LANDSCAPE GENETICS OF BOBCATS (*LYNX RUFUS*) ACROSS WESTERN TEXAS

by

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ABSTRACT

Various environmental, ecological, and anthropogenic factors impact the genetic structure of natural populations. I compared the heterozygosity of a highly mobile, generalist species, the bobcat (*Lynx rufus*), to environmental variables throughout western Texas to determine whether landscape-level changes impact population structure. Tissue samples from 102 bobcats were collected from 5 diverse ecoregions throughout western Texas. Bobcats were genotyped for 9 microsatellite loci to estimate genetic variation and population structure. I observed high genetic variation with little population structure (K=2) throughout western Texas. Bobcats were significant for isolation by distance ($P = 0.009$), but also exhibited significant differences in allele frequencies throughout the sample set and in comparisons AMONG ecoregions. Bobcats also demonstrated a relatively high inbreeding coefficient ($F_{IS} = 0.1002$), possibly as a result of philopatry and sensitivity to habitat fragmentation. Factorial detrended correspondence analysis indicated weak affinity for ecoregion locations within a single cluster, which is likely the result of habitat affinities and local adaptations to a highly variable landscape. To examine specific environmental variables, I performed local and regional scale ordination analyses. Canonical correspondence analyses indicated that ~25% of the variation in heterogeneity was explainable by the nine environmental variables used. At the local level, mesquite-lotebush plant associations most strongly impacted bobcat heterozygosity, while mesquite-lotebush and urbanization were the variables most strongly correlated to increased heterozygosity at the regional scale. Mean patch size,

mean patch edge, and Shannon's diversity index of patch size were also selected as meaningful variables for both scales, suggesting the relevance of spatial heterogeneity to bobcat gene flow. Bobcat genetic structure in western Texas appears to be partially impacted by habitat variation, vegetation composition, and spatial heterogeneity, though more information is needed to determine if other direct or indirect environmental gradients influence gene flow. There was very little variation in the genetic data, however, which reduced the meaningfulness of any correlations. My study suggested that local variables can impact population structure even when species are highly mobile and occupy variable ranges. As a result, managers should consider a myriad of landscape factors instead of individual barriers before making assumptions about the adaptations and flexibility of ecological generalists. Identifying landscape variables relevant to population structure has important management and conservation implications for maintaining genetic variation in highly mobile populations.

KEY WORDS*: bobcat, Canonical Correspondence Analysis, ecology, generalist, landscape genetics, microsatellites, ordination.*

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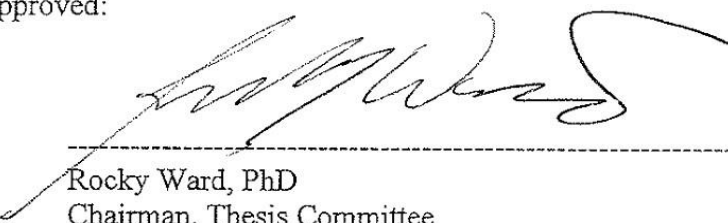
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DEDICATION

In memory of my grandfather, Dr. Hugh Harrison Hayes, Jr. (July 15, 1926-May 10, 2013), whose love and respect for science knows no bounds.

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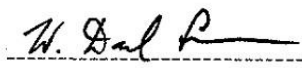Jan E. Janečka, Phd   Date
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CHAPTER 1

LANDSCAPE-MEDIATED GENETIC STRUCTURE OF BOBCATS ACROSS WESTERN TEXAS

INTRODUCTION

Landscape genetics is a scientific approach that investigates spatial genetic data by combining population genetics with landscape ecology (Manel et al. 2003). Landscape ecology gauges landscape patterns and measures structural connectivity, while population genetics examines genetic structure and current gene flow at a fine scale (Holdregger and Wagner 2008). The benefits of combining these techniques include being able to analyze spatial genetic data without identifying discrete populations ahead of time, as well as combining approaches from other disciplines, including spatial statistics and geography. The technique can also be non-invasive. This method helps population geneticists and biologists facilitate an understanding of the microevolutionary processes that drive genetic structure across space by testing the influence of landscape features on genetic population structure, gene flow, and genetic disruptions (Manel et al. 2003; Storfer et al. 2007). Landscape genetics was coined by Manel et al. (2003) to identify the process of analyzing genetic and spatial data at finer scales than in phylogeography, which focuses on diversity patterns and phylogenetics at broad spatial and temporal scales. Indeed, this synthesis of methods assists population geneticists, biologists, and evolutionary ecologists identify the processes that delineate genetic structure across space and draw appropriate conclusions for wildlife management, conservation, and evolution.

Identifying how landscape characteristics structure populations allows researchers and wildlife managers to provide information about microevolutionary processes such as gene flow, genetic drift, and natural selection. As a result, analyzing spatial genetic structure may contribute to an understanding of how individual movements structure populations (Manel et al. 2003). At the heart of this technique lies 2 components of measurement: detecting genetic interruptions and correlating these changes to environmental features (Manel et al. 2003). Specifically, these features can be barriers to or influencers of gene flow, and can include mountains, rivers, habitat changes, topography, etc. This technique can also identify promotors of gene flow. Because gene flow ultimately represents movement and reproduction across space, landscape genetics is a useful tool researchers can employ to generate information on ecological and evolutionary processes (Manel et al. 2003, Holdregger and Wagner 2008, Storfer et al. 2010). Understanding gene flow, then, is essential to the investigative process of determining factors that constrain or promote local adaptation, as knowledge of genetic discontinuities facilitates an understanding of how individual movements structure populations (Manel et al. 2003). The amalgamate technique of landscape genetics has been used to address an array of landscape questions, including identifying barriers to dispersal (Riley et al. 2006, Latch et al. 2008, Broquet and Petit 2009), estimating source-sink dynamics (Martinez-Solano and Gonzalez 2008), managing disease (DeYoung et al. 2003, Root et al. 2009), identifying areas of connectivity (Schwartz et al. 2002, Ernest et al. 2003), and examining historic and contemporary effects on gene flow (Reding et al. 2012, Tucker et al. 2013).

The study of landscape genetics explains the correspondence between landscape characteristics and population structure, where the interpretation of genetic data can suggest functionally relevant landscape features to the species of interest. Further, the geographical distribution of taxa often corresponds to major ecological events and gradients, resulting in landscape-mediated patterns of genetic diversity (Avise 2000). As such, landscape genetics uses 2 methods to study gene flow among populations. The first approach is an individual-based examination of fine-scale genetic structure, where the researcher assesses population relationships by first delineating genetically similar clusters and then computing their genetic relationship (Allendorf and Luikart 2008, Holderegger and Wagner 2008). The second method considers recent or current gene flow, where individuals are clustered into 'predescribed populations' on either side of a landscape feature and assigned to a cluster via assignment tests (Holderegger and Wagner 2008). In both approaches, genetic pairwise data are statistically correlated with landscape variables or environmental features.

Several environmental features and landscape patterns have been examined in landscape genetics studies, including historical and contemporary aspects. Research efforts have linked the genetic structure of species to evolutionary history, where issues such as climate oscillations during the ice ages drove species contraction and expansion, therefore affecting genetic variation (Hewitt 1996). Historical examples include Pleistocene range expansion leading to genetic diversity in the Jerusalem cricket (*Stenopelmatus mahogani*; Vandergast et al. 2007), Northern cardinal genetic lineage differentiation dating back to the Pliocene (*Cardinalis cardinalis*; Smith et al. 2011), and boreal forest glacial cycles from the Pleistocene correlating to genetic variation observed

in North American tree squirrels (*Tamariscus hudsonicus, Tamariscus douglasii, Tamariscus mearnsi*; Arbogast et al. 2001). Specific ecological processes, such as topographical distance, elevation, linear water bodies, habitat fragmentation, and cover type, to name a few, have also been examined in landscape genetics studies across numerous taxa, e.g. blotched tiger salamander (*Ambystoma tigrinum melanostictum*; Spears et al. 2005), western toad (*Bufo boreas;* Murphy et al. 2010), saddle-back tamarin (*Saguinus fuscicollis*; Peres et al. 1996), and roe deer (*Capreolus capreolus;* Coulon et al. 2004). Further, landscape genetics studies have correlated genetic variation to anthropogenic features, such as highways reducing genetic diversity in desert bighorn sheep (*Ovis canadensis nelson*i; Epps et al. 2005) and human-mediated habitat fragmentation resulting in genetically distinct Florida black bear populations (*Ursus americanus floridanus*; Dixon et al. 2007). The results of these studies both illuminate species history as well as draw pertinent conclusions for management needs, making landscape genetics widely applicable across several disciplines.

Landscape genetics studies have more recently included a focus on highly mobile species, which are those species that range widely throughout their distribution. Identifying the variables involved in structuring populations are challenging in abundant, widely distributed species, particularly if they are wide-ranging, highly mobile, and/or generalists. The evolution of a species across large distances likely results in several factors, sometimes making discernment of the causes of genetic structure complex. Specifically, the influence of landscape-level changes on populations is partially dependent upon the ecology and life history of the species. For highly mobile species, gene flow is not expected to be limited by environmental features because of the ecology

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and dispersal abilities of these organisms; populations are often genetically panmictic, where dispersal is not geographically limited and unrestricted gene flow limits the development of population genetic structure. For these species, genetic structure is presumed to fall within a pattern of isolation by distance, where populations exhibit differentiation via local dispersal and restricted interbreeding because of geographical distance (Wright 1943). Indeed, several studies have documented a lack of genetic structure in organisms persisting across large distances, including gray foxes (*Urocyon cinereoargenteus*; Bozarth et al. 2011), wolverines (*Gulo gulo*; Schwartz et al. 2009), and raccoons (*Procyon lotor*; Root et al. 2009). While this information is extremely useful to wildlife managers and conservation biologists, one must be careful when extrapolating this data onto other populations or sympatric or congener species, as panmictic genetic structure can be overestimated because of isolation by distance patterns and local adaptations (Reding et al. 2011, J.E. Janečka, Duquesne University, unpublished data). For example, despite being a continuously ranging species, bobcats can exhibit sensitivity to habitat fragmentation, where smaller habitat patches result in lower probability of occurrence as compared to areas with less fragmentation (Crooks 2002). With this in mind, increased testing for genetic structure in wide-ranging species has elucidated how population structure can occur without geographic isolation as the dominant factor. Further, genetic subdivision can impact conservation, ecology, and management decisions, making this area of research important for wildlife managers focusing on highly mobile species.

Notably, the landscape factors that can affect genetic variation in any species depends on the ecology, behavior, and habitat selection of the organism. However, the

genetic processes that structure vagile, highly mobile species are not well understood, in

part because of the behavioral flexibility of such an organism as well as the challenge of

identifying populations *a priori* when clear geographic barriers are not present.

Numerous examples can be found in the literature to illustrate the need for considering

the landscape effects on the genetic variation and structure of highly mobile species.

Barton and Wisely (2012) determined that late Pleistocene glacial melting was

responsible for genetic admixture in the striped skunk (*Mephitis mephitis*). Similarly,

Reding et al. (2012) found that Pleistocene climate oscillations contributed to significant

genetic substructure in United States bobcats, and data on the North American cougar

suggest that the Pleistocene extirpations throughout the United States may have included

the North American cougar (*Puma concolor*), with contemporary populations recolonized

with migrants from Central and South America (Culver et al. 2000, McRae et al. 2005).

Contemporary effects of landscape features on genetic structure include the Straits of

Mackinac forming a natural barrier to bobcats and resulting in genetically distinct

populations between the upper and lower peninsulas of Michigan (Millions and Swanson

2007), mountain ranges impeding gene flow between American mink populations in

Scotland (*Neovison vison*; Zalewski et al. 2009), differences in coastal and inland habitat

coupled with differences in habitat use driving population differentiation in Louisiana

river otters (*Lontra canadnsis*; Latch et al. 2004), pattern changes in climate and habitat

promoting genetic distance between gray wolf populations (*Canis lupus*; Geffen et al.

2004), and macrohabitat breaks correlating with coyote population structure in the

western United States (*Canis latrans*; Sacks et al. 2004). Highly mobile species have also

exhibited genetic structure as a result of anthropogenic presence and growth, such as U.S.

grizzly bear populations experiencing demographic fragmentation and population subdivision due to highways (*Ursus arctos*; Proctor et al. 2005), a California highway creating marked genetic differentiation and distinct populations in bobcats and coyotes (Riley et al. 2006), and population isolation and reduced genetic variation in ocelots because of human activity, development, and resulting habitat loss (*Leopardus pardalis*; Janečka et al. 2011). The underlying trend in these studies identifies the degree to which landscape variables limit movement and gene flow in abundant, highly mobile, often generalist species. In the absence of clear geographic barriers, determining functionally relevant landscape features involved in genetic structuring of populations is challenging, but necessary, as the structure that develops from landscape interactions can adversely contribute to population effects such as local adaptation, spread of disease, immigration and emigration, genetic drift, reproductive success, and extirpation. Examining population genetic metrics yields insights into demographic and evolutionary processes (Schwartz et al. 2007), which can be helpful in discerning how highly mobile species perceive and respond to a landscape.

Lynx rufus

The bobcat (*Lynx rufus*) is a highly mobile, generalist species that ranges throughout North America and persists across a variety of habitat (Anderson 1987). Bobcats are one of the most predominantly-distributed native mammalian species in North America, ranging from southern Canada to central Mexico and from California to Maine (Anderson and Lovallo 2003). This medium-bodied felid is found throughout the United States in an array of habitats, including coastal swamps, northern boreal forests,

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and arid deserts, as habitat type is not limiting to the distribution of the species (Anderson 1987).

Bobcats are obligate carnivores with highly varied diets, comprising lagomorphs, deer, birds, reptiles, and fish (Anderson 1987, Anderson and Lovallo 2003). Bobcats are typically solitary and territorial, and exhibit a social system that includes residents, transients, and kittens (Anderson 1987). Reproduction is seasonal and polygynous, and both sexes are capable of traveling great distances (Anderson and Lovallo 2003, Newbury 2013). Dispersal and movements vary across habitats and study areas, and, like most mammals, bobcats exhibit male-biased dispersal (Janečka et al. 2007). The U.S bobcat population is estimated between 2-3 million individuals (Roberts and Crimmins 2010), with a majority of states reporting growth in numbers in 2008. Currently, 38 states allow harvest of this economically important furbearer.

The behavior, ecological flexibility, and distribution of this generalist felid assumes a lack of genetic structure across its range, yet several studies contradict this. Bobcats have exhibited sensitivity to habitat fragmentation, anthropogenic presence, and agriculture (Crooks 2002, Riley et al. 2006, J.E. Janečka, Duquesne University, unpublished data), and respond to landscape heterogeneity (Reding et al. 2013), suggesting the importance of landscape structure to genetic structure in even generalist species. Further, Hall (1981) accounted for bobcat regional variations by delineating 12 subspecies. Reding (2012) noted that Hall's subspecies correlates with transitions between major ecological regions, which suggests that environmental variables, climatic variation, and habitat clines may play a role in structuring bobcat populations. Veritably, Reding's study (2012) on North American bobcat genetic structure suggested that bobcat

populations are bisected into 2 main groups, with the Great Plains region of the United States following the suture zone for this differentiation (Fig. 1, Reding et al. 2012). However, the genetic structure of bobcats in western Texas, parts of which are in the southern Great Plains, is not known. Specifically, there are two subspecies of bobcat in Texas, *Lynx rufus texensis* and *Lynx rufus baileyi* (Hall 1981). This transition occurs in central Texas, but is not known where or how bobcat populations are structured across western Texas, or if the genetic structure supports this change in subspecies.

Understanding the relationship between bobcat movement and the landscape is important for bobcat management, as this species is both economically and ecologically important. Bobcats play a role in ecosystem stability by regulating prey communities and, as the apex predator in some regions, suppressing mesopredators. Knowledge of population structure can impact decisions related to community-level interactions, identifying landscape features that suppress or promote movement, predicting the spread of disease, and managing recolonization following local extinction. The ecology, movement, and perception of a landscape can be manifested in genetic diversity, suggesting that an examination of bobcat genetic structure in western Texas will be beneficial to wildlife management and maintaining biodiversity.

Roberts and Crimmins (2010) reported that bobcat populations in Texas are declining. Further, bobcats in South Texas exhibited evidence of genetic differentiation in because of a lack of habitat connectivity (J.E. Janečka , Duquesne University, unpublished data). Because landscape features have been shown to correspond with bobcat genetic structure in other regions (Riley et al. 2003), identifying the genetic structure of bobcats in western Texas is important to their management in terms of

understanding movement and genetic variation, as the bobcat is both ecologically and economically valuable. Western Texas comprises a variety of ecoregions and concordant macrohabitat breaks, yet the majority of Texas is privately owned and bobcats are managed very differently throughout the state. Specifically, the bobcat is listed as a nongame animal in Texas and has no closed hunting season, resulting in no regulations for bobcat management on private land. Identifying bobcat genetic structure and determining whether genetic architecture correlates to landscape changes will provide insight on how bobcats interact with this landscape as well as assist managers in maintaining healthy, complete ecosystems. My objective was to examine the genetic structure of bobcats across 5 ecoregions in western Texas by examining variation in 9 autosomal microsatellites.

METHODS

Ecoregions and Bobcat Samples

Muscle tissue was collected from 103 bobcats throughout 5 ecoregions in western Texas from January 2013 to March 2015. Specifically, bobcat samples covered 5 ecoregions: the High Plains (Fig. 2), Rolling Plains (Fig. 3), Edward's Plateau (Fig. 4), Trans-Pecos (Fig. 5), and South Texas Plains (Fig. 6) ecoregions. These ecoregions differ by habitat type, elevation change, plant community structure, and topographic relief, though there is some overlap in these artificial designations.

The vegetation across all ecoregions has been dramatically altered in the last two hundred years through a combination of agriculture, cultivation, and changes within and between zones, but the following represents a contemporary description of each ecoregion (Correll and Johnston 1979). The High Plains ecoregion, the southern extent of

the Great Plains, is a high plateau of 8,100,000 hectares. This region, heavily cultivated, was classified as mixed prairie and is generally devoid of trees and brush, with elevations ranging from 914 to 1371 meters. The Rolling Plains ecoregion is adjacent to the High Plains and is also part of the Great Plains; together they form the Texas Panhandle. These ecoregions are dissected by the Caprock Escarpment, a series of fingered canyons running north to south. The Rolling Plains comprises 9,720,000 hectares of gently rolling to moderately rough topography, with a varied plant community that includes prairie vegetation, shinnery oak, and mesquite. Elevation ranges from 244 to 914 meters. The Edward's Plateau ecoregion comprises about 9,720,000 hectares in west-central Texas that are characterized by dense juniper, scrub oaks, and mesquite. Canyons interfinger with other regions and the elevation ranges from 304 to 914 meters. The Trans-Pecos ecoregion, approximately 7,685,000 hectares, is one of diverse habitats and vegetation within mountains and arid valleys, with elevation varying between 762 and 2590 meters. It is perhaps the most variable ecoregion in western Texas. The South Texas Plains ecoregion consists of about 8,100,000 hectares and is characterized by level to rolling topography and open prairies consisting of mesquite, cacti, catclaw, and small trees and shrubs of varying degree and composition at sea level to 304 meters in elevation (Correll and Johnston 1979).

Samples from all ecoregions were collected through an array of collaborative efforts, including opportunistic sampling of road-killed animals, tissues collected from both commercial and private trappers, and samples provided by Texas Parks and Wildlife biologists, game wardens, and Texas Wildlife Services personnel. Muscle tissue was harvested postmortem from either a front or hind leg using scalpel blades or knives.

These tissue samples were either stored immediately in 70% ethanol and frozen, or frozen and later stored in ethanol. Five samples are over ten years old and were harvested in similar conditions. Location information was not exact for many samples, and as a result GPS locations were approximated. Specifically, some locations were identified only by their county or town of origin; in these cases a GPS location representing the county center was used.

Two samples were obtained from live animals, which were live-trapped with approval obtained from the West Texas A&M University Institutional Animal Care and Use Committee (IACUS # 04-12-12). For both bobcats, DNA samples were collected with a 2 mm ear punch sample collector and the animals were released at the site of capture.

Microsatellite Genotyping

Tissue samples were either frozen or stored in 70% ethanol. I performed DNA extractions on all samples using a modified Gentra Puregene tissue kit protocol (QIAGEN Corporation, Valencia, CA). The primary modification concerned tailoring the amount of elution buffer based upon the quantity of DNA in the final EtOH wash as revealed by visualization of a 1% agarose gel following electrophoresis. Tissue was manually homogenized, with 300 ul of cell lysis solution and 3.0 ul of proteinase K added to each sample tube. Samples were heated in a bath overnight at 55 °C to maximize yields. The extraction was completed on the second day by adding 100 ul of protein precipitation solution to each tube and vortexing samples prior to centrifugation for 3 minutes at 13,000 rpm. The supernatant was added to a new tube containing 300 ul of 100% isoproponal and inverted 50 times, centrifuged for 2 minutes at 13,000 rpm, and

carefully poured off. The remaining DNA pellet was washed a second time with 70%

ethanol and centrifuged again for 2 minutes at 13,000 rpm. This supernatant was

discarded, and either 50 ul or 75 ul of DNA hydration solution was added to each tube

once dry. Samples were briefly vortexed and incubated at 65 C for 1 hour and maintained

at 2.7 C.

Nine autosomal microsatellites (FCA026, FCA043, FCA045, FCA077, FCA082,

FCA090, FCA096, FCA132, and Lc120) were used to genotype 95 bobcats. Five of these

loci (FCA043, FCA045, FCA077, FCA090, and FCA096) were previously found to be

informative for 1 bobcat population in South Texas (Janečka et al. 2006, 2007). Four

additional loci (FCA026, FCA082, FCA096, and Lc120) were screened against 8 bobcats

and observed to have sufficient genetic variability for population analyses and genotyped

across all animals. All loci but Lc120 were originally isolated in the domestic cat (*Felis*

catus) by Menotti-Raymond et al. (1999). The primer Lc120 was isolated in the Canadian

lynx (*Lynx canadensis*) by Carmichael et al. (2000).

Microsatellites were amplified in a 12.5 ul reaction containing 6.5 ul of

GeneMate Taq 2X Mastermix (concentrations proprietary information), 2.5 ul ddH$_2$O, 1.0

ul of Well-Red fluorescently-labeled oligonucleotide (Sigma-Genosys, The Woodlands,

Texas, USA), 1.0 ul forward primer, 1.0 ul reverse primer, and 0.5 ul genomic DNA.

PCR reaction conditions included initial denaturing step of 94 C for 1 min, 10 cycles of

94 C for 15 s, 53 C for 15 s and 72 C for 45 s, followed by 50 cycles of 89 C for 15 s, 53

C for 15 s and 72 C for 45 s and a final extension of 72 C for 30 min. The denaturing

temperature was lowered to 89 C after 10 cycles to decrease the amount of *Taq*

inactivated by the high temperature of each denaturing step (Menotti-Raymond et al.,

1999). All amplifications were performed on an Eppendorf Mastercycler (Eppendorf, Hamburg, Germany). Samples were fractionated with a Beckman Coulter CEQ8000 DNA Analyzer (Beckman Coulter, Indianapolis, IN, USA) and the sizes identified by the CEQ8000 software were confirmed by visual inspection of tracings.

Microsatellite Data Analysis

Measures of genetic variability, such as allele frequency (A_N), observed heterozygosity (H_O), expected heterozygosity (H_E), and polymorphic information content (PIC) values for each locus were determined with Microsatellite Toolkit for Microsoft Excel (Park 2001). Tests for Hardy Weinberg Equilibrium (HWE) and linkage disequilibrium (LD) were performed using GENEPOP 4.2 (Raymond and Rousset 1995, Rousset 2008). I examined allele frequency-based correlations with F_{ST} and F_{IS} estimates (population subdivision and inbreeding coefficients, respectively), which were derived for each locus using GENEPOP 4.2. I tested for deviations from equilibrium within ecoregions at each locus and across all loci. The Bonferroni method was used to correct p-values for multiple comparisons in the HWE and LD tests (Rice 1989).

Fine-scale population genetic structure was examined for all bobcats using the program STRUCTURE 2.1 (Pritchard et al. 2000), which uses individual genotypes to determine the optimal number of populations (K) and to explore admixture across the landscape (Rosenberg et al. 2002). In order to determine the optimal K, which minimizes Hardy Weinberg and linkage disequilibria (Pritchard et al. 2000), I first estimated the number of subgroups (K) by initiating 5 independent runs of K = 1-5 with 300,000 Markov Chain Monte Carlo (MCMC) repetitions and 1,000,000 burn-in steps. This was done without *a priori* knowledge of population delineation information. Program

parameters were set to default values as suggested by Pritchard and Wen (2003). When subtle population structure is expected, the options to correlate allele frequencies and the admixture model were utilized as recommended by Falush et al. (2003). The posterior probability was then calculated for each value of K using the estimated log-liklihood of K to select the optimal K (Evanno et al. 2005). I chose the number of clusters suggested by inflection in the rate of change in log probability of successive K values (ΔK). Once K was chosen, individuals were assigned to each of the K groups based on sample locality and its relation to the apparent shift in population affinity determined by STRUCTURE.

The genetic structure of the tentative populations was examined using a number of exploratory and inferential genetic analyses to quantify differences between putative populations and to determine the strength of genetic structure. To account for any correlation between genetic distance and geographic distance, a factorial Detrended Correspondence Analysis (Lebart et al. 1984) was performed on the multilocus microsatellite genotypes of all individuals using the '2D' 132 module of the software program GENETIX 4.04 (Belkhir 2001). A measure of differentiation in allele frequencies, G_{ST} (Hedrick 2005), was derived for all individuals as well as in ecoregion comparisons using GENEPOP 4.2 (Raymond and Rousset 1995). I examined population subdivision using a hierarchal analysis of molecular variance (AMOVA) with ARLEQUIN 3.5 (Excoffier et al. 2005). Isolation by distance, where populations exhibit differentiation via local dispersal and restricted interbreeding because of geographical distance (Wright 1943), was analyzed and implemented for all individuals using GENEPOP 4.2 (Raymond and Rousset 1995).

RESULTS

Patterns of Genetic Diversity

Multilocus genotypes from 102 samples were included in the analyses. Bobcats were grouped by county based on GPS location. When multiple individuals were from the same general locale, one was randomly selected for inclusion in the analyses. The resulting individuals were assigned to ecoregions based on GPS location: 11 cats in the High Plains, 64 cats in the Rolling Plains, 12 cats in the Edwards Plateau, 12 cats in the Trans-Pecos, and 3 cats in the South Texas Plains ecoregions (Fig. 7). Samples with less than 50% of loci amplified were not included in analyses. Each of the 9 microsatellite loci was polymorphic with 8-13 alleles per locus. 39 of the 102 cats had incomplete genotypes, with 1, 2, 3, or 4 missing loci. Expected heterozygosity exceeded observed heterozygosity in all nine loci, and the phylogenetic information content (PIC) of each loci ranged from 0.768-0.857. No loci were found to be approaching statistical significance for linkage disequilibrium following Bonferroni correction ($\alpha = 0.0011$). Only one loci (Lc120) was out of Hardy Weinberg Equilibrium (HWE) within the Rolling Plains ecoregion following Bonferroni correction ($P = 0.0011$). When all bobcats were examined as one group, HWE was highly significant ($P < 0.0001$).

Using GENEPOP, F_{ST} across all 5 ecoregions suggested minimal structure and little divergence with the overall $F_{ST} = 0.0095$. The overall $F_{IS} = 0.1002$, with higher positive values supporting deviations from HWE in some loci (FCA045).

An overall G'_{ST} value of 0.007 was obtained for bobcats across all ecoregions, suggesting panmixia may not be widespread throughout western Texas because of high variation of allele frequencies. I also compared differences in allele frequencies between adjacent ecoregions and for all paired ecoregion comparisons to examine admixture

throughout the landscape (see Table 3 for G'_{ST} values between all ecoregion pairs). Of 10 ecoregion pairings, the ones that were significantly different were the High Plains/Edward's Plateau, the Rolling Plains/Edward's Plateau, the High Plains/Trans-Pecos, and the Rolling Plains/Trans-Pecos ecoregion combinations (Table).

Population Structure

The Bayesian analysis identified subtle population structure. A mode was observed at $K = 2$ (Fig. 8), but was similar to the likelihood at $K = 1$ and unlike analyses employing $K = 4$ or 5. The mode at $K= 3$ was weak (Fig. 9). The STRUCTURE analysis (Pritchard et al. 2000) found no distinct population subdivision correlated to geographic location, with bobcats being equally likely to assign to cluster 1 in the High Plains as bobcats assigning to cluster 1 in the South Texas Plains. When $K=2$ for the total sample, 45.7% of bobcats identified most strongly with cluster 1, while 54.3% identified most strongly with cluster 2 (Fig. 8). When $K=3$, 29.6% of bobcats identified most strongly with cluster 1, 34.3% identified with cluster 2, and 36.2% of cats in cluster 3 (Fig. 9). The genetic discontinuity when $K = 2$ did not correspond to sample location or to ecoregion, but $K = 3$ identified a weak north-south gradient for one population (Fig. 9).

The factorial Detrended Correspondence Analysis for all samples (Fig. 10) found little structure across the five ecoregions, with 5 outliers not falling within the single cluster determined by GENETIX (Belkhir 2001). These outliers were isolated points outside of the main cluster and were not associated with a second cluster. Outliers were located in the High Plains (1), Rolling Plains (3), and Trans-Pecos (1) ecoregions. Three of these were samples over 10 years old. In order to more clearly identify fine-scale differentiation in the main cluster, these outliers were removed from the dataset and the

analysis was run again. The correspondence analysis for the remaining 97 samples again detected one primary cluster, but some weak ecoregion affinity was present because of animals plotting nearer to animals from the same ecoregion (Fig. 11). Bobcats from the Rolling Plains, however, exhibited the greatest genetic variability and were evenly distributed throughout the primary cluster. I performed this analysis a third time, this run sans both outliers as well as all samples from the Rolling Plains ecoregion, in order to examine the clustering relationship among the samples with less genetic variability. This analysis revealed a weak trend for ecoregion affinity, but little structure was still apparent in the clustering.

Bobcats across all ecoregions showed significant isolation by distance ($P = 0.009$). This is unsurprising given the movement capabilities of this species, yet the significant differences among allele frequencies suggested that local phenomena impacted gene flow throughout the entire region.

DISCUSSION

Impact of Bobcat and Landscape Ecology on Genetics

The variation in multilocus microsatellite genotypes for 102 bobcats sampled throughout 5 ecoregions in western Texas corroborates minimal genetic structure throughout the landscape. However, differences in allele frequencies between bobcats sampled from different ecoregions suggested that some substructuring occurred within the putative populations across all 5 ecoregions. Expected heterozygosity exceeded observed heterozygosity. This finding was supported by the homozygote excess identified with a high overall inbreeding coefficient ($F_{IS} = 0.1002$), where a heterozygote deficiency suggested possible population subdivision. However, the genetic structure

throughout western Texas, as evidenced by the likelihood that K =1 and K=2 in the STRUCTURE analysis, was weak (Fig. 8). For example, some bobcats in the Trans-Pecos ecoregion, a highly variable landscape, exhibited the same likelihood of falling into cluster 2 as individuals from the Rolling Plains ecoregion, which is physically separated from the Trans-Pecos by the Edwards Plateau ecoregion. When I mapped bobcats geographically by population from STRUCTURE, however, there was no geographic pattern when K = 2 (Fig. 8). When I created a map for K=3, some bobcats in the southern portion of the study area exhibited stronger affinity for one population (Fig. 9). This suggested a north-south gradient influence for population structure, though the trend was weak. In the absence of clear genetic divergence, isolation by distance is not unexpected for this species. In concert, I did find significant isolation by distance for all bobcats. Bobcat gene flow, then, appears to be limited by distance, especially in the company of habitat changes, possibly associated with ecoregion transitions. Despite bobcat movement capabilities, however (Newbury 2013, Anderson and Lovallo 2003), responses to spatial heterogeneity and habitat composition in this region may play a role in underlying genetic structure. Similar results have been seen in other highly mobile species such as coyote (Sacks et al. 2004) cougar (McRae et al. 2005), and brown bear (*Ursos arctos*; Kopatz et al. 2012). Definitively, despite the heavy influence of isolation by distance on bobcat genetic structure, the detected intrapopulation variability seems to be the result of unobstructed boundaries, where the variation across the distribution may be impacted by local environmental or landscape gradients.

Indeed, these results suggested that functionally relevant environmental or anthropogenic features, in addition to distance, exist across this landscape that may be

impacting bobcat movement and genetic structure. Previous studies on wildlife

movements and population structure, for example, have reported sensitivity to human

development (Tigas et al. 2002), landscape and local fragmentation variables (Crooks

2002), and correspondence between genetic structure and ecological differences within

species (De Leon et al. 2010). It seems likely that the slight genetic discontinuity of

bobcats in western Texas can be attributed to habitat affinities or limitations rather than

physical dispersal barriers, and that the level of impact varies within ecoregion based on

bobcat ecology and landscape composition. Research has suggested that highly mobile

species can be structurally impacted by habitat factors and environmental gradients, such

as vegetation and climate structuring grey wolf populations, genetic subdivisions in

coyotes associated with unobstructed boundaries between contiguous habitat (Sacks et al.

2004), and habitat fragmentation negatively impacting bobcats (Riley et al. 2003)

Further, the Detrended Correspondence Analysis illustrated a primary genetic cluster

with minimal grouping by ecoregion. The weak sub-structuring by ecoregion suggests

that regional bobcat genetic structure may be impacted by local environmental variables

within and among ecoregions rather than a consistent genetic trend across all ecoregions.

Bobcat landscape responses can be impacted by innumerable factors, comprising

energetic demands, mating opportunities, and hunting and escape cover (Anderson 1987).

Throughout western Texas, environmental variables are diverse and include transitions in

temperature, vegetation composition, elevation, prey availability, and habitat

heterogeneity. The likelihood of 1 or more of these factors influencing bobcats within any

ecoregion is high, where the level of impact has the potential to mediate bobcat

population structure on a regional scale.

Influences of Bobcat Sympatry on Genetic Structure

Most evidence of bobcat social structure has resulted from studies on home range patterns and use, where the level of organization varies based on population density, food resources, habitat configuration, and climate (Anderson 1987). As a result, territoriality, tolerance, avoidance, and overlap in bobcats may be impacted by the habitat mosiac. The consequent behavior can impact gene flow by way of altering a species' movements.

Studies have hypothesized that philopatry, the tendency of an animal to remain in or return to an area, mediates genetic structure in some populations. For example, Sacks et al. (2004) hypothesized that the genetic structure seen in coyotes in the absence of clear, physical barriers was the result of natal-biased habitat dispersal, where animals disperse into habitat similar to natal habitat. This habitat affinity likely generates habitat-adapted genetic structure. Similarly, Pilot et al. (2006) linked habitat type, climate, and diet to natal-biased habitat dispersal as the underlying cause for nonrandom spatial genetic structure in European grey wolves. Philopatry has already been suggested in bobcats as an explanation for kinship and social structure in South Texas (Janečka et al. 2006). If aspects of philopatry exist for bobcat populations in western Texas, the macrohabitat breaks throughout the 5 ecoregions could mediate genetic structure and diversity by way of natal-biased habitat dispersal. Indeed, the significance in allele frequencies detected in the sample set as well as in ecoregion pairs suggests that factors within ecoregions are impacting movements. If bobcats are partial to familiar habitat, this would increase positive assortative mating and serve as one hypothesis for the high inbreeding coefficient found in the sample set. This is an important consideration in light of the lower F_{ST} value (P = 0.0095), which suggests little population divergence.

The apparent nonrandom genetic structure of bobcats in western Texas may also be symptoms of sympatry, where populations occupy the same geographic space and encounter one another. In terms of evolution, sympatry can lead to speciation. Sympatric speciation involves several mechanisms that result in the emerging of new species independent of geographical isolation (Via 2001, Bolnick and Fitzpatrick 2007). Specifically, the factors thought to drive sympatric speciation include habitat choice affecting mate choice, large population size, small population size, and positive assortative mating (where individuals mate with like individuals), among others (Dieckmann 1999, Via 2001). While these events do not explicitly mean speciation is occurring, it is more likely that bobcats are experiencing symptoms of sympatric divergence. Sympatric divergence assumes that local extrinsic barriers isolate populations and promotes genetic changes and stochastic divergence (Johannesson 2001). The above factors can be the result of resource partitioning within a population, making sympatric divergence an important consideration in genetics studies. Previous studies have examined niche partitioning in highly mobile, sympatric carnivore communities, including bobcats (Neale and Sacks 2001, Thornton et al. 2004, Horne et al. 2009, Booth-Binczik et al. 2013). Further, while some research suggests that spatial exclusivity among bobcats is not an ecological necessity (Cochrane et al. 2006), the tendency for bobcats to avoid one another temporally might contribute to efficient resource use and therefore impact population structure. In habitat of varying quality, bobcat movements might especially be altered.

Anthropogenic Influence on Bobcat Population Structure

Human-mediated habitat fragmentation can impact carnivore populations by limiting movements and altering population structure. Bobcats are known to be sensitive to extreme habitat fragmentation (Crooks 2002, Riley et al. 2006, Poessel et al. 2014). The sample locations from this study occurred outside of areas with heavy human traffic, specifically areas with high road density. While every effort was made to obtain an even distribution of samples, the areas I was unable to collect samples from are primarily areas with high agriculture impact and high road density. The allele frequencies for bobcats in western Texas suggested some substructuring. Of particular interest were the results from the G-test comparing allele frequencies from bobcat in the High Plains and Rolling Plains ecoregions. The High Plains ecoregion is defined by heavy agriculture/rangeland use and associated road infrastructure. Both Tucker et al. (2008) and Reding et al. (2013) corroborated bobcat avoidance of agriculture lands, which suggests that bobcats perceive this habitat as suboptimal. Based on fur-trapper success throughout this study in the Rolling Plains ecoregion, it is possible that bobcat d ensity is high throughout this area. Indeed, bobcat genetic variation was high in the Rolling Plains, yet the 11 samples obtained from the High Plains ecoregion had no significant differences in allele frequencies. One hypothesis is that bobcats perceive the habitat in the High Plains as suboptimal, and dispersing individuals from the Rolling Plains are not reproductively successful in the High Plains. This could create a source-sink population effect between the 2 ecoregions, where within-habitat reproduction in the High Plains is not sufficient to counteract local mortality (Pulliam 1986). Seemingly lower bobcat density in the High Plains, possibly because of suboptimal habitat quality and anthropogenic presence and persecution, could promote continued immigration from the more reproductive Rolling

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Plains and promote the genetic similarities between the 2 ecoregions. A more thorough examination of bobcat movements and genetic structure is needed for these 2 regions.

A final consideration for the results of this study is that the sample distribution is not adequate to delineate meaningful insight into the population structure of bobcats in western Texas. Specifically, more samples from the South Texas Plains would be beneficial. The current low sample yield from this ecoregion (n = 3) makes identifying correlations weak. Finally, bobcats have been shown to avoid fragmented habitat, yet the genetic structure of those same animals indicated no impact on population structure (Reding et al. 2013). This introduces the possibility that gene flow can be unrelated to local adaptation when ecological variables are concerned, particularly in highly mobile species with long generation times, making ascertaining of meaningful landscape variables more difficult.

CONCLUSIONS

My research reveals that bobcats throughout western Texas are indeed vagile, though some isolation and resistance may be affecting admixture and inbreeding across the landscape. The population structure found in western Texas is likely the result of historical processes that include repeated expansions and contractions of a highly mobile species ranging across areas with distinct (and non-distinct) macrohabitat breaks, but over time contemporary landscape and ecological processes may have impacted historical events and microevolutionary processes. The high genetic variation reflects bobcats' wide use of habitat throughout the western portion of the state, though it is likely that a myriad of environmental variables impact bobcat movements and gene flow to varying degrees. More research is needed to determine what specific ecological and

anthropogenic gradients are important to bobcat population structure in addition to isolation by distance. The results of this study have important management and conservation implications, as highly mobile, generalist species around the world can be negatively impacted by many anthropogenic and environmental gradients (Cain et al. 2003, Ernest et al. 2003, Dixon et al. 2007, Haag et al. 2010, Kobmuller et al. 2010). As a result, managers should be considerate of local variables that might affect microevolutionary processes and population structure, particularly when species cover large ranges. Overestimating the adaptative abilities of generalist species, especially in areas of increasing habitat fragmentation and anthropogenic pressure, can have detrimental effects on dispersal, structure, and populations success. My study illustrates that even abundant, widespread species can respond to the landscape in complex ways that manifest in variable genetic diversity.

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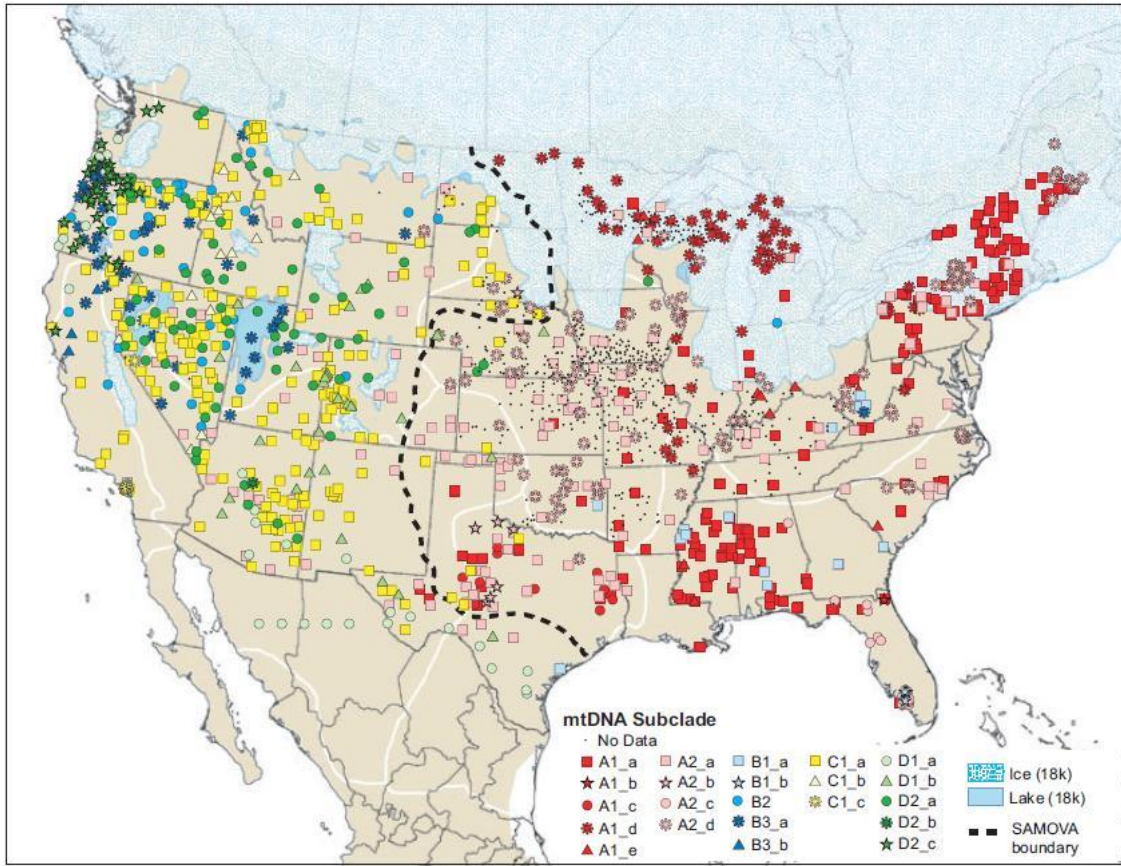
Figure I.1. Continental bobcat genetic structure bissected by the Great Plains. The black line denotes the Great Plains and the sharp transition in bobcat genetic structure, where bobcats across the United States are divided into two main groups (From Reding 2012).

Figure I.2. Characteristic High Plains Ecoregion, Potter County, Texas. A relatively level high plateau, this ecoregion is dominated by shortgrass prairie, little topographic relief, and agriculture. Photo by R.T. Kazmaier.

Figure I.3. Characteristic Rolling Plains Ecoregion, Cottle County, Texas. This ecoregion transitions from shortgrass prairie to a landscape dominated by mesquite plant associations and low, rolling hills. Photo by R.T. Kazmaier.

Figure I.4. Characteristic Edward's Plateau Ecoregion, Real County, Texas. This ecoregion is dominated by mesquite prairie plant associations. Springs, stony hills, and steep canyons dominate this landscape. Photo by R.T. Kazmaier.

Figure I.5. Characteristic Trans-Pecos Ecoregion, Brewster County, Texas. This highly variable ecoregion ranges from arid desert to mountain habitat. Photo by R.T. Kazmaier.

Figure I.6. Characteristic South Texas Plains Ecoregion, Dimmit County, Texas.

This ecoregion is characterized by plains of thorny shrubs and trees and

intermittent patches of palms and subtropical woodlands. Photo by R.T. Kazmaier.
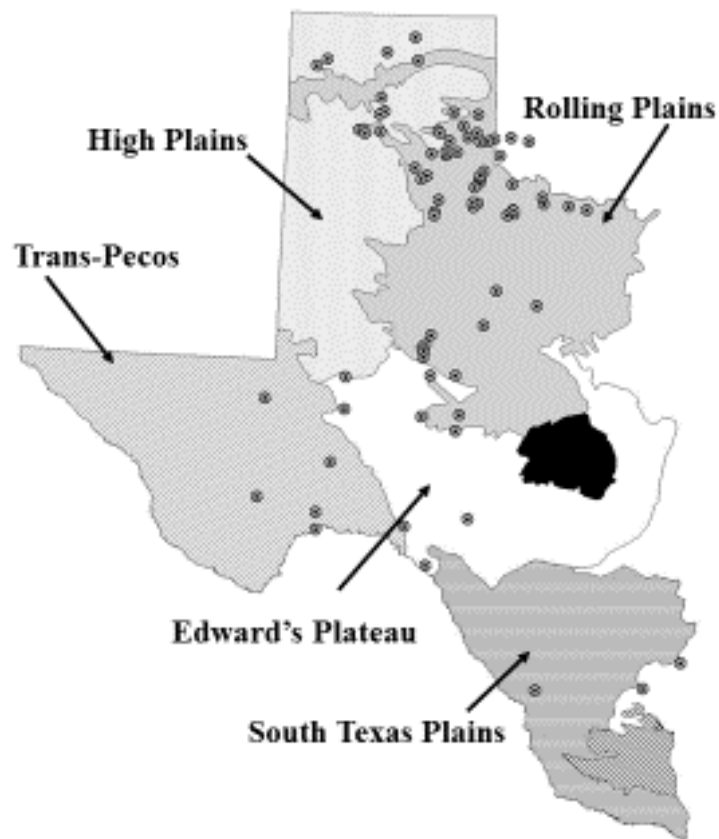
Figure I.7. GPS locations of 102 bobcat samples across five ecoregions in western Texas. Bobcat samples are denoted by gray circles.
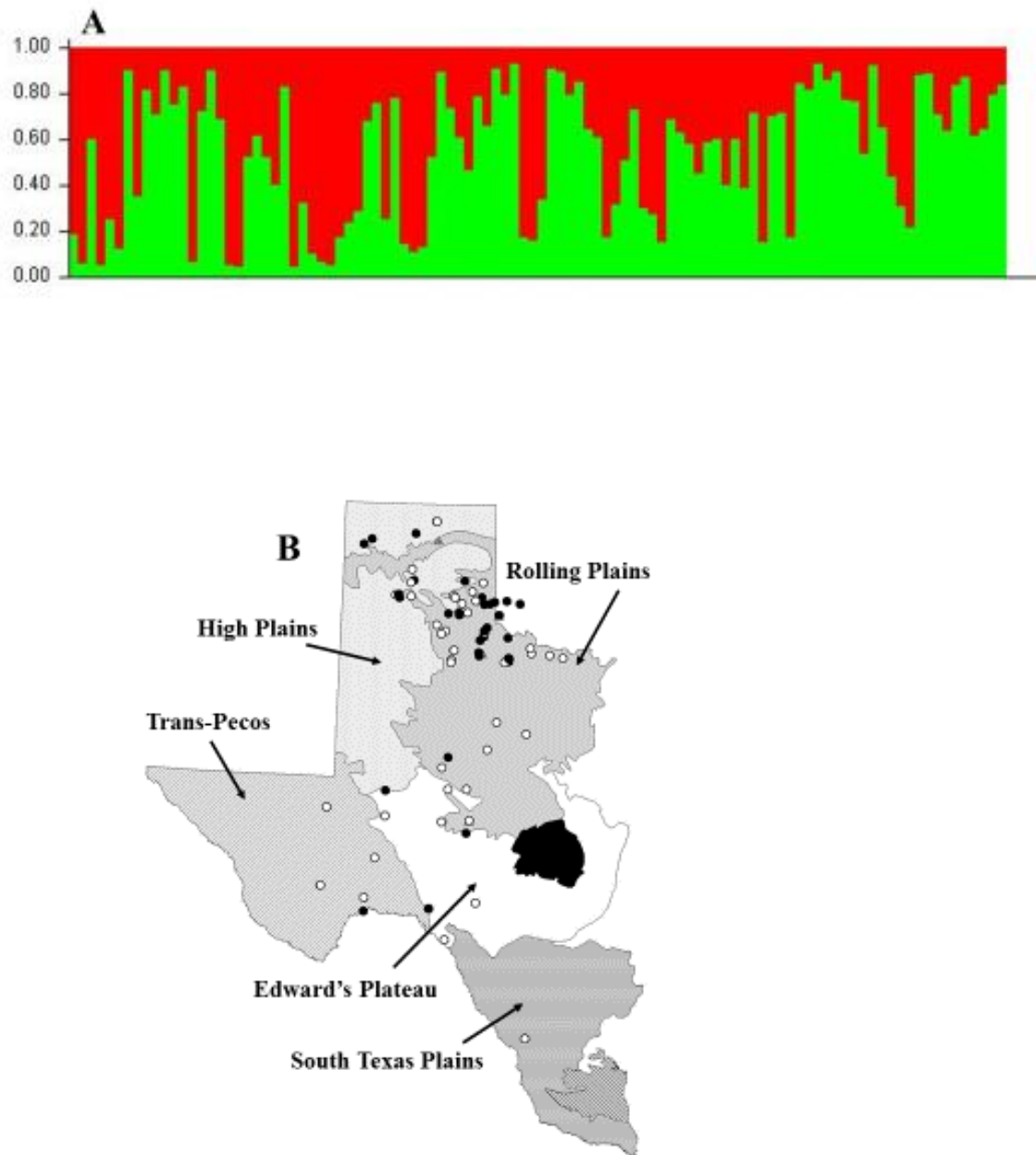
Figure I.8. Bobcat populations when K=2 from the STRUCTURE analysis (A) and when organized by population across my study site (B). Black circles = population 1, white circles = population 2, gray triangles = admixed individuals. See text for full description.
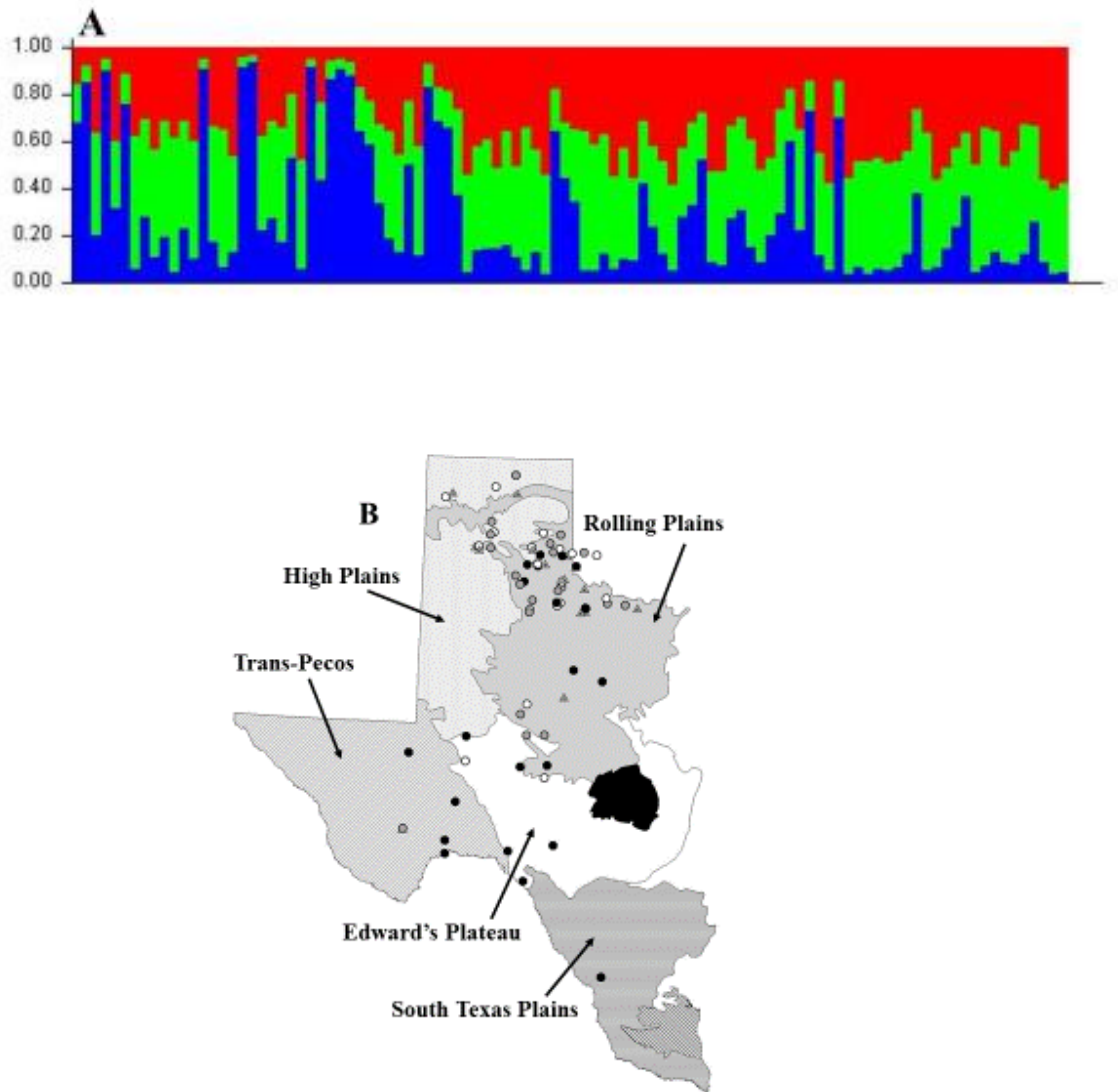
Figure I.9. Bobcat populations when K = 3 in the STRUCTURE analysis (A) and when organized by population across my study site (B). White circles = population 1, gray circles = population 2, black circles = population 3, and gray triangles = admixed individuals. See text for full description.
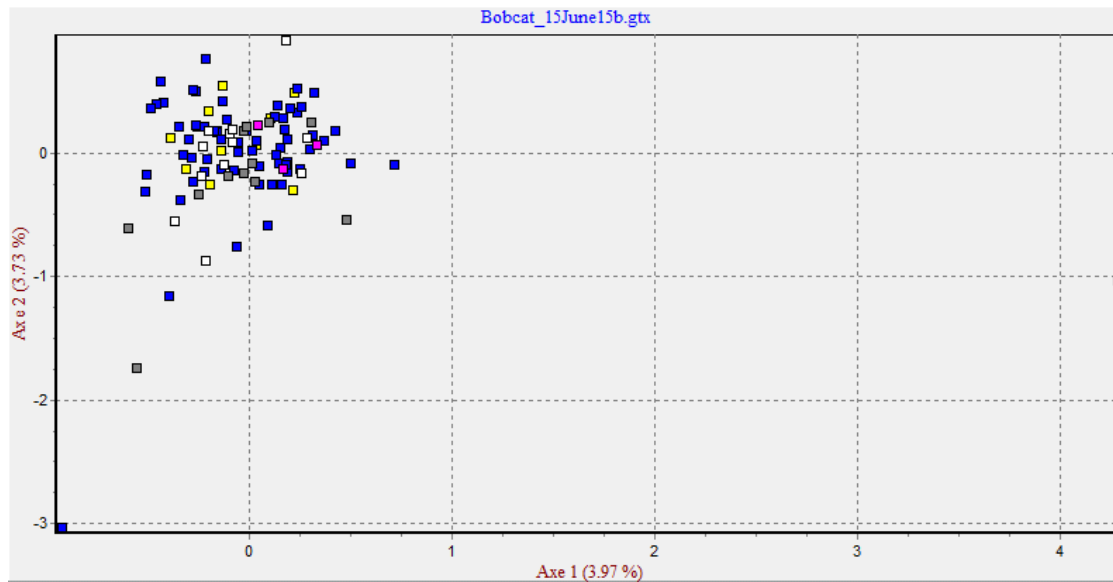
Figure I.10. Results from the Detrended Correspondance Analysis with all cats. Bobcats fell into 1 cluster and are individually represented by colored squares. Yellow = High Plains cats, Blue = Rolling Plains cats, White = Edward's Plateau cats, Gray = Trans-Pecos cats, Pink = South Texas Plains cats.

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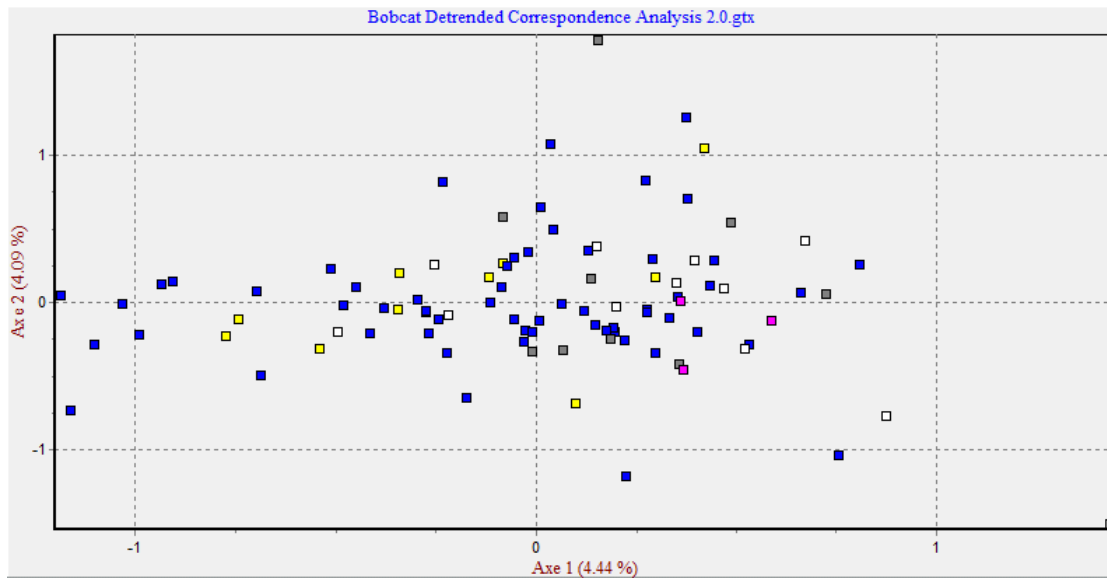
Figure I.11. Results of the Detrended Correspondance Analysis sans outlier bobcats. Bobcats fell into 1 cluster and individually represented by colored squares. Yellow = High Plains cats, Blue = Rolling Plains cats, White = Edward's Plateau cats, Gray = Trans-Pecos cats, Pink = South Texas Plains cats.

CHAPTER 2

LOCAL AND REGIONAL SCALE ORDINATION AND THE INFLUENCE OF

SPATIAL HETEROGENEITY AND VEGETATION COMPOSITION ON

BOBCAT GENETIC STRUCTURE IN WESTERN TEXAS

INTRODUCTION

The way an animal perceives landscape features and disperses across that
landscape may influence the genetic structure of populations. Understanding the
relationship between environmental variables and genetic structure provides information
on biological processes that impact microevolution and local adaptation (Manel et al.
2003, Storfer et al. 2007, 2010). Landscape genetics is the interdisciplinary field that
merges landscape ecology with population genetics to explore the impact of landscape
composition and configuration on gene flow, genetic discontinuities, and genetic structure
(Manel et al. 2003, Holderegger and Wagner 2006, Storfer et al. 2007). This approach has
enabled researchers to address an array of objectives related to wildlife-landscape
relationships, including measuring the effects of habitat fragmentation on population
structure (Proctor et al. 2005, Haag et al. 2010), identifying corridors for disease
transmission (DeYoung et al. 2008, Root et al. 2009), quantifying areas of landscape
connectivity (Murphy et al. 2010, Rabinowitz and Zeller 2010) and identifying barriers to
gene flow (Mcrae et al. 2005, Epps et al. 2005, Zalewski 2009), and determining
environmental variables and gradients that impact movement and dispersal (Geffen et al.

2004, Sacks et al. 2004, Stenseth et al. 2004). The results of landscape genetic studies are broadly applicable to wildlife management and conservation because of the ability to better understand population movement and behavioral ecology by way of gene flow. Specifically, these studies aid in our understanding of the factors driving microevolutionary processes across spatial scales.

The amalgamate technique of landscape genetics has created a plethora of options in both experimental design as well as analysis. Manel et al. (2003) reviewed various spatial patterns of genetic diversity that can be addressed from a landscape genetics perspective: random and clinal patterns, boundaries to gene flow versus isolation by distance, and metapopulations. Further, several statistical tests exist that are directly useful in landscape genetics applications and include assignment tests (determines genetic population of origin, identify barriers), least-cost path analyses (spatial autocorrelation to identify corridors, clinal patterns), linear regression (gene flow boundaries), isolation by distance (measures the relationship between genetics and geographic distance), Mantel and Partial Mantel tests (matrix correlation), and ordination (determines relative influence of landscape variables on gene flow; Manel et al. 2003, Storfer et al. 2007, Manel and Holderegger 2013). Studies have demonstrated the importance of testing multiple environmental variables in order to address landscape genetics objectives (Angers et al. 1999, Spear and Storfer 2010, Moore et al. 2011), making ordination a common analytical method.

Ordination is an analytical method used to ascertain the relative influence of variables and gradients on variation in samples (Storfer et al. 2007). One ordination technique useful in detecting patterns in the spatial arrangement of individuals is

Canonical Correspondence Analysis (CCA), which is ideal for examining relationships between species and their environment with the goal of identifying gradients in environmental variables (ter Braak and Verdonschot 1995). Indeed, this 2-step approach (ordination followed by environmental gradient identification) is a multivariate analysis technique that relates community composition to known environmental variation (ter Braak 1986). Researchers employ CCA to measure species and environmental data across axes (gradients), whereupon if the measured environmental variables correlate strongly to the first few ordination axes, the environmental variables are sufficient as predictors for the sample variation seen in the species composition (ter Braak 1986). These tests can be used in combination with Geographic Information Systems (GIS), which effectively represent spatial features of landscapes in a myriad of ways. Geographic information systems can be employed in landscape genetics studies by overlaying land use maps, topographical maps, or climatic effects with genetic information (Ewers et al. 2010, Manel and Holderegger 2013). Combining these analytical methods helps researchers identify existing correlations between potentially relevant landscape features and genetics patterns.

Analyzing spatial genetic structure correlates landscape connectivity with dispersal and gene flow between habitat patches (Holderegger and Wagner 2008). Examinations that consider several landscape variables, however, may be necessary for certain populations, as the anthropogenic, ecological, or environmental processes that affect genetic diversity can be varied or difficult to identify. Furthermore, factors such as habitat use and population dynamics can impact the level of landscape-mediated population changes, making species-specific hypothesis testing useful for determining how species ecology corresponds to patterns of genetic diversity (Storfer 2007). An

approach that combines ordination with GIS, then, can be useful for species that cover wide ranges with varying degrees of habitat heterogeneity, as spatial genetic structure may not be consistent across populations or regions.

Generalist species can have adverse effects in genetic diversity and connectivity as a result of habitat fragmentation (Riley et al. 2003, Proctor et al. 2005, Riley et al. 2006, Dharamarajan et al. 2009, Ruell et al. 2012), population structuring via macrohabitat and vegetation (Geffen et al. 2004, Sacks et al. 2004), and genetic impacts from physical barriers (Millions and Swanson 2007, Croteau et al. 2012, Reding et al. 2012). These are important considerations for the management of generalist species, as the ecological vagility of these organisms often supports panmixia, where genetic structure is generally patterned by isolation by distance and mate choice is limited only by geographical distance (Wright 1943). In contrast, other studies have found little population structure in generalist species across great distances (DeYoung et al. 2009, Bozarth et al. 2011, Talbot et al. 2012). This is to be expected for those species that cover wide ranges across variable habitat. However, when population structuring is apparent without distinct physical barriers, the question becomes, what gradients influence gene flow?

The bobcat (*Lynx rufus*) is a highly mobile, generalist species that ranges throughout North America and inhabits an array of habitat (Anderson 1987). Bobcats have also been found near human modified areas, roads, and agricultural fields (Larivier and Walton 1997). Despite their ecological flexibility, several studies have been conducted on bobcat landscape genetics and found population structure impacted by fragmented habitat (Riley et al. 2006, Millions and Swanson 2007, Ruell et al. 2012,

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Reding et al. 2012). Croteau et al. (2012) identified regional population structure in bobcats across 16 states in North America, and Janečka (J.E. Janečka, Duquesne University, unpublished data) found evidence of reduced dispersal and connectivity between populations of bobcats in South Texas. In western Texas, the impact of landscape-level change on the genetic structure of bobcats is not understood. In particular, it is not known whether specific environmental variables or gradients influence bobcat gene flow and dispersal in this region. The spatial arrangement of resources across the landscape is a dominant factor that affects where individuals occur (Azevedo and Murray 2007), making an investigation into the influences on bobcat genetic patterns valuable for habitat, resource, and population management. Because bobcats range throughout public and private land in Texas, understanding the spatial organization of genetic data can be useful in determining the most appropriate level of management for this ecologically and economically important furbearer. In order to investigate variation in the genetic structure of bobcats throughout western Texas, I evaluated bobcat heterozygosity with respect to 68 environmental variables.

METHODS

Study Area and Sampling

Muscle tissue from 64 bobcats throughout 5 ecoregions in western Texas were collected from January 2013 to March 2015 (Fig. 1). Specifically, bobcat samples covered 5 ecoregions: the High Plains, Rolling Plains, Edward's Plateau, Trans-Pecos, and South Texas Plains ecoregions. These ecoregions differ by habitat type, elevation change, and plant community structure, though there is some overlap in these ecotone designations (Correll and Johnston 1970).

Samples from all ecoregions were collected through an array of collaborative efforts, including opportunistic sampling of road-killed animals, tissues collected from both commercial and private furtrappers, and samples provided by Texas Parks and Wildlife biologists, game wardens, and Texas Wildlife Services personnel. Some sample locations were identified only by county of origin and therefore a GPS location of the county center was created for mapping purposes.

Microsatellite Genotyping and Analysis

Tissue samples were either frozen or stored in 70% ethanol. I performed DNA extractions on all samples using modifications of the Gentra Puregene tissue kit protocol (QIAGEN Corporation, Valencia, CA). The primary modification involved tailoring the amount of elution buffer based upon the quantity of DNA in the final EtOH wash as revealed by visual examination of the extraction pellet following the final ethanol wash.

Nine autosomal microsatellites (FCA026, FCA043, FCA045, FCA077, FCA082, FCA090, FCA096, FCA132, and Lc120) were used. Five of these loci (FCA043, FCA045, FCA077, FCA090, and FCA096) were previously found to be informative for 1 bobcat population in South Texas (Janečka et al. 2006, 2007). Four additional loci (FCA026, FCA082, FCA096, and Lc120) were screened against 8 bobcats and observed to have sufficient genetic variability for population analyses and genotyped across all animals. All loci but Lc120 were originally isolated in the domestic cat (*Felis catus)* by Menotti-Raymond et al. (1999). The primer Lc120 was isolated in the Canadian lynx (*Lynx canadensis*) by Carmichael et al. (2000).

Microsatellites were amplified in a 12.5 ul reaction containing 6.5 ul of GeneMate Taq 2X Mastermix (concentrations proprietary information), 2.5 ul ddH$_2$O, 1.0

ul of Well-Red fluorescently-labeled oligonucleotide (Sigma-Genosys, The Woodlands, Texas, USA), 1.0 ul forward primer, 1.0 ul reverse primer, and 0.5 ul genomic DNA. PCR reaction conditions included initial denaturing step of 94 C for 1 min, 10 cycles of 94 C for 15 s, 53 C for 15 s and 72 C for 45 s, followed by 50 cycles of 89 C for 15 s, 53 C for 15 s and 72 C for 45 s and a final extension of 72 C for 30 min. The denaturing temperature was lowered to 89 C after 10 cycles to decrease the amount of *Taq* inactivated by the high temperature of each denaturing step (Menotti-Raymond et al.., 1999). All amplifications were performed on an Eppendorf Mastercycler (Eppendorf, Hamburg, Germany). Polymerase chain reaction products were visualized with a Beckman Coulter CEQ8000 DNA Analyzer (Beckman Coulter, Indianapolis, IN) and the sizes identified by the CEQ8000 software were confirmed by visual inspection of tracings.

Statistical Methods

Canonical Correspondence Analysis (CCA; ter Braak 1986) was used as a direct gradient analysis technique, where species composition is directly related to environmental variables. In my case, the species matrix was created by defining each bobcat as either homozygous or heterozygous for each locus. I constructed my matrix of environmental variables by buffering individual bobcat sample locations by 2 different distances representing different landscape scales that might influence the heterozygosity of each loci. For a more local scale, I buffered sample locations by a radius of 2.69 kilometers, which represented the average radius of several reported bobcat home range sizes in this region of the U.S. (Rolley 1985, Kamler and Gipson 2000, Elizalde-Arellano et al. 2012). For a more regional scale, I buffered sample locations by a radius of 5.58

kilometers, which represented typical dispersal distances for bobcats reported from the literature (McCord and Cardoza 1982). These buffer polygons were then intersected with GIS layers for each environmental variable to determine scale-specific information for each sample. Nine environmental variable classes were selected based on the likelihood of meaningfulness to bobcat behavior and ecology and examined at both scales. Each environmental variable category was represented by a GIS layer, which was used to calculate proportions and densities of variables within individual buffer polygons. Each environmental variable category GIS layer was used to delineate several different environmental variables within its respective category. The **land use land cover (LULC)** layer identified land throughout Texas by LULC classification, which included levels of urban, agriculture, rangeland, wetland, frozen tundra, and forest land use. I calculated the proportion of land use land cover (LULC) categories from the intersected GIS layer by dividing the hectares of individual cover classes into the total hectares of each buffer polygon for each cat. At both spatial scales I used Patch Analyst 3.1 (Rempel et al. 2012) to develop fragmentation statistics (number of patches, mean patch size, mean patch edge. patch density, edge density, total edge, and Shannon's diversity index of patch size) for the LULC layer to be included in the environmental matrix. I also included latitude and longitude. The **vegetation composition** GIS layer categorized Texas by plant community associations. I calculated the proportion of vegetation composition types by again dividing the hectares of each plant type into the total hectares of each buffer polygon. I used a GIS layer denoting **ecoregion designation** across Texas to determine proportions of buffer polygons in each of the 5 ecoregions. I intersected this GIS layer with individual buffer polygons to calculate the proportion of each ecoregion type within.

The **stream density** GIS layer mapped stream bodies throughout Texas. I edited this layer to only include streams above an order of magnitude of 5. Stream density was determined by dividing total stream lengths (in km) by the total hectares of the buffer polygons and converted to km/km^2. The **railroad density** GIS layer mapped railroads across Texas. Within my study site, railroad density was calculated by dividing total railroad length (m) by the total hectares of each buffer polygon and converted to km/km^2. Similarly, the **road density** GIS layer mapped roads across Texas, which I used to calculate road density for each buffer polygon. I divided total road length (m) for each buffer polygon by the total hectares and converted values to km/km^2. The GIS layers for both **maximum temperature** and **minimum temperature** indicated the average maximum and minimum temperatures for areas across Texas, respectively. I used these layers to calculate the weighted average of maximum temperature and minimum temperature within each polygon by adding the products of the number of hectares at each temperature value and dividing this sum by the total hectares of each polygon. The last environmental variable category was **annual precipitation.** Annual precipitation was also represented by a GIS layer, which indicated annual precipitation levels moving across Texas. I converted annual precipitation to a weighted average by intersecting this layer with buffer polygons and adding the products of the number of hectares at each rain average value (in) and divided by the total hectares of each buffer polygon.

All spatial analyses were performed using ArcView 3.3 (Environmental Systems Research Institute, Redlands, California, USA). The resulting genetic (= species) and environmental (= 9 environmental variable categories) matrices were then processed using CANOCO version 4.5 to evaluate local and regional influences on heterozygosity.

For each of the 2 scale-dependent CCAs, I used forward selection with Monte Carlo

Analyses (1000 permutations) to select the best 16 environmental variables that explained

the most variation in the heterozygosity data.

RESULTS

Statistical Analyses

For the local scale analysis, the ratio of the sum of canonical eigenvalues (0.014)

to the sum of unconstrained eigenvalues (0.048) suggested that the environmental

variables in the CCA model justified 24.6% of the variance in the data. The first and

second axes accounted for 60.0% of the explainable variation; the third axis added

another 13.9% of explanatory power, and the fourth axis only 9.4% more. The 16

environmental variables selected by the CCA were latitude, number of patches, mean

patch size, total edge, mean patch edge, Shannon's diversity index of patch size,

proportion of the High Plains ecoregion, proportion of the Rolling Plains ecoregion,

proportion of the Edward's Plateau ecoregion, proportion of the Trans-Pecos ecoregion,

proportion of the South Texas Plains ecoregion, mean minimum temperature, mean

maximum temperature, railroad density, proportion of mesquite juniper associations, and

proportion of mesquite lotebush associations.

The pattern of heterozygosity for each locus was weakly influenced by the

majority of environmental variables selected (Fig. 2). However, based on the relative

length of biplot arrows, proportion of the mesquite lotebush association had the greatest

influence on heterozygosity, with increased proportion of the vegetation associated with

increased heterozygosity for microsatellites FCA026, FCA077, and FCA090. FCA077

had the weakest degree of association with proportion of mesquite lotebush associations,

and was instead more influenced by proportion of mesquite juniper associations and total edge. Latitude, the proportion of the Rolling Plains ecoregion, and Shannon's diversity index of patch size were also equally correlated with the heterozygosity of FCA026, FCA077, and FCA090, though the degree of association seemed minimal. Railroad density was associated with some of the variation in FCA045, and to a lesser degree for FCA096. The heterozygosity observed in locus FCA096 was more associated with the increased proportions of the Edward's Plateau and South Texas Plains ecoregions. Interestingly, the number of patches as well as the proportion of the High Plains ecoregion were both associated with increased heterozygosity of Lc120, though these variables were not distributed close to one another. FCA132 fell close to axis 2 on the graph, suggesting a combined influence by total edge and patch number. Both minimum and maximum temperature influenced the heterozygosity of FCA082, and vaguely influenced FCA043. The biplot arrows for mean patch edge, mean patch size, and proportion of the Trans-Pecos ecoregion also influenced the variation in FCA043and FCA082 (Fig. 2).

For the regional scale analysis, the percentage of variance explained by the environmental data was 23.9%. The sum of the canonical eigenvalues was 0.014, and the sum of the unconstrained eigenvalues totaled 0.048. The 16 environmental values selected by the CCA were latitude, longitude, number of patches, mean patch size, total edge, edge density, mean patch edge, Shannon's diversity index of patch size, urban land use, agricultural land use, herbaceous rangeland, shrub rangeland, forest, creosote, mesquite lotebush, and the proportion of the Trans-Pecos ecoregion.

Similar to the local scale ordination, the length of the biplot arrows in the regional analysis suggested that mesquite lotebush had the greatest influence on microsatellite heterozygosity (Fig. 3). Mesquite lotebush, latitude, longitude, and agriculture land use were most closely associated with increased variation in FCA090, while edge density had minimal effect. Creosote was the only other vegetation association to explain variation in heterozygosity, and accounted for variation in FCA082, FCA090, and FCA132. Heterozygosity in these same loci was also influenced by urban land use, which was the second most influential variable at this scale based on biplot arrow length. However, number of patches and total edge may have more explanatory power for FCA096 and FCA132. FCA082 fell near the biplot arrow for Shannon's diversity index of patch size and was near the biplot arrow for shrub rangeland, suggesting a relationship between both variables. Herbaceous rangeland weakly impacted the heterozygosity of FCA026, FCA045, and FCA077, but it is likely that a decrease in urban land use also influences heterozygosity for all three. Finally, a combination of forest cover and mean patch edge was correlated with Lc120, while mean patch edge, shrub rangeland, and Shannon's diversity index of patch size (weakly) were associated with the variation in FCA043 (Fig 3).

DISCUSSION

Local Scale Canonical Correspondence Analysis

Bobcat heterozygosity throughout western Texas exhibited weak levels of genetic variability with regards to the 16 selected environmental variables. At the local scale, road density and urban land use were not selected by the CCA. Similarly, Millions and Swanson (2007) did not find significance between roads and genetic structure of bobcats

in Michigan. For my study, this was interesting given that all bobcat sample locations fell in areas with low road density. Sampling pressure was likely uneven because of furtrapper preference, however, and as a result I cannot ascertain the relevance of roads by bobcats. Agriculture was also not selected as having an influence on genetic variation at the home range level, but mean patch size, mean patch edge, and Shannon's diversity index of patch size were correlated with increased heterozygosity. Heterozygosity levels of 5 of the 9 loci were impacted by these fragmentation statistics. This suggests that the type of habitat fragmentation may be less important to bobcats than the amount of spatial heterogeneity across the landscape. Specifically, while examples in the literature suggest that agricultural land use may result in suboptimal habitat for bobcats (Tucker et al. 2008, Thurmond 2014), it is possible that agricultural land use is not as important to bobcats as is the amount of fragmentation within that landscape. This would suggest that the degree of habitat fragmentation, regardless of it being natural or artificial in nature, impacts bobcat heterozygosity. Further, bobcats utilize edge habitat (Tigas et al. 2002), making spatial heterogeneity functionally relevant. This could also explain the association between number of patches and heterozygosity in Lc120 and FCA132. It is noteworthy, however, that Reding et al. (2013) observed physical avoidance of agriculture land by bobcats, but were unable to detect a genetic pattern reflecting those movements.

Of particular interest was the influential relationship between the High Plains ecoregion and the number of patches. Both Lc120 and FCA132 were influenced by these 2 variables. The High Plains ecoregion is characterized by agricultural land use and is highly fragmented. As the number of patches as well as proportion of this ecoregion increased, heterozygosity also increased. If agricultural land is suboptimal habitat for

bobcats, whether due to lack of escape cover, competition with coyotes (*Canis latrans*), or reduced prey availability, animals on this landscape may be poorly adapted. One hypothesis for high genetic variability in suboptimal habitat involves the High Plains ecoregion being a sink population, where a local demographic deficit is occurring (Dias 1996). Because Canonical Correspondence Analysis does not test for indirect gradients, it is difficult to determine what features of the High Plains ecoregion are functionally relevant to bobcats in the absence of agricultural land use as a meaningful variable. More information on this landscape is needed to determine how land use in this region impacts bobcat genetic structure.

The higher degree of influence on bobcat heterozygosity by mesquite lotebush can be explained ecologically by resource use, where spatial organization is associated with habitat selection and use and prey availability (Janečka et al. 2006). The CCA results suggested that factors pertaining to this plant association, be it soil type or plant species infrastructure, impacts heterozygosity. Specifically, factors associated with mesquite lotebush vegetation may provide increased prey availability, cover, and landscape connectivity for bobcats, which promotes movement, reproductive success, and genetic variation.

The influence of ecoregions was more significant in the Edward's Plateau and South Texas Plains ecoregions. The transition between the Edward's Plateau from the High Plains, Rolling Plains, and South Texas Plains ecoregions is sharp, whereas the transition between the Trans-Pecos and Edward's Plateau ecoregion is gradual (Correll and Johnston 1970). This sharp transition may be important for bobcat connectivity and gene flow. Only 3 samples came from the South Texas Plains ecoregion, however, so

more samples are needed to determine how influential components of this ecoregion are on bobcat heterozygosity. Increasing temperature was also associated with increased heterozygosity, which may or may not be coincidental to the 2 ecoregions being farther south. Similarly, Pilot et al. (2006) found that genetic variation in European grey wolves (*Canis lupus*) was strongly influenced by latitude.

Regional Scale Canonical Correspondence Analysis

The 16 variables selected for the regional scale ordination had low explanatory power for the percentage of genetic variation correlated to environmental variables. There was little variation to explain initially, and with such low explanatory power by the selected environmental variables, the associations are extremely weak as a result. Because the buffer zone polygons at this scale were much larger, the landscape features that were relevant to bobcats are expectedly different than the local scale CCA. Once again, proportion of mesquite lotebush associations was selected as a meaningful variable, suggesting that this type of plant association impacts bobcat gene flow in western Texas. Vegetation type has been previously demonstrated as an important variable in the genetic structure of coyotes and grey wolves (Sacks et al. 2004, Pilot et al. 2006), with vegetation explaining 43% of the genetic variation seen in one European grey wolf population (Pilot et al. 2006). This cryptic genetic structure reflects the strong influence of environmental variables, such as latitude and longitude, both of which were selected by the CCA. Doebeli and Dieckmann (2003) demonstrated that local adaptation occurs along environmental gradients, and because the north-south gradient involves notable transition in direct and indirect environmental variables, it is likely that local

adaptation drives bobcat gene flow by way of filtering landscape perception based on individual location.

It has been suggested in the literature that urban development impacts bobcat connectivity by reducing population numbers and genetic diversity (Ruell et al. 2012), and bobcats have demonstrated altered behavior in areas with high human traffic (George and Crooks 2006) and shown tendency to completely avoid urban areas (Riley 2006). My results indicated that bobcats are not adversely affected by all aspects of urban land use, though the CCA cannot account for all factors involved with urbanization, making it difficult to identify what aspect of urban activity promotes heterozygosity. It is possible that bobcat genetics in western Texas do not reflect the landscape effects that may be present in bobcat movement, though in the absence of roads as a significant variable, it is likely that urbanization and associated human activity is not significantly limiting bobcat movement or connectivity. This could be because of the significance of edge density, total edge, and number of patches being associated with urbanization and heterozygosity for 3 loci, which suggests that periphery habitat may be important for bobcat connectivity and movement.

Unlike the local scale CCA, agriculture was selected as a meaningful variable. The regional CCA was more likely to detect patterns in the spatial data than in the local scale CCA because of the increased buffer polygon size. For the 1 locus that was influenced by agriculture, this could again be the result of local demographic deficits, where bobcats on an agriculture-dominated landscape are moving across the landscape at a high rate with little success. Janečka et al. (2006) explained that establishing a home range is necessary for bobcats to breed. If bobcats avoid agriculture based on a lack of

resources, it is likely that this analysis is detecting genetic variation from transient bobcats or residents of a sink population. In contrast, 2 different types of rangeland were associated with bobcat heterozygosity. Because rangeland generally comprises more diverse plant communities than agricultural land, both the shrub and herbaceous rangeland may offer more resources for bobcats. Forest land would offer similar advantages in terms of escape cover and prey availability, though this variable's effect on heterozygosity was weaker, perhaps because of the homogeneity of forest types available. This notion is supported by Cushman et al.'s (2012) findings that homogenous landscapes with low fragmentation and higher levels of suitable habitat are less likely to exhibit significant impacts on gene flow. Relative to both forest and shrub rangeland, mean patch size and mean patch edge were both important variables for bobcat heterozygosity, though mean patch size had a stronger influence, suggesting once again that landscape heterogeneity is functionally relevant as well as important to bobcat ecology.

Factors Impacting Bobcat Spatial Genetic Data

Results at both scales indicated very little genetic variation in bobcats, and less than 30% of that genetic variation was attributed to the environmental variables included in the analyses. As a result, the influential power of the chosen environmental variables was weak throughout. The results of this study demonstrate that bobcats in western Texas are impacted by environmental gradients, but the level of significance for some variables was not high. The factors most likely to have contributed to not detecting a strong landscape effect include the need for a larger spatial scale. It is possible that, at this level, the chose environmental variables do not have enough effects on gene flow to detect

landscape correlations in the absence of clear physical barriers. Alternatively, I may have not selected the environmental variables most likely to have a strong landscape effect. Further, the genetic information used in this analysis may not have been adequate to detect a pattern. Finally, the direct gradient analysis does not account for those "invisible" indirect gradients, and unknown covariables could impact bobcat genetic structure in western Texas. It is likely that local adaptation across a myriad of habitat, coupled with repeated contractions and expansions of this highly mobile generalist species contributes to the genetic variation not explained by the CCAs.

Three trends emerged from the CCAs. The first was the relative influence of the mesquite lotebush vegetation association, where untested factors such as soil type, escape cover, or prey availability may be significant to bobcat connectivity throughout this study site. The second important observation may be the usefulness of spatial heterogeneity and variety of patches and edge habitat for bobcat gene flow. This study suggests that bobcats may benefit from access to a variety of habitat as well as demonstrate an increase in genetic variation when habitat borders promote connectivity. Finally, bobcats in the High Plains ecoregion may be part of a sink population, as evident by the increased heterozygosity in uniformly fragmented habitat. Dispersing individuals may arrive in the area, but may have less reproductive success because of suboptimal habitat. Previous studies that have reported bobcat sensitivity or avoidance to agriculture may not adequately account for the degree of fragmentation within that habitat type; specifically, bobcats may not be avoiding agriculture as much as landscape homogeneity. This landscape perception may be represented in regional scale CCA via meaningfulness of rangeland versus agriculture land, where bobcat heterozygosity was more influenced

overall by diverse rangeland as well as mean patch size. In this case, the homogeneity of the High Plains ecoregion may be detrimental to bobcat population success in that area. In the absence of clear physical barriers, however, it is unlikely that bobcats in this area will diverge significantly from other populations. Instead, these bobcats are at an increased risk for negative effects on genetic and demographic processes. More research is needed on this landscape to determine what factors drive this spatial relationship.

CONCLUSIONS

Despite the weak relative influence of environmental variables on bobcat genetic variation detected by the CCAs, my study demonstrates that a generalist species can perceive the landscape in meaningful ways and be impacted by various anthropogenic and ecological gradients. Unfortunately, I was not able to identify more functionally relevant landscape features. However, this approach demonstrates the importance of spatial heterogeneity for bobcats in western Texas. Wildlife managers should be cautious when considering direct environmental gradients, as indirect variables may be silently impacting animal movements and microevolutionary processes.

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Table II.1. Descriptions of abbreviations used in the environmental space of the local scale CCA. The proportions and densities of each described environmental variable was used in the CCA.

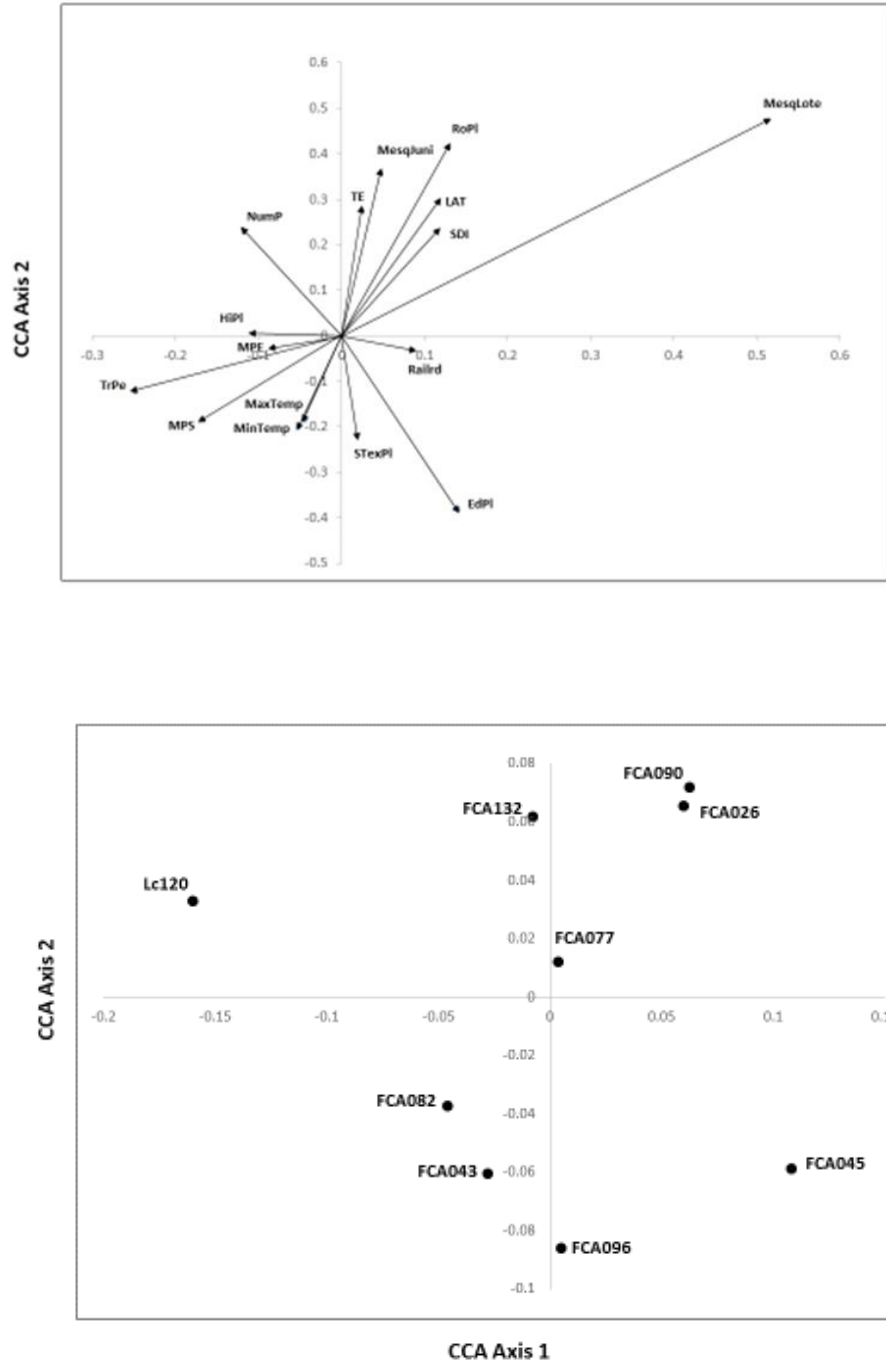| CCA Environmental Variables	Description
EdPl	Edward's Plateau ecoregion
HiPl	High Plains ecoregion
RoPl	Rolling Plains ecoregion
TrPl	Trans-Pecos ecoregion
STexPl	South Texas Plains ecoregion
LAT	Latitude
MPE	Mean patch edge
MPS	Mean patch size
NumP	Number of patches
SDI	Shannon's diversity index of patch size
TE	Total edge
MesqJuni	Mesquite juniper
MesqLote	Mesquite lotebush
MaxTemp	Maximum temperature
MinTemp	Minimum temperature
Railrd	Railroad density

Table II.2. Descriptions used in the environmental space of the regional scale CCA. The proportions and densities of each described environmental variable was used in the CCA.
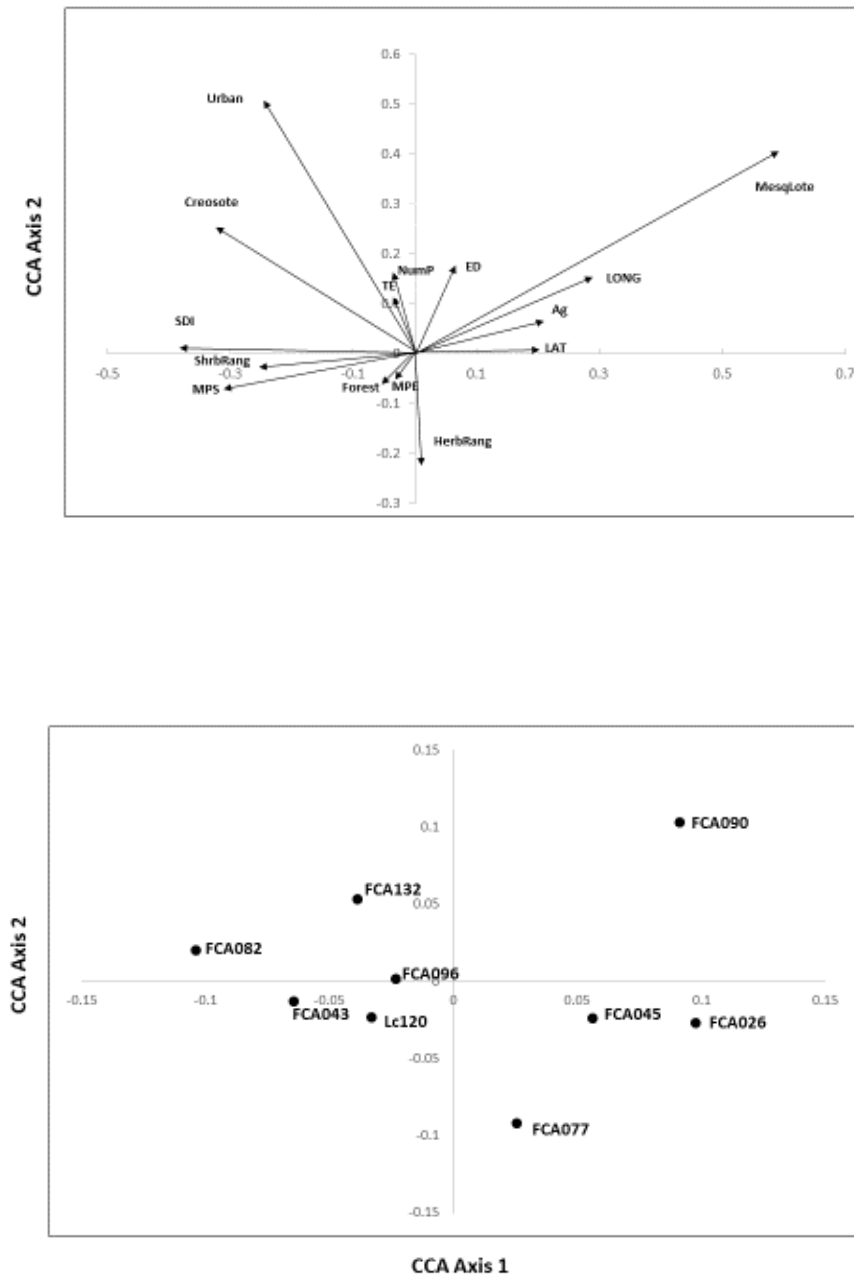
CCA Environmental Variables	Description
ED	Edge density
LAT	Latitude
LONG	Longitude
MPE	Mean patch edge
MPS	Mean patch size
NumP	Number of patches
SDI	Shannon's diversity index of patch size
TE	Total edge
Ag	Level 1 Agriculture LULC
Forest	Level 1 Forest LULC
HerbRang	Herbaceous Rangeland
ShrbRang	Shrub Rangeland
Urban	Level 1 Urban LULC
Creosote	Creosote
MesqLote	Mesquite Lotebush
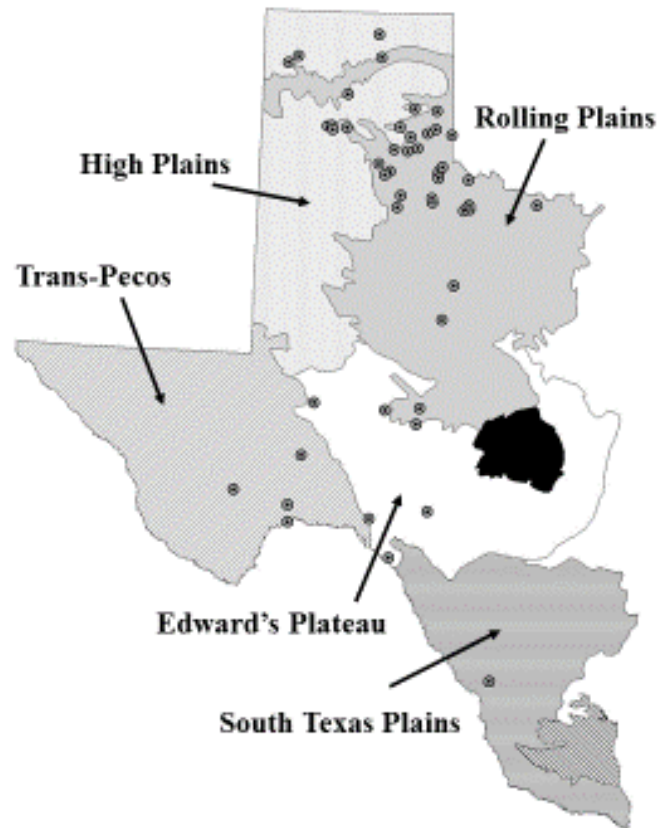
Figure II.1 GPS locations of 64 bobcat samples across five ecoregions in western Texas. Bobcat samples are denoted by gray circles.

Figure II.2. Distribution of environmental variables (top) and species scores (bottom) derived from the local scale CCA. For environmental variables, arrows are biplot arrows for continuous variables (see Table 1). For species score, black circles denote heterozygosity of each locus.

Figure II.3. Distribution of environmental variables (top) and species scores (bottom) derived from the local scale CCA. For environmental variables, arrows are biplot arrows for continuous variables (see Table 2). For species score, black circles denote heterozygosity of each locus.

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