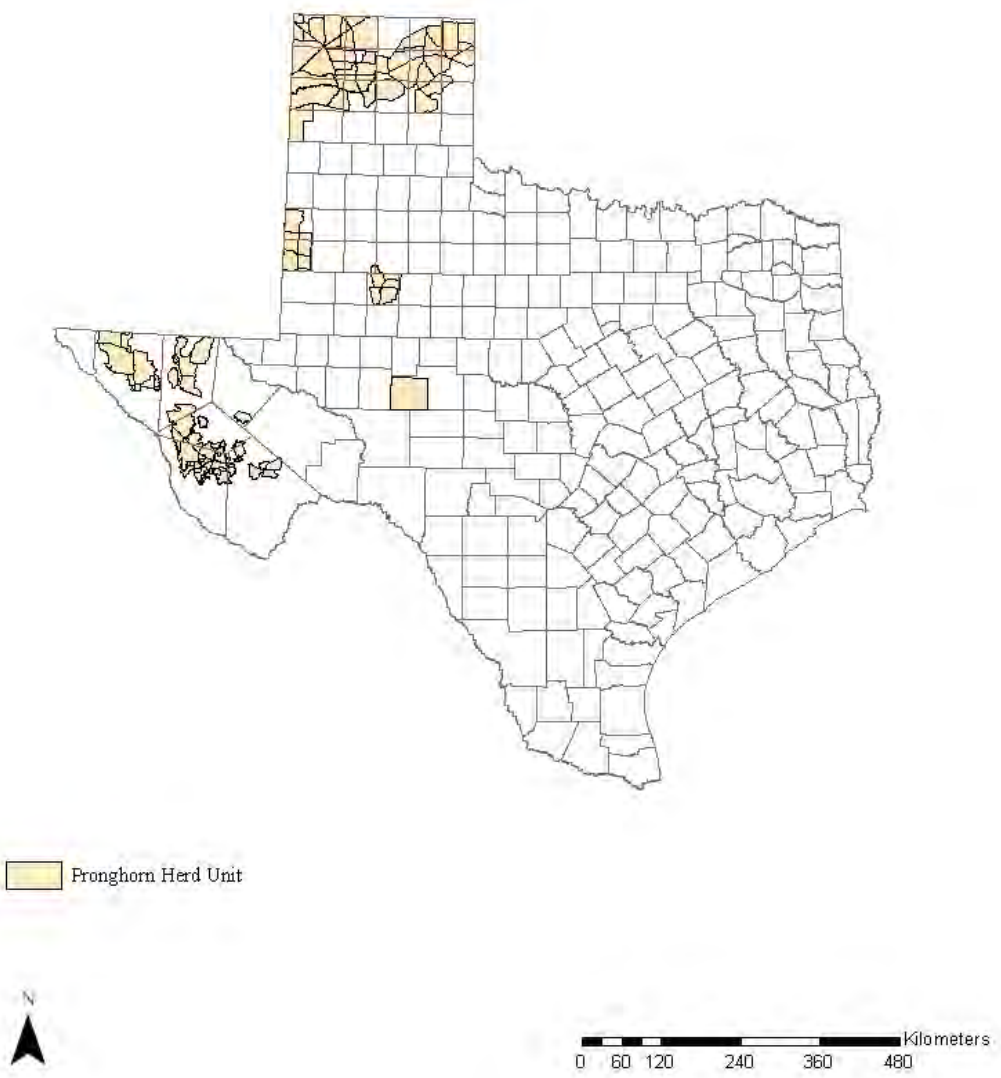

Figure 3. Pronghorn herd units in Texas established by the Texas Parks and Wildlife Department.

holdings, historic habitat conditions, survey data, and suggested movement barriers (e.g., canyons, certain types of fences, mountain ranges, and roads) to define each herd unit.

Today, these same herd units are the basis of Texas' pronghorn survey and harvest management program. Aerial surveys of herd units are flown in line transects throughout late July-August which provide data such as herd density, buck-doe ratios, fawn ratios, and provide harvest quotas for permit issuance. Buck only permits have been conservatively issued and permit utilization remains close to 50% (Tarrant 2006).

Recent trend data indicate a decline in pronghorn numbers in the Trans-Pecos. In fact, pronghorn numbers reached one of the lowest recorded population estimates of 5,919 individuals in 2009 (Figure 4). A number of theories have been suggested to explain these declines in population size and distribution. Among them are persistent droughts, increased predation on fawn crops, long-term habitat deterioration, and depleted range resources (Buechner 1950, Hailey 1979, Sullins 2002). Simpson et al. (2006) evaluated the relationship between pronghorn population trends and precipitation indices in the Panhandle and Trans-Pecos regions of Texas. They also examined the relationship of fawn production and precipitation indices in the 2 regions. Their data suggest the population in the Trans-Pecos is closely related to long-term moisture conditions. Further, the relationship between fawn production and precipitation is closely related to immediate moisture conditions. However, population trends and precipitation were not closely correlated in the Panhandle. They concluded that the amount of precipitation influences the quality of habitat, which in turn determines the production and abundance of pronghorn in the Trans-Pecos. Further, net-wire fences to control domestic sheep are disadvantageous for pronghorn seeking forage in the arid southwest

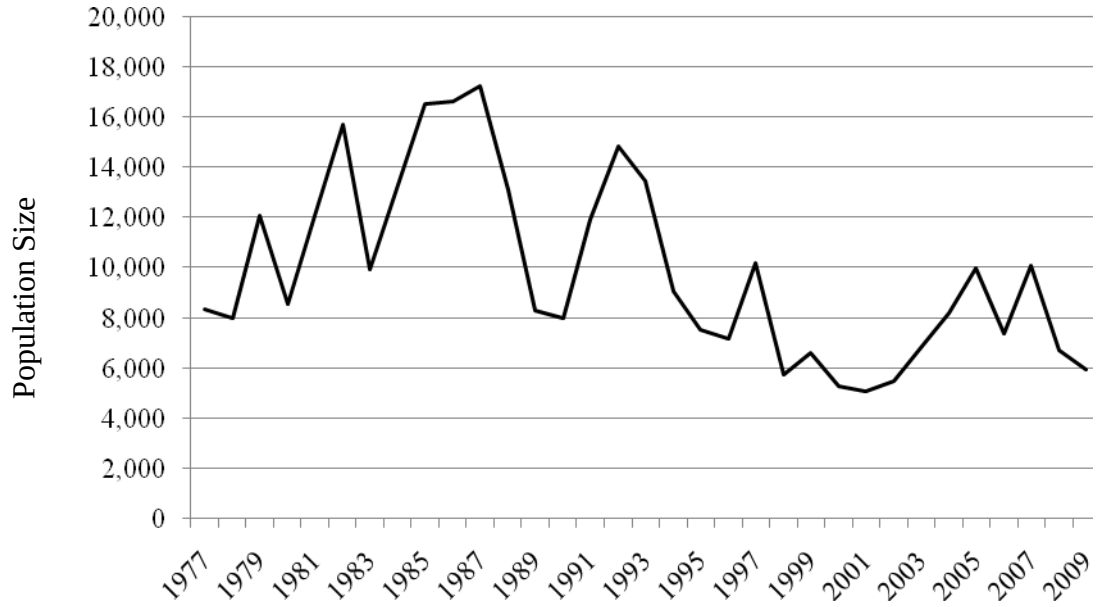

Figure 4. Estimated pronghorn population for Trans-Pecos, Texas, 1977–2009.

because of their movement restrictions (Buechner 1950, Van Riper and Ockenfels 1998, O'Gara and Yoakum 2004, Autenrieth et al. 2006). In fact, net-wire fences in the Trans-Pecos prevented pronghorn from moving into areas with available forage during a severe drought in the 1960s. The population experienced a 60% die off from June 1964 to June 1965 due to malnutrition (Hailey 1979). Consequently, creating travel corridors and fence modifications that facilitate pronghorn movements are a management priority in Texas.

Genetic Applications in Wildlife Management

Although short-term survival of populations is influenced more by demography and stochastic events, genetic variation is considered an important factor in the long term persistence of populations (Lande 1998). Advances in molecular genetics, including the development of the polymerase chain reaction (PCR), the advent of new molecular markers, and spatial autocorrelation have increased the integration of genetic methods into wildlife science. As a result, genetic techniques are being combined with demographic, geographic, and ecological data in wildlife studies (Honeycutt 2000). For example, Latch et al. (2006) evaluated the subspecific status and degree of hybridization of individuals within an introduced population of Merriam's turkeys in the Davis Mountains, Texas.

Genetic variation is believed to be important for maintaining fitness and adapting to environmental change within populations (Soule and Wilcox 1980). Patterns of genetic variation can be used to evaluate gene flow and the degree to which populations have been isolated over time. Gene flow decreases genetic variation and homogenizes populations. If gene flow between populations is limited, increased levels of genetic

differentiation and population structuring can occur. Genetic variation within individuals is described as the percentage heterozygous loci (Beebee and Rowe 2004). At the population level, genetic variation is determined by the percentage of polymorphic loci, average number of alleles per locus, or by expected heterozygosity assuming Hardy-Weinburg equilibrium (DeYoung and Honeycutt 2005). Genetic data used to measure structure of populations can be applied to designate management units (Palsboll et al. 2006, Holycross and Douglas 2007). Moritz (1994) defines management units as populations with significant divergence of allele frequencies at nuclear or mitochondrial loci. Populations with divergent gene frequencies likely exchange so few migrants that they exhibit demographic independence and should be managed appropriately (Moritz 1994). Patterns of genetic variation overlaid on geographical distribution allow for the delineation of management units.

Molecular markers are used to detect genetic variability of individuals and populations. Molecular markers are sections of a genome that can be identified and compared across individuals to measure variation (Beebee and Rowe 2005). Levels of polymorphism vary between markers; therefore, markers can be combined or selected individually to investigate genetic variation. The selection of marker(s) to use often depends on the research question, sample size, cost, and the degree of information desired. Microsatellites are useful genetic markers for studies focusing on gene flow, dispersal, and geographic structuring of populations because they are widely dispersed in eukaryotic genomes, have a high mutation rate, and are highly polymorphic (Jarne and Lagoda 1996, Beaumont and Bruford 1999). Microsatellites are composed of short nucleotide repeats of noncoding DNA at a single inherited locus. Variation within

microsatellites is determined by the number of nucleotide repeats at a locus. Because microsatellite markers have shown to be selectively neutral and have higher variation, their use may provide greater resolution among closely related populations than other classes of markers (Lou 1998, Honeycutt 2000). Microsatellites have been used to study phylogenetic relationships (Beaumont et al. 2001, Chan and Arcese 2002) and genetic diversity in many wildlife species, such as *Ursus arctos* (Waits et al. 1998), *Puma concolor* (Walker et al. 2000), *Gulo gulo* (Cegelski et al. 2003), and *Cervus elaphus nannodes* (Williams et al. 2004).

Pronghorn populations are often described by demographic features with little regard for genetic characterizations. It is well documented that natural (e.g., mountains and canyons) and manmade obstacles (e.g., fences, railroads,and roads) can curtail pronghorn movements and isolate populations (Yoakum 1975, van Riper and Ockenfels 1998, Gavin and Komers 2006). Yet, there are few genetic studies on the effects of fragmentation on pronghorn populations. If populations become further reduced and isolated, it is important to determine if genetic depletion will limit pronghorn recovery. In the future it may be necessary to augment populations with individuals from other areas. Thus, knowledge of genetic variation among populations can aid in successful management strategies and long term population monitoring.

Pronghorn Genetic Studies

Phylogeography, gene flow, and population structure have been assessed across a wide portion of the pronghorn's range. However, the majority of the studies have

concentrated on populations in the northern latitudes of North America (Jenks et al. 2006, Lee et al. 1994, Lou 1998, Reat et al. 1999).

A pioneering study of genetic structure in pronghorn populations was performed by Lee et al. (1989). Lee et al. (1989) used allozyme markers to assess genetic relationships and patterns of genetic variation in 6 geographically isolated pronghorn populations (n = 65) in west Texas. Their study suggested that west Texas pronghorn populations were characterized by relatively low levels of genetic variation within populations (mean multilocus heterozygosity = 0.027) and moderate levels of genetic differentiation among populations (Roger's genetic distance ranged from 0.010 to 0.064). Allozyme variation in southern Trans-Pecos (Presidio, Jeff Davis, and Brewster counties) populations were divergent from northern Trans-Pecos and Panhandle (Hudspeth, Irion, and Dallum) counties. Further, they concluded that the Marathon Basin herd (Brewster county) was significantly different from the other 5 populations and recommended no translocations into the Marathon Basin population.

Lee et al. (1994) expanded his analysis of pronghorn populations by assessing allozyme and mitochondrial DNA (mtDNA) variation in 330 pronghorn from 29 populations throughout North America. Analysis of their data suggested low levels of genetic diversity (mean multilocus heterozygosity = 0.024). Genetic variation was generally higher in the southern populations (Trans-Pecos region of Texas and in New Mexico) with a mean heterozygosity of 0.031 compared to a mean of 0.015 from northern populations (ranging from Montana to the Texas Panhandle). Moderate differentiation among pronghorn populations across North America was detected. Clustering of Rogers' distance values (ranging from 0.01 – 0.07) using unweighted pair group method analysis

indicated close affinities between *A. oregona* and *A. americana*. Populations from southwestern Texas and southern New Mexico clustered in several groups outside of the Oregon-American subspecies cluster. Their analysis supported the geographic range of 2 subspecies of pronghorn in Texas, *Antilocapra a. americana* and *Antilocapra a. mexicana* (Lee et al. 1994). The study's data also suggested intermediate populations that include genetic characteristics of both the Mexican and the American pronghorn subspecies.

To estimate the genetic diversity within and among pronghorn populations, Lou (1998) examined nucleotide sequences from mtDNA and measured genetic variation with 4 microsatellite loci in 196 pronghorn from 14 North American pronghorn populations. Similar to Lee et al. (1994), he found 2 genetically differentiated groups of populations: a southern group (Texas and Arizona populations) that are representative of the Mexican and Sonoran subspecies and a northern group (Wyoming, Montana, Oregon, California, Colorado, Idaho and Kansas populations) representing the American and Oregon subspecies. His analysis indicated moderate genetic differences among populations and suggests that gene flow across the continent is restricted. Updated genetic analyses of pronghorn populations in Texas will allow researchers to further examine the structure being detected by Lou (1998) and assist biologists with population management in a scale-appropriate manner.

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INTRODUCTION

Pronghorn are an important big game species in Texas. Their economic value and aesthetic popularity generates interest in management and research that focuses on increasing or maintaining pronghorn populations. Historically, pronghorn were distributed over two-thirds of Texas including all areas west of the 97th meridian (Buechner 1950). However, Texas' pronghorn populations have steadily declined in numbers and geographic range since the westward expansion of human settlement (Buechner 1950). In fact, pronghorn numbers in the Trans-Pecos region reached the lowest recorded population estimate of 5,061 individuals in 2001. Population declines have been linked to habitat loss, prolonged drought, increased predation, movement restrictions caused by fences, and disease (Buechner 1950, Hailey 1979, Simpson et al. 2006, Texas Parks and Wildlife Department unpublished data).

A thorough understanding of pronghorn population structure is vital to effective management, especially in light of recent trends in pronghorn numbers. Thus, it is important to understand the spatial structure of pronghorn populations to determine if declining pronghorn herd units are genetically isolated. The reduction of gene flow can lead to inbreeding and loss of genetic diversity within populations. Traditional wildlife research involving tagging and radio telemetry can provide valuable information on wildlife populations but are time-consuming and constrained by sample size. Fortunately, analyses based on genetic markers offer a highly useful alternative to traditional methods (DeYoung and Honeycutt 2005). Studies of gene flow in relation to landscape structure can give valuable information about terrain features that influence effective movements

(i.e., movements followed by successful reproduction), which are often difficult to assess by direct methods such as observation or telemetry (Coulon et al. 2006).

Microsatellites are short, highly repetitive sequences of DNA. Microsatellites that are located in non-coding segments of DNA are typically used to measure genetic variation (Frankham et al. 2004). They are useful genetic markers for wildlife population studies, especially those which focus on gene flow and dispersal, geographic structuring, and recent population history. Microsatellites have been used to detect fine-scale genetic structure in many ungulates (DeYoung et al. 2003, Whittaker et al. 2004, Williams et al. 2004, Coulon 2006). The pioneering genetic studies of pronghorn populations were done over 20 years ago (Lee et al. 1989, Lee et al. 1994). These 2 studies focused on broad-scale comparisons of genetic diversity and similarity among populations in North America among a relatively small number of individuals and populations in western Texas. While these studies provided important information, the use of this data in refining pronghorn management in Texas is limited. First, there have been significant changes in pronghorn census sizes and distributions since their publication. Second, the genetic markers (allozymes and restriction analysis of mtDNA haplotypes) used in their studies are somewhat limited in terms of genetic variation. New genetic markers such as DNA microsatellites have greater resolution for detecting fine scale population structure.

Previous genetic studies used molecular markers with little variation and limited analytical tools for addressing fine scale questions. Each genetic distance parameter has unique evolutionary and statistical properties. Evolutionary relationships inferred from each genetic distance can be quite different. The inferred structure often depends on assumptions concerning historical and demographic population characteristics, which are

reliable to an unknown extent (Coulon et al. 2006). Also, the idealized models of population structure, migration, demographics, and evolution on which these methods are based are far from realistic and unlikely to occur in nature (Pearse and Crandall 2004). New analytical techniques (e.g., Bayesian clustering methods) and GIS applications have increased the inferential power of genetic markers for understanding population structure across landscapes (Pearse and Crandall 2004, Latch et al. 2006*a*). Thus, an updated study of population genetic structure of pronghorn in Texas is warranted.

Texas' pronghorn population is managed within about 100 herd units throughout the Panhandle, Trans-Pecos, and southern Rolling Plains regions (Figure 3). The herd units were delineated in the 1970s by factors such as population estimates, habitat quality, large land holdings, roads, mountain ranges, and fences. Today, these same herd units are used for pronghorn management purposes, such as population surveys and harvest permit issuance. However, herd units delineated over 30 years ago do not represent the current pronghorn population structure. Texas Parks and Wildlife Department (TPWD) has discussed combining some herd units into metaherd units to increase the efficiency of pronghorn management in Texas. Before TPWD can proceed, important information regarding pronghorn population structure is needed.

The goal of this study was to evaluate herd units to determine if pronghorn management reflects contemporary patterns of population structure. I used several landscape-scale analyses based on genetic data from nuclear DNA microsatellite markers to characterize patterns of population structure and dispersal among populations of pronghorn in Texas. My objectives were to: (1) identify genetic diversity and similarity among sites, (2) assess population structure with respect to putative barriers, and (3)

integrate the information from objectives 1 and 2 to inform TPWD with biologically based management units. The identification of genetically distinct groups can help identify biologically meaningful management units, indicate dispersal corridors or barriers, and assess the level of connectivity or fragmentation among populations (DeYoung and Honeycutt 2005). Information from this project will provide a foundation for future pronghorn translocations and population monitoring efforts.

STUDY AREA

Pronghorn populations are distributed throughout grasslands in the Trans-Pecos and Panhandle regions of Texas with a small population occurring in the northern Edwards Plateau region.

Trans-Pecos

The Trans-Pecos region is about 7.3 million ha and is bordered to the east by the Pecos River, to the west and south by the Rio Grande, and to the north by New Mexico. There is tremendous vegetation diversity in the region, which includes at least 268 grass species and 447 species of woody plants (Hatch et al. 1990). More than 95% of the region constitutes rangeland. Grasslands persist on gentle slopes with deeper, sandstone-derived soils. Water is scarce; the few streams that originate from springs at higher elevations do not persist beyond the mouths of major canyons. The climate is mostly arid with scant precipitation and rapid evaporation (Buechner 1950). Mountain ranges occur throughout the Trans-Pecos and receive more precipitation (30-46 cm), primarily in the form of monsoonal rains, than the lowlands and basins (20-30 cm).

Putative pronghorn movement barriers.—Pronghorn populations occur in inter-mountain grasslands throughout this region. Mountain ranges, Highway 90, Interstate 10, urban sprawl associated with cities, and sheep and goat net-wire fencing are likely movement barriers in the Trans-Pecos (Figure 5). Most of the rangelands in the Trans-Pecos are privately owned, and the ranch boundaries and interior pastures are often fenced. Major roads and highways are also fenced on both sides in order to keep livestock

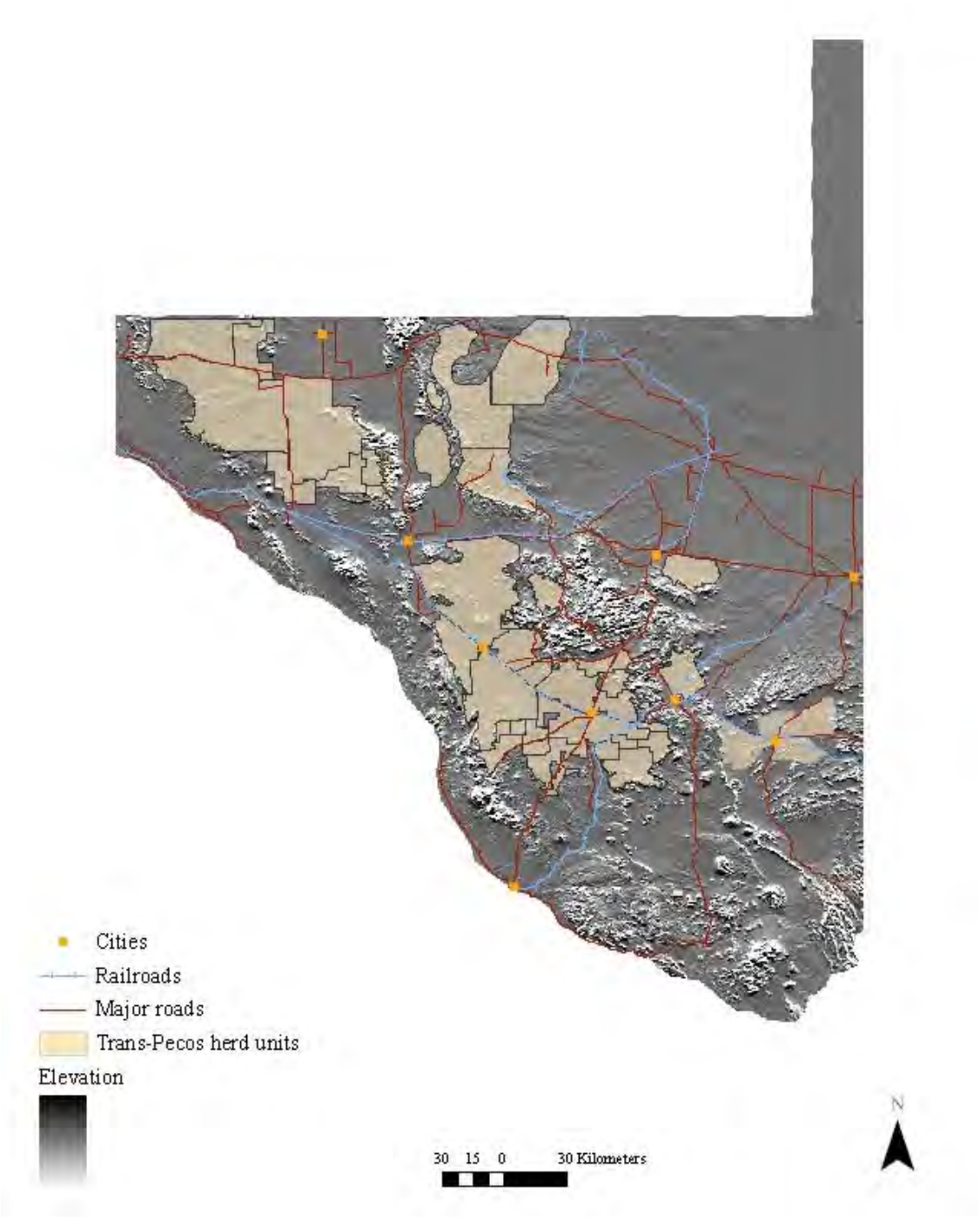


Figure 5. Putative movement barriers for pronghorn populations occurring in the Trans-Pecos region of Texas.

off. Pronghorn avoid crossing highways and mountains and are reluctant to cross fences with woven wire or not modified for wildlife passing. I did not explicitly evaluate movement barriers in the Panhandle or Edwards Plateau region. A more detailed analysis of barriers will be done only in the Trans-Pecos region.

Panhandle

Pronghorn populations; Northwest Panhandle, Northeast Panhandle, Southwest Panhandle, and Southeast Panhandle occur in the Panhandle region. The High Plains and the Rolling Plains of the Texas Panhandle extend north from the Edwards Plateau region to western Oklahoma. The Rolling Plains compromises 9.7 million ha and is located in the eastern half of the Texas Panhandle and is a part of the Great Plains of the central United States. Two-thirds of the Rolling Plains is combined cropland and rangeland (Hatch et al. 1990). Topography is flat to rolling with elevations of 243-914 m. Average annual precipitation ranges from 56-76 cm; most rainfall occurs in May and September. Hatch et al. (1990) described the Rolling Plains with tall and mid-grasses with increasing shrub species. The High Plains region of Texas compromises approximately 8 million ha of the Great Plains eco-region. About 60% of the area is cropland, half of which is irrigated (Hatch et al. 1990). The region consists of a relatively high and level plateau of sandy to heavy, dark calcareous clay soils over a layer of caliche known as the Caprock Escarpment. The plateau is dissected by the Canadian River and its associated riparian topography. Elevation in the High Plains ranges from 914-1,371 m and receives 38-53 cm of precipitation annually. Hatch et al. (1990) describes the vegetation of the High

Plains with mixed-grass plains, shortgrass high plains, shinnery oak grasslands, and mesquite grasslands.

Edwards Plateau

The Rocker b ranch, located in Irion County, is part of the northwestern section of the Edwards Plateau known as the Stockton Plateau area. The Rocker b Ranch pronghorn population has declined in recent years to the point where few harvestable males are present. The Edwards Plateau is 566,559 ha and 98% of the area is rangeland. This area is bordered to the north by the Panhandle and to the west by the Trans-Pecos. The Stockton Plateau supports short to midgrass mixed vegetation and redberry juniper (*Juniperus pinchotti*) (Hatch et al. 1990).

METHODS

Sample Collection

In Texas, pronghorn population management is regulated with buck-only harvest permits. Permits are issued to landowners whose properties fall within TPWD pronghorn management herd units. The number of permits issued is determined by annual population surveys and habitat assessment of herd units. I mailed packets of data cards and instructions for tissue collection to 1,531 landowners who received 3,369 harvest permits issued by TPWD during the 2007 and 2008 hunting seasons (Adkins 2009). Hunters were asked to collect a portion (~2.5 cm) of the tongue from harvested pronghorn. Successful hunters placed tissues in a plastic zip lock bag, and recorded the date, location, and permit number. Tissues were frozen until collection by TPWD biologists. Samples were kept frozen -20 °C until further analysis. Samples were grouped into populations based on sampling region, geographic proximity, and putative movement barriers (Figure 6).

DNA Extraction and Amplification

I extracted DNA from tissue samples using the DNeasy Tissue Kit and Protocol (Qiagen, Valencia, CA). I amplified eight microsatellite loci (Aam1, Aam2, Aam3, Aam4, Aam5, Aam6, Aam7 and Aam8) described by Carling et al. (2003) using the polymerase chain reaction (PCR). Amplification was performed in 20-µL reaction volumes. Forward primers for all microsatellite loci were fluorescently labeled on the 5′ end with a dye (NED, 6-FAM, or HEX) for detection and separation on an ABI 3130*xl*

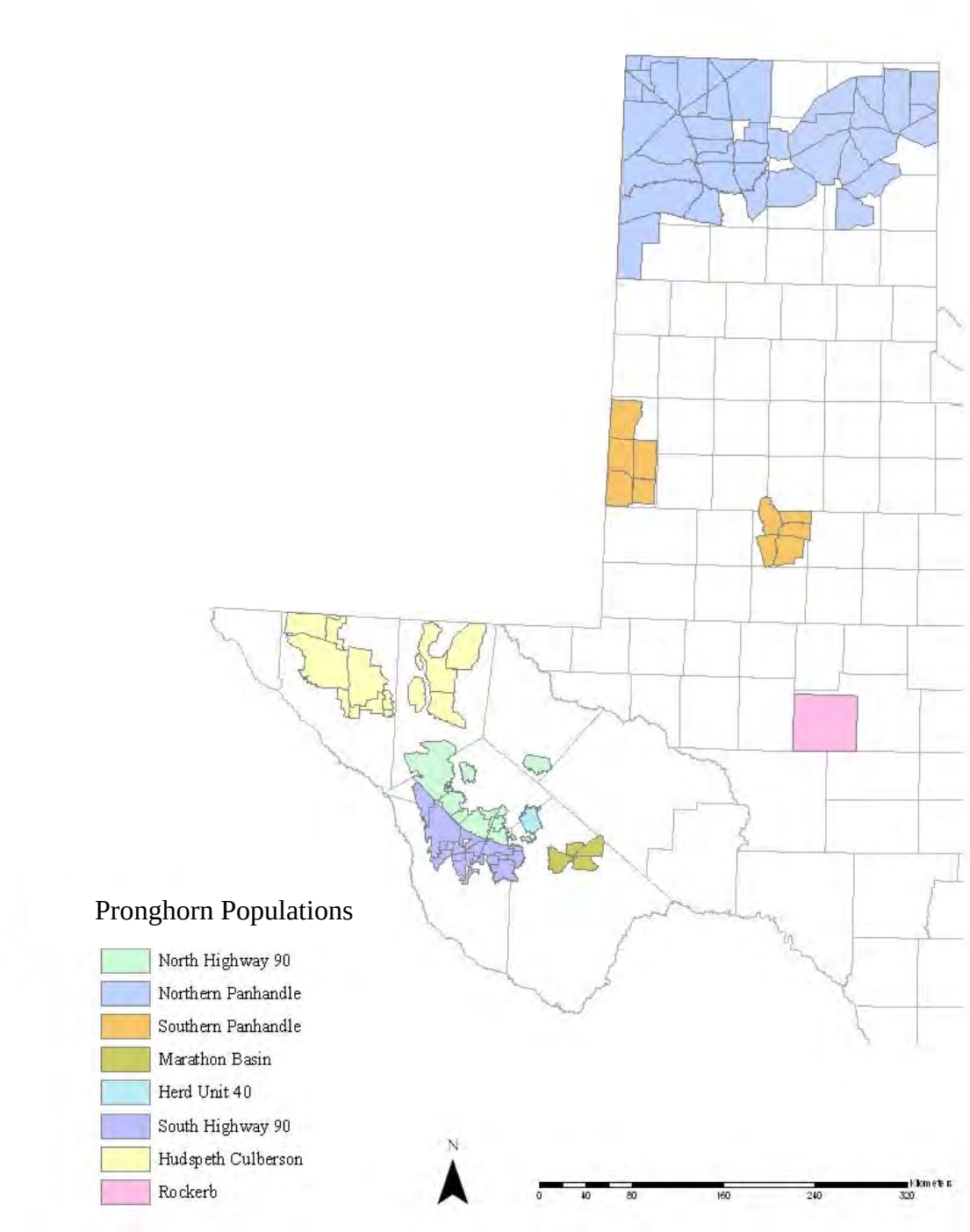


Figure 6. Locations of pronghorn population based on region, geographic proximity, and putative movement barriers.

DNA sequencer. The PCR products were then sized and genotypes were scored using GENEMAPPER 4.0 software (Applied Biosystems, Foster City, CA.).

Genetic diversity

Samples were grouped into 8 populations based on geographic region, and putative movement barriers: Hudspeth and Culberson Counties, North Highway 90, South Highway 90, Herd Unit 40, Marathon Basin, Rocker b Ranch, North Panhandle, and South Panhandle. I examined expected and observed heterozygosities (Nei 1972) among pronghorn population sites to estimate any differentiation in allele frequencies that may be occurring between populations. Heterozygosity values were calculated with the computer program ARLEQUIN 3.1 (Excoffier et al. 2005). Significant departure from Hardy-Weinberg expectations was assessed by 100,000 permutations in a Markov chain reaction. I calculated allelic richness using Kalinowski's method (Kalinowski 2005) to compare genetic diversity among sites and account for differences in sample size.

Genetic structure and differentiation

I assessed population structure and differentiation among pronghorn populations using F-statistics, exact tests, analysis of molecular variance (AMOVA), and Bayesian clustering. Genetic structure among and between the 8 populations was quantified with F_{IS} and pairwise population comparisons of F_{ST} (Weir and Cockerham 1984). Statistical significance was determined by 1,200 permutations of multilocus genotypes among sampled sites in a Markov chain procedure. I performed a hierarchical analysis of molecular variance (AMOVA; Weir and Cockerham 1984, Excoffier et al. 1992, Weir 1996) to evaluate the extent of genetic structure occurring among the 8 regional

population sites with the computer program ARLEQUIN 3.1 (Excoffier et al. 2005). The significance of the results was assessed with 1,020 permutations of genotypes within and among regions using the Markov chain procedure. The AMOVA partitioned the genetic variation and provided an estimation of F_{ST} (Weir and Cockerham 1984) over the dataset.

I performed exact tests of population differentiation to further investigate genetic structure across likely barriers with the computer program ARLEQUIN 3.1 (Raymond and Rousset 1995, Goudet et al. 1996). The exact tests tested the hypothesis of random distribution of genotypes among populations. Samples were grouped into 8 populations in order to test for a random distribution of genotypes among the populations. Statistical significance of the exact tests was assessed by 1,000 permutations in a Markov chain procedure.

I used 2 Bayesian clustering methods to further explore genetic structure among sites. The translocation history of pronghorns suggests that some or many populations may contain admixed individuals. Standard analyses based on F-statistics are a useful and widely understood means of quantifying population structure. However, F-statistics assume an underlying evolutionary model violated to an unknown degree by the presence of admixture (Nei and Kumar 2000). Bayesian analyses can probabilistically group individuals into genetic clusters that minimize Hardy-Weinberg and linkage disequilibrium as well as detect changes in allele frequencies among populations as a function of spatial locations (Pritchard et al. 2000, Corander et al. 2003). Simulation and empirical studies have demonstrated that Bayesian clustering algorithms can overestimate the number of clusters (Swartz et al. 2008, Frantz et al. 2009), so I compared clustering solutions derived from both spatially implicit and spatially implicit clustering methods.

First, I used a spatially implicit Bayesian algorithm (Pritchard et al. 2000) implemented in the computer program STRUCTURE to assess the number of genetic clusters (*K*) among the data. No prior information on population sampling design or spatial location of the samples is needed. This aspatial approach allows the researcher to let the data define the populations, rather than making definitions of populations prior to the analysis (Pearse and Crandall 2004). Samples were grouped into 10 populations based on their sampling location (Hudspeth County, Culberson County, North Highway 90, South Highway 90, Marathon Basin, Rocker b Ranch, Northwest Panhandle, Northeast Panhandle, Southwest Panhandle, and Southeast Panhandle) without consideration of barriers (Figure 7). I used the admixture model which allows individuals to be from more than one of the *K* clusters and I allowed correlated allele frequencies. I performed a 100,000 repetition burn in period followed by 200,000 Markov chain Monte Carlo (MCMC) repetitions of data collection. I performed 5 independent runs of $K = 1\text{-}11$. Real and simulated data have shown determining the optimal number of clusters (*K*) is not straightforward when complex population structure is present (Pritchard et al. 2000, Evanno et al. 2005, Bergl and Vigilant 2007). Therefore, I inferred the optimal *K* value by calculating the ΔK statistic (Evanno et al. 2005). This procedure identifies the appropriate number of clusters using the ad hoc statistic ΔK, which is based on the second order rate of change in the log probability of the data between successive values of *K*. In cases where the STRUCTURE program finds clustering solutions with similar probabilities at different values of *K*, the lowest value is typically the most conservative (Pritchard et al. 2000). Samples were then placed into a respective cluster based upon the highest percentage of membership values (*q*) they were allocated. The *q* value describes

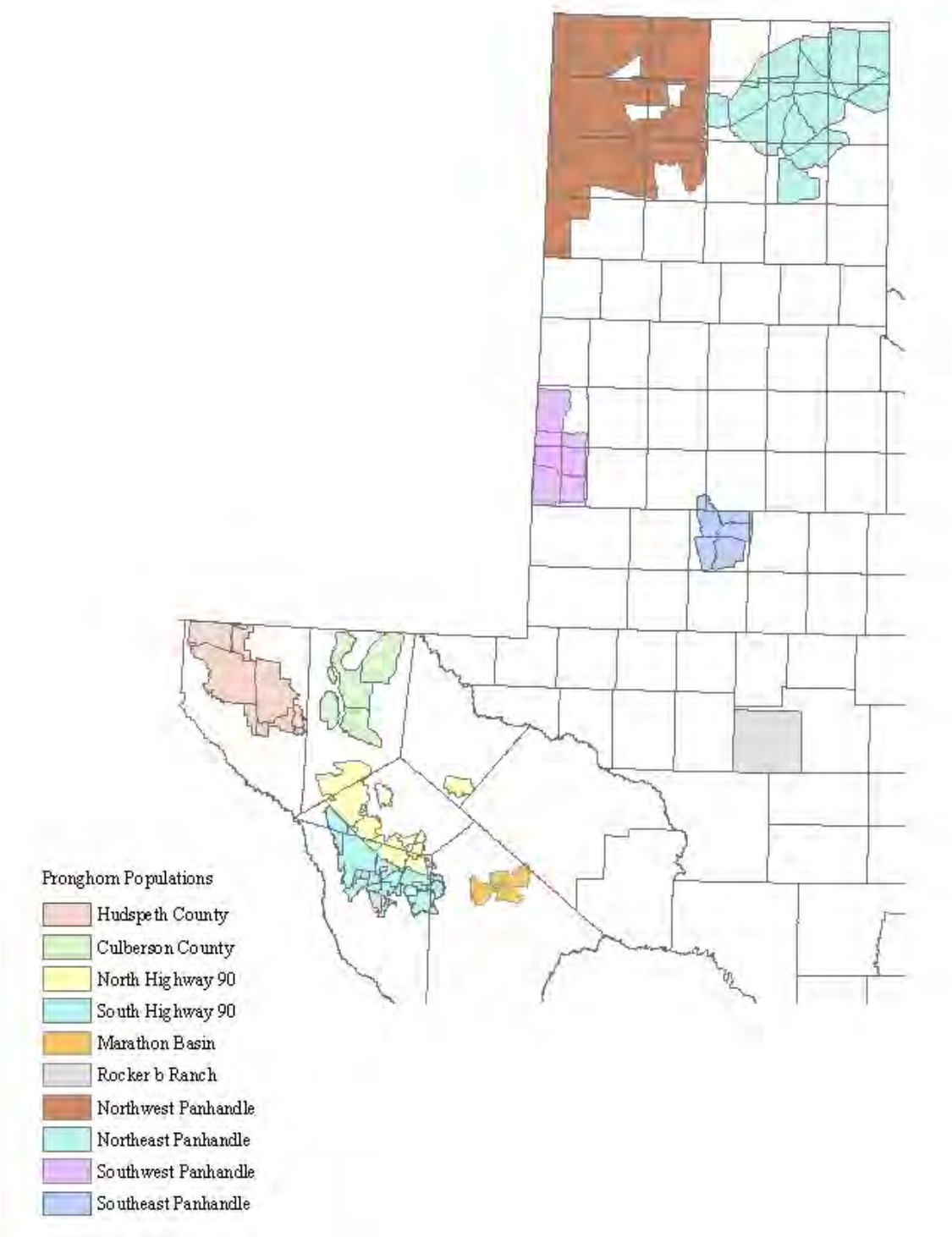


Figure 7. Locations of pronghorn populations sampled in Texas during the 2007-2008 harvest seasons using the software STRUCTURE.

the proportion of an individual's genotypic ancestry that can be attributed to each identified genetic cluster. I averaged *q* over all runs of the top two optimal *K* values with the computer program CLUMPP (Jakobsson and Rosenberg 2007) to correct for between run discrepancies common to cluster analyses. The results of CLUMPP were then visualized in the computer program DISTRUCT 1.0 (Rosenburg 2004) which represents membership values (q) of each cluster in a graphical display. Individuals were assigned to a cluster if they had >50% membership to a given cluster. Finally, I overlaid the inferred clusters defined by the Bayesian method onto GIS coverages of the region to identify terrain, habitat, land-use, and other environmental features that may affect the spatial structure of populations.

I also used a spatially explicit Bayesian method with the computer program BAPS 5.3 (Corander et al. 2008). The BAPS approach uses a spatial model that uses individual georeferenced multilocus genotypes to assign a biologically relevant nonuniform prior distribution over the space of clustering solutions, thereby increasing the power to correctly detect the underlying population structure (Fuentes-Contreras et al. 2008). I used the option 'clustering of groups of individuals' with 10 independent runs of K = 1-11. Spatial coordinates were selected in ArcGIS with the UTM (Universal Transverse Mercator) coordinate system. A centroid location point was arbitrarily taken from each of the 8 populations as a geographic reference. The number of detected clusters was inferred from the optimal number of clusters and the probability associated with each cluster size associated by BAPS. Voronoi tessellation of pronghorn genetic structure was plotted for visual analysis.

Fine-scale population structure and detection of barriers to migration

I evaluated fine-scale population structure and exchange among populations using fixation indices and the Bayesian clustering procedures. I also conducted 2 additional analyses to further characterize movements among adjacent sites. First, I used an assignment test procedure to test for individual migrants across proposed movement barriers (Highway 90, Interstate-10, mountains, geographic distance) to better understand movement between populations. Data on recent migration from assignment indices may be more representative of current population processes than F_{ST} or clustering algorithms (Bergl and Vigilant 2007). I used a likelihood-based assignment test to calculate probabilities of individual migrants with the GeneClass 2 computer software program (Paetkau et al. 1995). I selected the 'detection of first generation migrants' function to explicitly identify first generation migrants. I used the likelihood-based test statistic Lh/Lmax described in Patkau et al. (2004) to identify migrant individuals. This test statistic calculates the ratio of the likelihood computed from the population where the individual was sampled (Lh) over the highest likelihood value among all population samples (Lmax); including the population where the individual was sampled. The ratio of Lh/Lmax has greater statistical power than comparable approaches when all source populations have been sampled (Paetkau et al. 2004). I used the Monte-Carlo resampling method described by Paetkau et al. (2004) with 10,000 simulated individuals. I selected an alpha level of 0.01 as my Type I error rate to determine critical values, as simulated data have shown this level to represent an appropriate balance between stringency and power (Paetkau et al. 2004). Individuals were identified as migrants if they were assigned to a population other than the one in which they were sampled.

Next, I performed a spatial autocorrelation analysis to investigate the spatial extent of genetic structure in pronghorn populations with the software SPAGeDi v. 1.2 (Hardy and Vekemans 2002). This analysis will help distinguish between ongoing gene flow (e.g. natural migration among sites across putative barriers) and recent historical separation (i.e. translocations). Spatial autocorrelation quantifies the degree to which individual genotype frequencies are correlated as a function of the Euclidian geographic distances between pairs of individuals (Manel et al. 2003). Many populations that are restored with translocations do not display a positive relationship between genetic and geographic distance due to genetic drift after the founding event, uneven founder population sizes, and differing founder stocks (DeYoung et al. 2003). A positive relationship between genetic and geographic distance implies some level of genetic exchange or movement/dispersal has occurred among sites. I performed a spatial autocorrelation analysis of 8 populations based on the geographic region and putative barriers of their sampled location. I used Nei's (1978) standard distance (D_S) as a measure of genetic differentiation among sites. A centroid location point was taken from each of the 8 sampling sites as a geographic reference. Spatial locations of populations were selected in ArcGIS 9.3 (ESRI, Redlands, CA.) with the UTM (Universal Transverse Mercator) coordinate system; geographic distance was then expressed in kilometers. Spatial genetic structure was tested by 1,000 permutations of genes, locations, and individuals. Permuting locations is equivalent to carrying out a Mantel test between the matrices of pairwise genetic statistics and pairwise spatial distances (Hardy and Vekemans 2002). Spatial distances between sites and pairwise D_S values were plotted to assess the relationship between genetic and geographic distances among sites.

RESULTS

Sample collection and DNA amplification

During the 2007-2008 pronghorn harvest seasons, 1,673 bucks were harvested state-wide (Adkins 2009). I genotyped 344 pronghorn samples at 8 polymorphic microsatellite loci.

Genetic diversity

Expected and observed heterozygosity.—All microsatellites were polymorphic and the number of alleles per locus ranged from 4 to 19 across the data set. Sampled pronghorn populations had moderate levels of genetic diversity in terms of observed and expected heterozygosity. Observed heterozygosities ranged from 0.523 to 0.670 (Table 1)

Allelic richness.—I detected 76 alleles across 8 microsatellite loci. Allelic richness ranged from 3.88 to 6.25 and was the highest in the southern Panhandle population and lowest in the Rocker b Ranch population (Table 1).

Genetic structure and differentiation

AMOVA, F_{ST}, pairwise $F_{ST,}$ and exact tests.—The AMOVA indicated that approximately 95.29% of genetic variation is within individuals, 3.40% among populations, and 1.31% among individuals within populations (Table 2). The overall F_{ST} among sampled areas was 0.034. F_{IS} ranged from -0.036 to 0.186 (Table 1) and the overall F_{IS} was 0.013. Pairwise comparisons among populations inferred significant levels of genetic structure and ranged from 0.006 to 0.118 (Table 3). All pairwise comparisons were significantly different from 0.0 except for 2.

Table 1. Genetic diversity (H_O and H_E), fixation indices (F_{IS}), and allelic richness (A) for 8 pronghorn populations sampled in Texas, during 2007-2008.

Population	N	H_O	H_E	A	F_{IS}
Hudspeth/Culberson	110	0.603	0.631	5.48	-0.007
North Highway 90	66	0.563	0.600	4.79	-0.001
South Highway 90	76	0.554	0.593	4.75	0.019
Marathon Basin	18	0.523	0.572	4.50	0.028
Herd Unit 40	21	0.601	0.619	4.85	0.001
Rocker b Ranch	7	0.661	0.670	3.88	-0.036
Panhandle North	26	0.600	0.634	5.94	0.014
Panhandle South	20	0.496	0.647	6.25	0.186

Table 2. Analysis of molecular variance comparing genetic variation in microsatellite data among 8 pronghorn populations sampled in Texas during 2007-2008.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	P value
Among populations	7	68.02	0.08	3.40	<0.001
Among individuals within populations	340	755.15	0.03	1.31	<0.001
Within individuals	348	760.50	2.18	95.29	<0.001

Table 3. Pairwise estimates of F_{ST} (Weir and Cockerham 1984) for 8 pronghorn populations in Texas based on 8 microsatellite loci. P values are presented above the diagonal and pairwise F_{ST} values are presented below the diagonal.

Population	Hudspeth/ Culberson	North HWY 90	Herd Unit 40	South HWY 90	Marathon Basin	Panhandle North	Panhandle South	Rockerb Ranch
Hudspeth/ Culberson		< 0.0001	0.0001	< 0.0001	< 0.0001	0.0185	< 0.0001	0.0001
North HWY 90	0.0497		< 0.0001	0.0283	0.3734	0.0659	< 0.0001	< 0.0001
Herd Unit 40	0.0290	0.0483		< 0.0001	0.0088	0.0208	0.0002	0.0037
South HWY 90	0.0352	0.0060	0.0327		0.0661	0.3789	< 0.0001	0.0001
Marathon Basin	0.0526	0.0017	0.0387	0.0114		0.0683	0.0004	0.0005
Panhandle North	0.0111	0.0088	0.0196	0.0014	0.0156		0.0041	0.0003
Panhandle South	0.0345	0.0507	0.0807	0.0456	0.0570	0.0326		0.0004
Rocker b Ranch	0.0777	0.1183	0.0672	0.0877	0.1051	0.08192	0.1098	

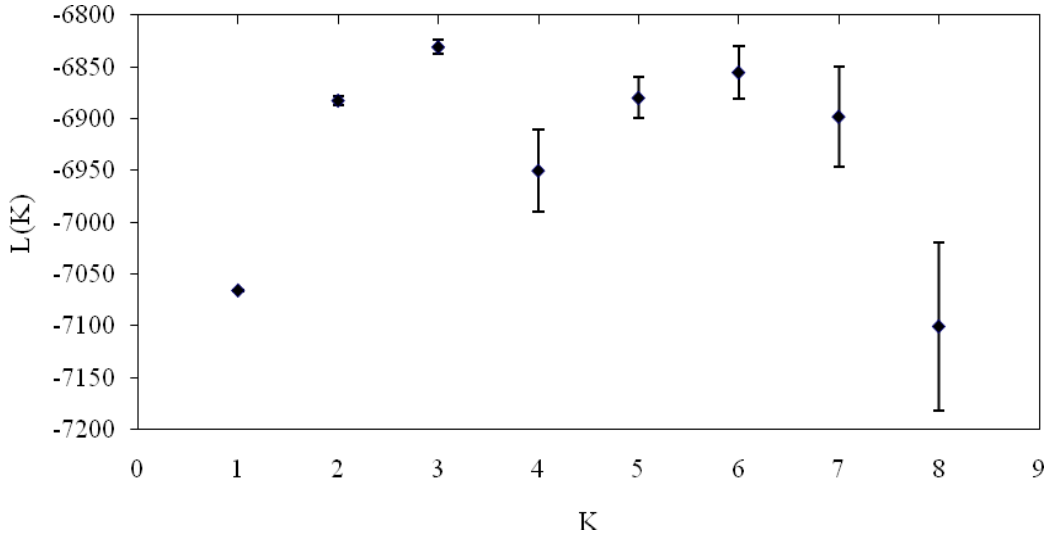


Figure 8. Log probability of data [L*(K)*] as a function of *K* averaged over 10 independent runs for sampled pronghorn populations in Texas, derived using a Bayesian clustering algorithm implemented in the computer program STRUCTURE. The Y-error bars are standard deviation and *K* is the assumed number of genetic clusters.

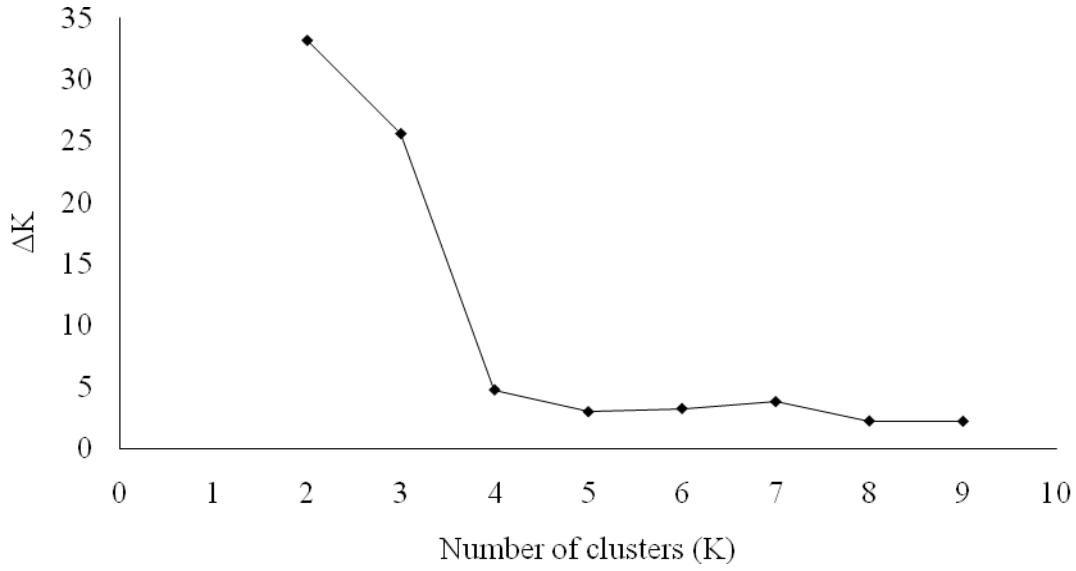


Figure 9. The number of clusters (*K*) vs. the second order rate of change in *K* (Δ*K*), derived using the Evanno et al. (2005) method for identification of genetic clusters.

Table 4. The mean Bayesian assignment probabilities of membership to cluster 1 and 2 detected in STRUCTURE.

Population	*P* (cluster 1)	*P* (cluster 2)	*N*
Hudspeth Co.	0.247	0.753	98
Culberson Co.	0.210	0.790	12
N HWY 90	0.687	0.312	87
S HWY 90	0.700	0.300	76
Marathon Basin	0.804	0.196	18
NW Panhandle	0.513	0.487	13
NE Panhandle	0.449	0.551	13
SW Panhandle	0.071	0.929	4
SE Panhandle	0.285	0.715	16
Rocker b	0.375	0.625	7

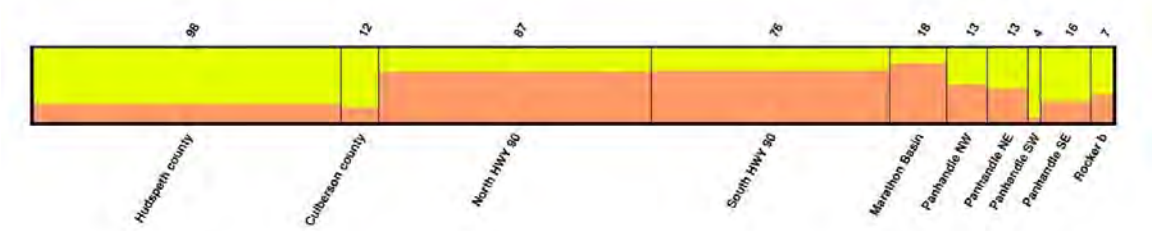
Bayesian clustering (aspatial and spatially explicit).—The aspatial Bayesian analysis revealed that sampled populations could be defined by 2 or 3 genetic clusters (Figure 8). Log probability of data was greatest for K = 3, while the Evanno et al. (2005) rate of change method suggested $K = 2$ as the optimal clustering solution (Figure 9). Samples were placed into a respective cluster, (1) Hudspeth County, Culberson County, Southwest Panhandle, and Southeast Panhandle or (2) North Highway 90, South Highway 90, Marathon Basin, Rocker b, Northwest Panhandle, and Southeast Panhandle based upon the highest percentage of membership values (*q*) they were allocated (Table 4). Results of the clustering analysis was also visualized for $K = 2$ and $K = 3$ (Figure 10A, 10B). There was evidence of admixture between the sampled populations based on individual membership values (q) to a certain cluster (Figure 11). Individual assignments for $K = 2$ were placed on a map to better identify patterns in cluster analysis (Figure 12)

The spatial analyses of genetic population structure with BAPS also identified 2 clusters: (1) Hudspeth and Culberson counties and (2) North Highway 90, Herd Unit 40, South Highway 90, Marathon Basin, Rocker b, Northern Panhandle and Southern Panhandle populations (Figure 13).

Fine-scale movements and detection of migrants

Assignment tests.—The detection of migrant procedures in GENECLASS identified 10 individuals as potential migrants among North Highway 90, South Highway 90, Herd Unit 40, Hudspeth, and Culberson County populations. However, the number of migrants was equal to the number expected using a Type I error rate $\alpha_{0.01}$. Closer inspection of likelihood plots (Figures 14, 15, 16, 17, 18, 19) indicated that populations

(A)



(B)

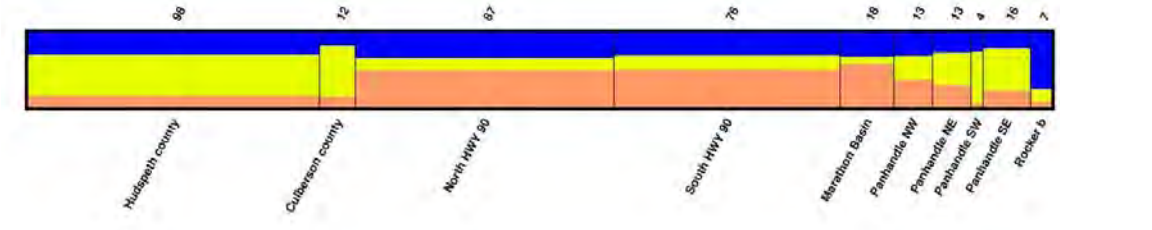


Figure 10. Results of the clustering analysis performed in STRUCTURE visualized with the computer program DISTRUCT 1.0 to represent membership values (q) of each cluster for sampled populations in a graphical display for (A) K = 2 and (B) K = 3.

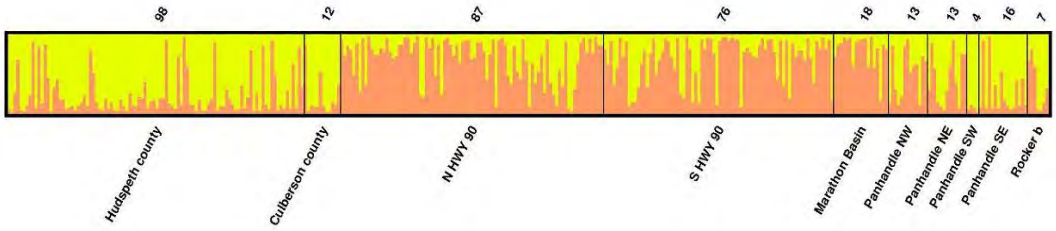


Figure 11. Results of the clustering analysis performed in STRUCTURE visualized with the computer program DISTRUCT 1.0 to represent membership values (q) of each individual in a sampled population in a graphical display.

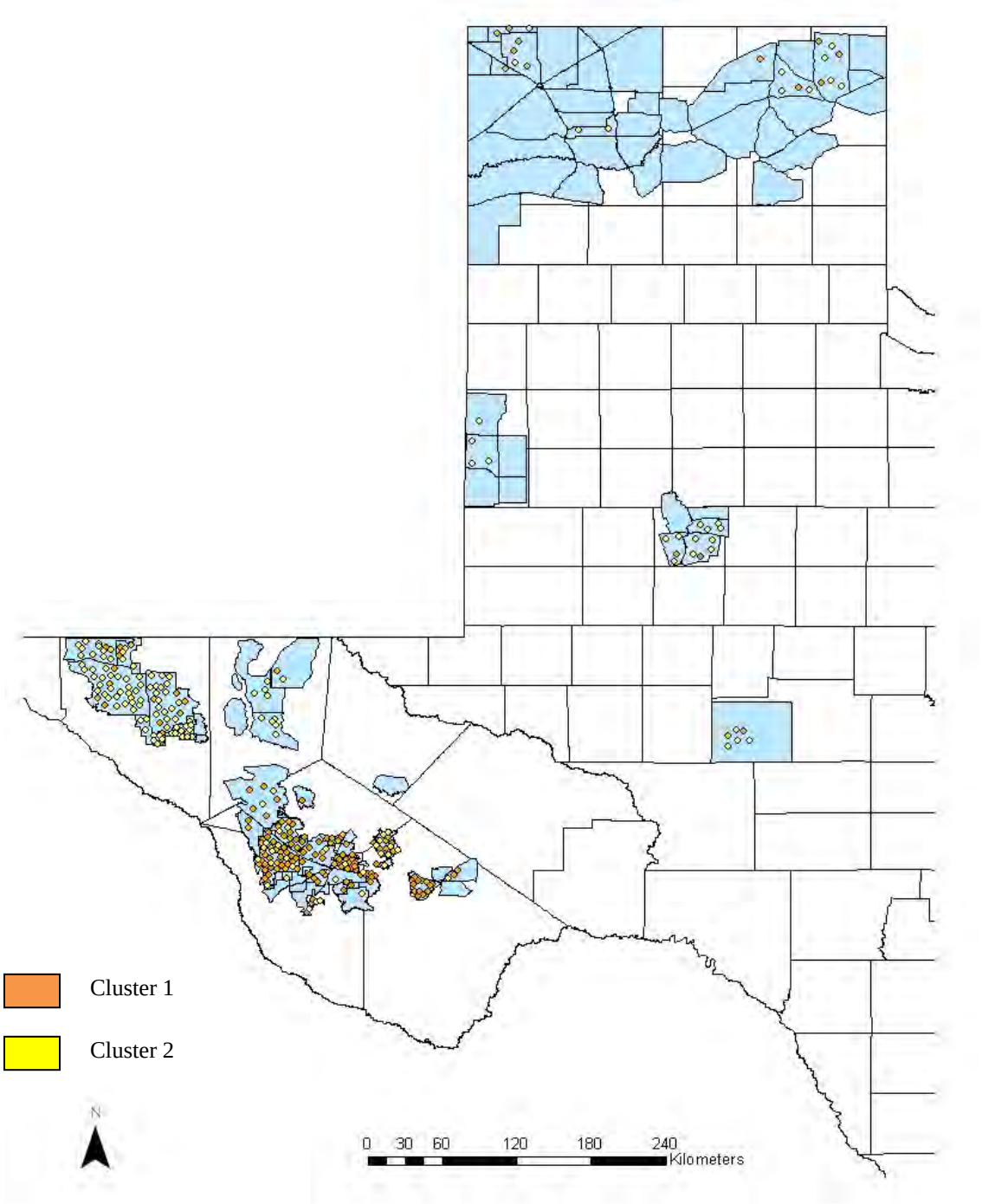


Figure 12. Population representation of individual pronghorn samples grouped into 2 clusters based on their genotypes using the software STRUCTURE.

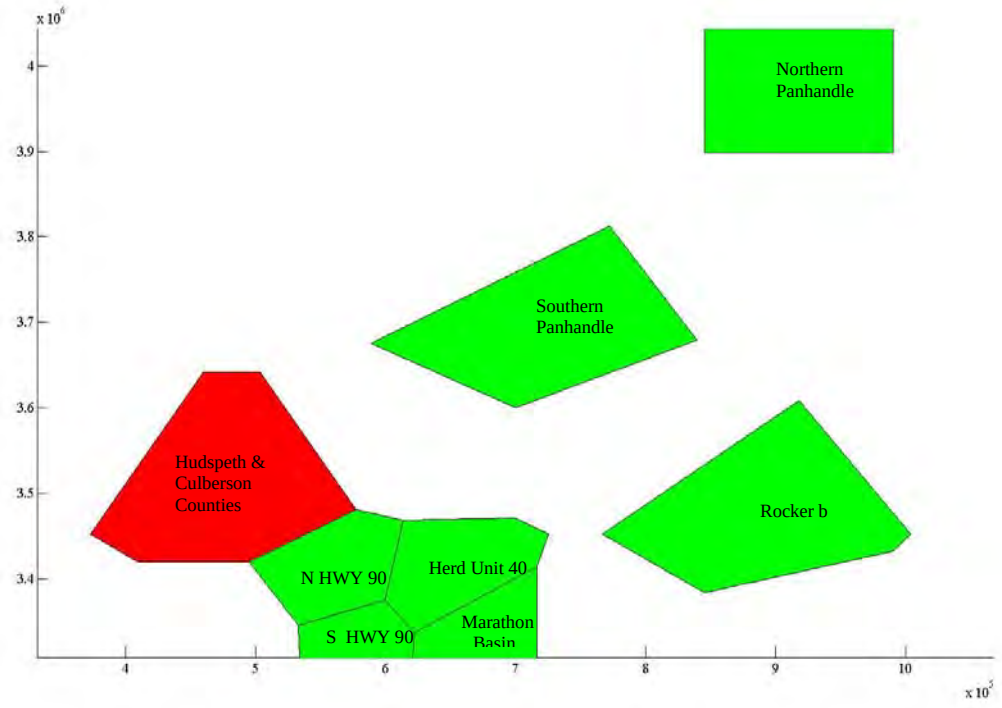


Figure 13. Voronoi tessellation of population structure in space of pronghorn in Texas, estimated using the spatially explicit clustering algorithm in BAPS. Each cell of the tessellation corresponds to the physical neighborhood of an observed data point, and is colored according to the cluster membership.

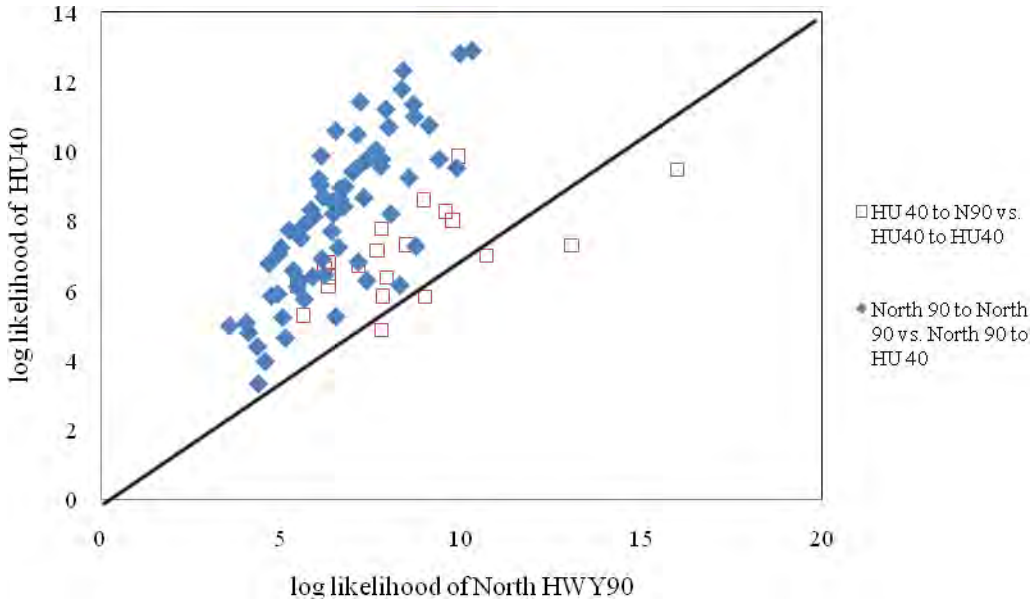


Figure 14. Plot of log likelihood for a frequency based assignment test between Herd Unit 40 and North HWY 90 populations.

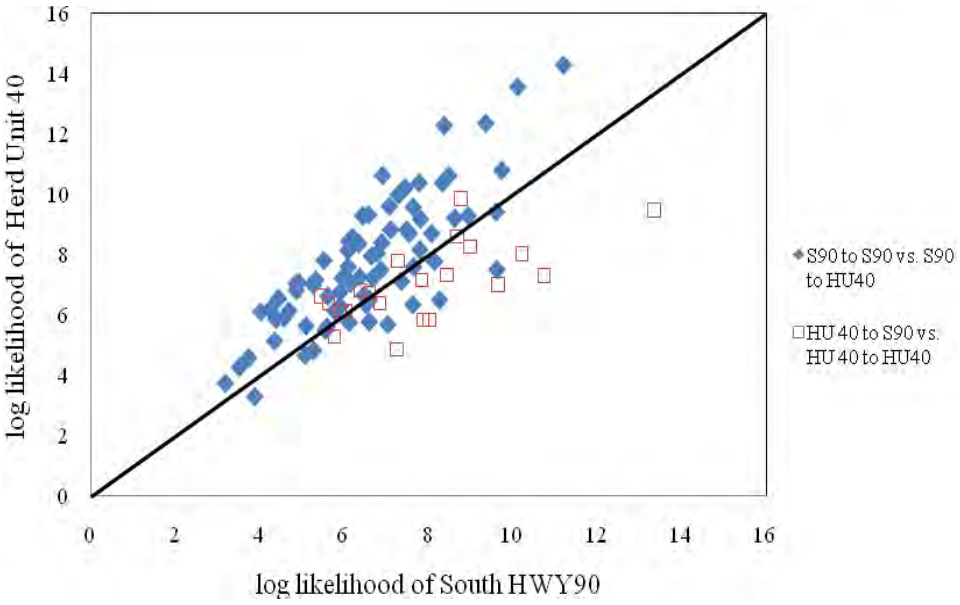


Figure 15. Plot of log likelihood for a frequency based assignment test between Herd Unit 40 and South HWY 90 populations.

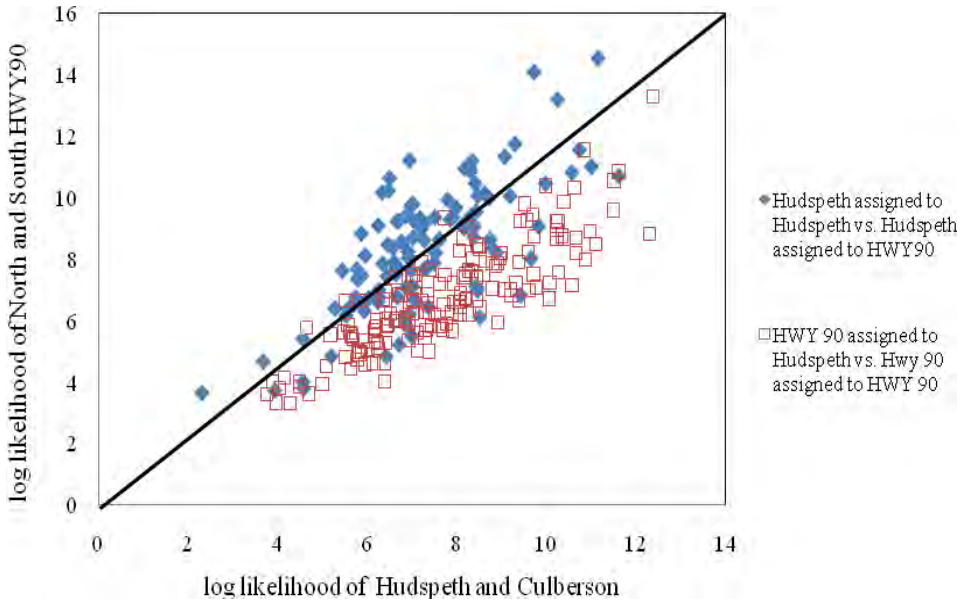


Figure 16. Plot of log likelihood for a frequency based assignment test between North and South of Highway 90 and Hudspeth and Culberson County populations.

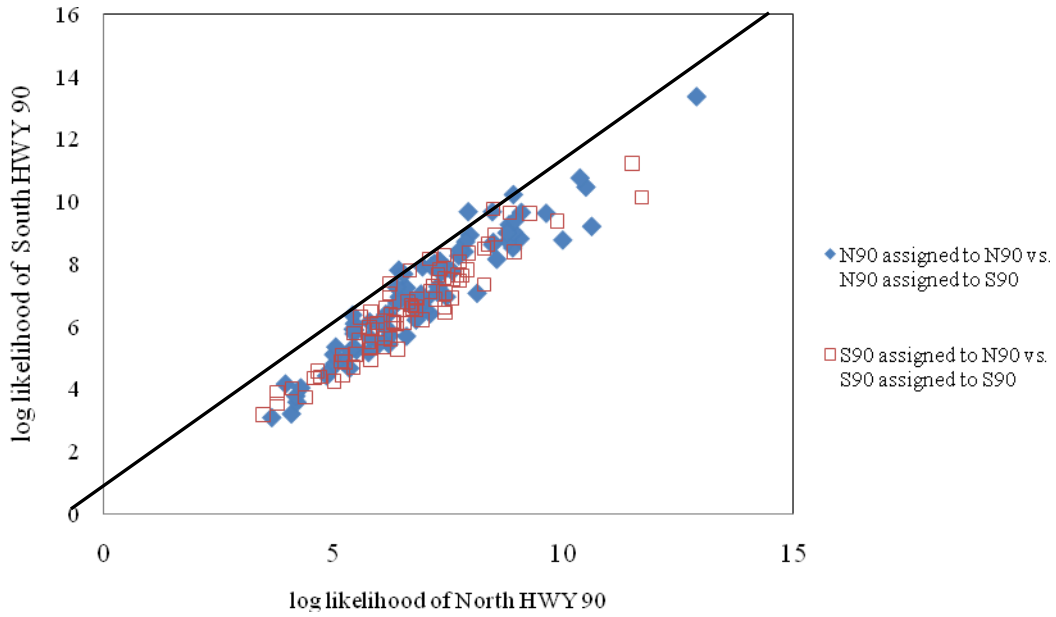


Figure 17. Plot of log likelihood for a frequency based assignment test between North of Highway 90 and South of Highway 90 populations.

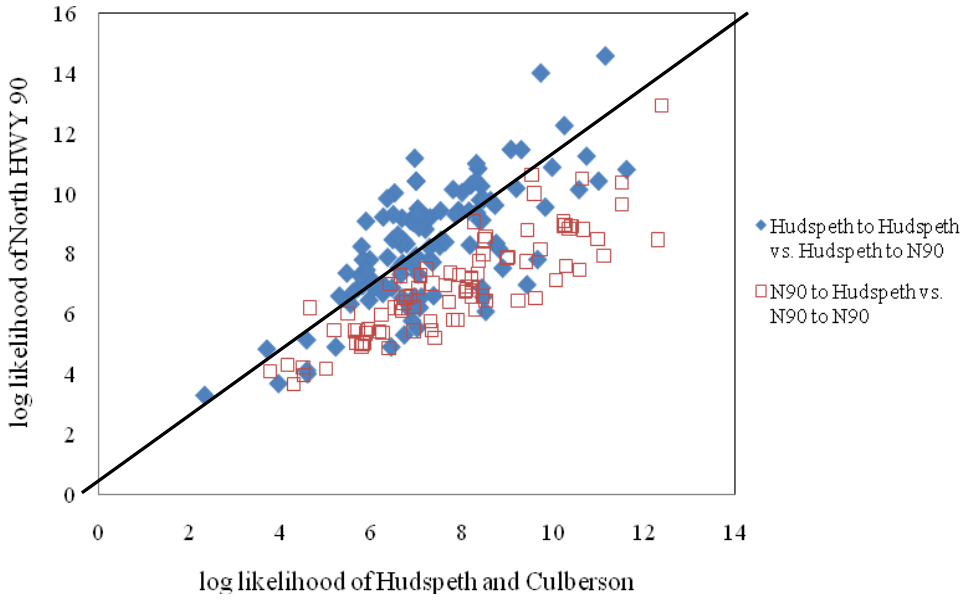


Figure 18. Plot of log likelihood for a frequency based assignment test between North of Highway 90 and Hudspeth and Culberson County populations.

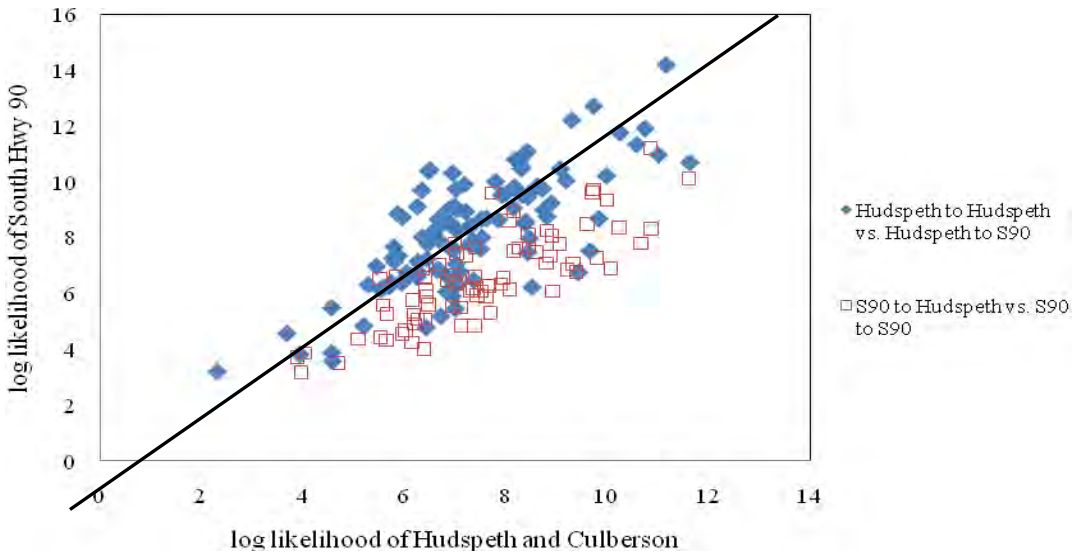


Figure 19. Plot of log likelihood for a frequency based assignment test between South of Highway 90 and Hudspeth and Culberson County populations.

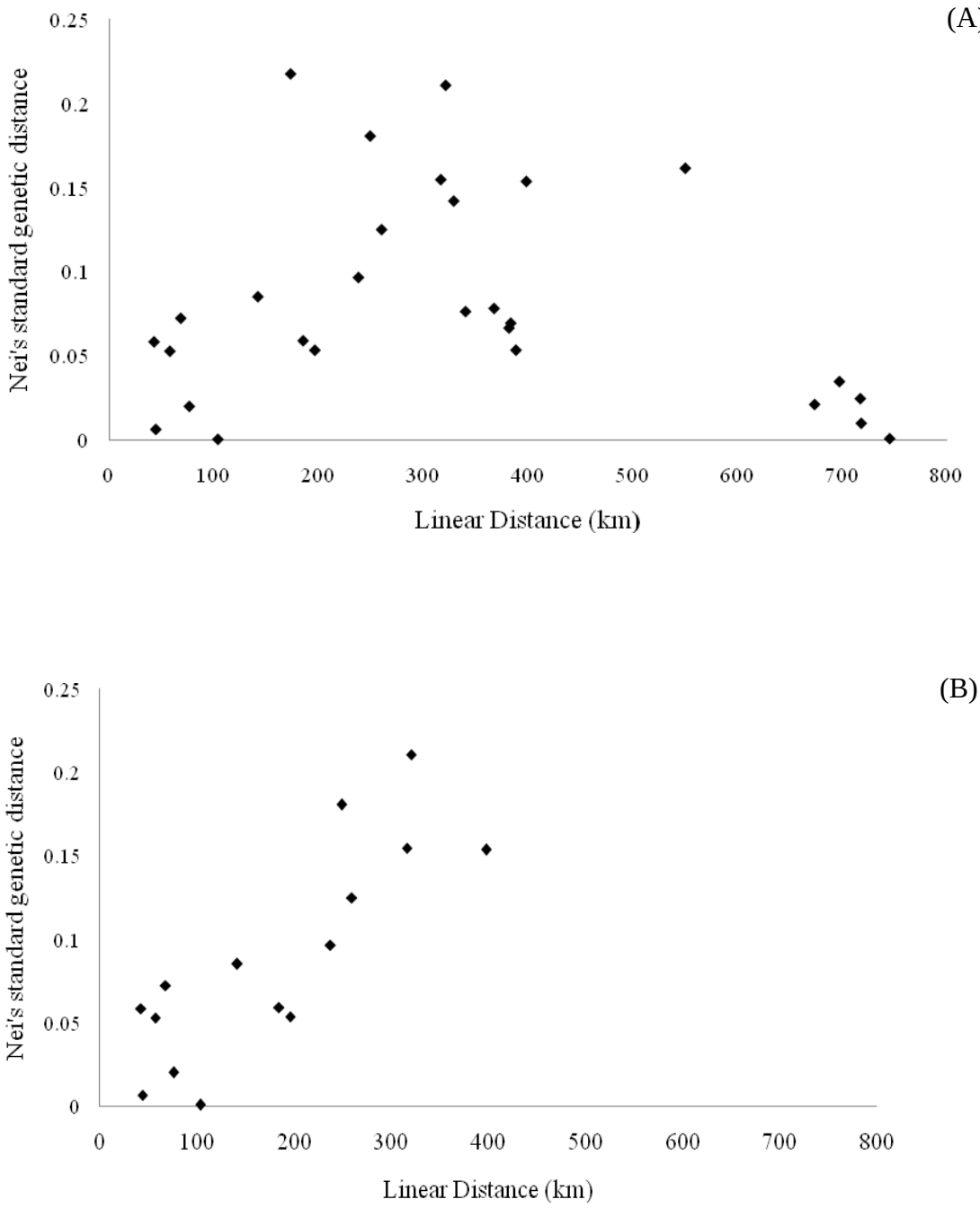


Figure 20. Relationship of genetic differentiation (pairwise differences) and geographical distance (km) between 8 Texas pronghorn populations (r^2 = 0.0243) (A), and between the Trans-Pecos region (B).

were not differentiated enough to detect migrants. A number of individuals were not classified as residents either, suggesting that these individuals are the products of admixture between localities or the descendants of translocations.

Autocorrelation.—Pairwise genetic distances and geographical distances between all populations did not have a strong correlation ($r^2 = 0.0243$, Figure 20A) but the populations in the Trans-Pecos region expressed patterns of isolation by distance when the Panhandle region was removed (Figure 20B).

DISCUSSION

Genetic diversity and differentiation

No evidence of reduced genetic diversity was found among sampled pronghorn populations. This could be a factor of successful pronghorn population restorations due to the high and similar levels of neutral diversity among sites, as well as the genetic similarity over large geographic distances (e.g. clustering between Panhandle and Trans-Pecos regions. A previous study of Texas pronghorn detected genetic diversity occurring among 6 populations (n = 65) using 3 polymorphic allozyme loci (Lee et al. 1989). Their results suggested that west Texas pronghorn populations were characterized by relatively low levels of genetic variation within populations (mean multilocus heterozygosity = 0.027) and moderate levels of genetic differentiation among populations (Roger's genetic distance ranged from 0.010 to 0.064). Their study used molecular markers with limited genetic variability and a smaller sample size. I detected larger diversity values most likely

attributed to larger sample sizes, numbers, and type of molecular marker used in my study.

More than 90% of genetic variation was partitioned within individuals opposed to among populations or among individuals within populations. The overall F_{ST} value (0.0339) among populations did not provide evidence for strong genetic differentiation. Further, F_{IS} values did not identify any substructure or patterns of isolated populations. If gene flow were restricted over time, I would expect to see larger F_{IS} values and decreased heterozygosity values within those restricted populations. The pairwise F_{ST} analyses indicated geographic patterns of differentiation. There was a significantly higher value of F_{ST} (0.0497) between the Hudspeth and Culberson County population and the North Highway 90 population. This may be due to Interstate 10 and mountain ranges bisecting the 2 populations, or results of previous restocking efforts. Lee et al. (1989) found a similar pattern of pairwise F_{ST} (Nei 1972) values within west Texas. For example, their greatest genetic distance value (0.064) was detected between a Marathon Basin population and a population in western Hudspeth County. I observed a high F_{ST} (Weir and Cockerham 1984) of 0.053 between the Marathon Basin and Hudspeth County populations.. Lee et al. (1994) further assessed genetic variation of pronghorn with mitochondrial DNA analysis of 29 populations (n = 330) in North America. Their cluster analysis of allozyme data grouped southwestern Trans-Pecos populations separate from northwestern Trans-Pecos and Rocker b pronghorn populations. These clusters were also found in my aspatial and spatially explicit Bayesian analyses.

Genetic structure between the Panhandle and Trans-Pecos regions was not strongly differentiated. Greater structure between the southern Panhandle and the Trans-Pecos region was detected when compared to the northern Panhandle population. Previous restocking efforts could have homogenized the populations, which would help explain why large levels of structure are not being detected among the Panhandle and Trans-Pecos regions. Genetic structure within the Panhandle region was slightly differentiated based on the pairwise comparison of northern and southern Panhandle populations (F_{ST} = 0.033). The Rocker b population was the most differentiated among all populations but had the lowest sample size (n = 7).

The spatially explicit and aspatial Bayesian analyses produced concordant results and grouped populations into 2 genetic clusters. The presence of admixture among all populations detected with the STRUCTURE analysis suggests movements between the populations or supports successful historical translocations. Gene flow between the first cluster located in the southeastern part of the Trans-Pecos (e.g. North Highway 90, South Highway 90, Herd Unit 40, and Marathon Basin populations) appeared to be restricted from the second cluster located in the northwestern part of the Trans-Pecos (Hudspeth and Culberson County populations). The genetic barrier between the clusters is most likely a combination of Interstate 10, mountains, and the restocking legacy.

The southern Panhandle region clustered strongly with the Hudspeth and Culberson populations located in the Trans-Pecos region. This is most likely due to the homogenization of populations that occurred during the restocking of both regions. I detected minimal genetic variation between the northern Panhandle and the Trans-Pecos region. In fact, the northern Panhandle was clustered with populations that were over 500

km away in the Trans-Pecos. One possible explanation of the observed genetic similarity between these two regions is presumably due to translocations of pronghorn in Texas.

The third cluster detected by STRUCTURE was not supported with any other analyses I performed and cannot be explained with biological meaning at this time. No patterns can be determined from the restocking records but increasing samples from the Rocker b ranch could help clarify the third cluster being detected.

Fine-scale movements and detection of migrants

There was a stronger pattern of pairwise F_{ST} values within regions than among regions. In the Trans-Pecos region, Herd Unit 40 appeared differentiated from populations that were closest to it geographically. Pairwise F_{ST} values were greater than 0.032 in 5 of the 7 populations that were compared to Herd Unit 40. This differentiation may be attributed to the location of herd unit 40. Herd Unit 40 lies on the peripheral of pronghorn distribution in the southeast Trans-Pecos and is bordered by 2 highways and 2 mountain ranges. The Marathon Basin population was differentiated ($F_{ST=}$0.052) from the Hudspeth and Culberson County populations. These two populations are the furthest from each other than any other population within the Trans-Pecos movement between these two populations is unlikely. Populations north and south of Highway 90 were not differentiated. A low pairwise F_{ST} value (0.006) across Highway 90 suggests minimal genetic structure and provides support that gene flow is occurring on both sides of the highway.

In addition to low F_{ST} values across barriers and cluster analyses, assignment tests and autocorrelations provided support for movement occurring across putative barriers. I

was unable to identify large amounts of differentiation occurring across barriers with the assignment tests. This implies that there is some level of migration occurring between these populations or that restocking was successful in maintaining a similar level of genetic diversity among sites. Similarly, Lee et al. (1989) found high migration rate of 6.80 individuals (*Nm*-statistics) per generation occurring in west Texas and attributed the results to restocking. A study on reintroduced pronghorn populations into extirpated areas of Arizona concluded that populations that shared common sources retained the genetic characteristics of those sources (Rhodes et al. 2001).

Genetic distance (*Ds*) did not have a strong correlation with geographical distance among the Panhandle and Trans-Pecos region. This pattern may be indicative of successful pronghorn translocations maintaining similar levels of genetic diversity among restocking sites. Lee et al. (1994) reported 4 pronghorn from the southwestern Trans-Pecos clustering with pronghorn populations outside of Texas. This observation may be a genetic signature of the Utah, Colorado, and Wyoming pronghorn used for restocking areas of Texas.

However, there appears to be a relationship between genetic and geographic distance occurring within the Trans-Pecos region. This suggests that movements between populations are occurring within the region. This pattern is slightly disrupted with the herd unit 40 population and is supported with the structure detected with pairwise F_{ST} and assignment tests.

MANAGEMENT IMPLICATIONS

My analyses indicate that natural movements are occurring between some populations in the Trans-Pecos and Panhandle regions. The analyses also support successful population restocking efforts. There is no firm evidence for isolated populations; but Herd Unit 40, Marathon Basin, and the Rocker b ranch are peripheral in pronghorn distribution in their regions and should be monitored with survey data for isolation trends in the future. Further, the effects of movement barriers on population structure may not be detectable for several generations. A follow up analyses of pronghorn population structure in Texas is warranted. Pronghorn management should continue to be aimed at preserving natural movements among sites. Fence modifications, construction of wildlife highway crossings, and limiting urban development in key travel corridors are recommended to facilitate pronghorn movements. Attention should be given to the supporting evidence of gene flow occurring between populations north and south of Highway 90. If pronghorn are crossing highways (e.g. direct observations and road kills), cooperation between the Department of Transportation and the Texas Parks and Wildlife Department should identify and protect major crossing zones through road signs and fence structures. Brush encroachment, urbanization, heavy livestock stocking rates, drought, predation, and agriculture could influence or restrict pronghorn movements, and may be detrimental for the recolonization of favorable habitat. A pronghorn habitat management priority should be one that that promotes healthy grasslands.

It does not appear that future translocations within Hudspeth and Culberson counties or within the Panhandle will disrupt unique genetic signatures of populations. Translocations between some of the Panhandle populations and some of the Trans-Pecos

populations could also be an option for future population restorations, if necessary. Consideration should be given to mixing individuals from different clusters within the Trans-Pecos region (e.g. Hudspeth/Culberson counties and North/South HWY 90) in order to protect genetic characteristics unique to those populations.

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VITA

Renee C. Keleher
6410 Tarna Lane
Houston, TX 77074
renee.keleher@gmail.com

Education

2007– 2010 Sul Ross State University, Alpine, TX

Major: Range and Wildlife Management
Master of Science
Genetic variation of pronghorn populations in Texas

2001 – 2006 Texas State University, San Marcos, TX

Major: Wildlife Biology
Minor: Animal Science
Bachelors of Science
Graduated Fall 2006

Experience

Research Assistant, Borderlands Research Institute, Sul Ross State University, Alpine, Texas, 2008 – 2010.

Wildlife Technician, Circle Ranch, Hudspeth County, Texas, 2006 – 2007.

Research Technician, Texas State University, San Marcos, Texas, 2006.

Private Lands Program Intern, Texas Parks and Wildlife Department, Austin, Texas, 2006.

Research Interests

Pronghorn ecology and behavior in desert grasslands, especially focusing on predator-prey relationships, home ranges, fawn survival, hierarchy in female bands, and mating strategies.