EXPLORING MOUNTAIN LION ECOLOGY IN TEXAS USING GENETIC TECHNIQUES

A Thesis

by

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ABSTRACT

EXPLORING MOUNTAIN LION ECOLOGY IN TEXAS USING GENETIC TECHNIQUES

(August 2011)

Joseph Dale Holbrook, B.S., University of Idaho

Co-Chairman of Advisory Committee: Dr. Randall DeYoung

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Large, territorial, and highly mobile carnivores such as mountain lions (*Puma concolor*) are difficult to study. I used genetic tools to address recent population characteristics and temporal changes in Texas mountain lions. My recent sample consisted of 245 individuals sampled from New Mexico, western Texas, and southern Texas during 1985–2010. My historical sample consisted of 69 museum specimens collected from western Texas during 1935–1989, and 34 specimens from southern Texas collected during 1934–1942. My contemporary results indicated that mountain lions in New Mexico (H_E = 0.61) and western Texas (H_E = 0.58) displayed moderate levels of genetic diversity, whereas estimates for southern Texas were lower (H_E = 0.47). These regions also exhibited moderate–high levels of genetic differentiation (New Mexico-western Texas F_{ST} = 0.06, New Mexico–southern Texas F_{ST} = 0.15, western Texas-southern Texas F_{ST} = 0.10). However, I identified long-distance movement across my sampling area. These findings indicate a metapopulation structure, and suggest western and southern Texas represent 2 management units. Populations in New Mexico and western Texas may be important for mountain lion recolonization in the southern U.S.

Comparisons including historical samples revealed a ≈10-20% decline in genetic diversity for southern Texas over time, while diversity in western Texas has remained stable. Genetic differentiation between western and southern Texas has increased 2.5 times, which is likely due to the temporal changes that have occurred within southern Texas (temporal $F_{ST} = 0.13$) rather than western Texas (temporal $F_{ST} = 0.02$). Effective size estimates indicated a lower historical population size in southern Texas relative to western Texas, and that southern Texas has declined > 50% over time. Effective size in western Texas has remained large and stable. My findings show substantial temporal declines and changes have occurred in southern Texas. Future research exploring reproduction and survival in southern Texas is essential. Management actions such as monitoring and harvest reduction may be needed to ensure the persistence of mountain lions in Texas. Overall, this study emphasizes the importance and utility of applying genetic tools to assist wildlife management and conservation.

DEDICATION

This thesis is dedicated to my parents (Chris and Dale), sister (Nicole), and dog (Chief). I would not be here today without their unmatched love, support, and encouragement. I am sincerely blessed to call them family! I also dedicate this work to my Grandpa (Marvin), a true woodsman. His love of the outdoors and wildlife has carried over 2 generations, and has largely influenced my career path in wildlife science. Thank you Grandpa, I love and miss you!

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First, I thank the Lord for giving me the strength, persistence, and ability to complete my Master's degree. Next, I sincerely thank my advisory committee Drs Randy DeYoung, Mike Tewes, and John Young for their considerable support throughout the project. My major professors, Randy DeYoung and Mike Tewes, deserve special thanks for providing valuable insight, opportunities, criticism, time and effort, and friendship during my stay at Kingsville. I very much enjoyed working under their tutelage and appreciated the flexibility they offered to explore my ideas.

My fellow graduate students and colleagues deserve many thanks for their support, as well as providing an environment that made the transition much easier for us out-of-staters. Special thanks go to the cat coalition for stimulating conversations regarding felid and carnivore ecology - thank you Arturo Caso, Lon Grassman, Chad Stasey, Jen Korn, and Sasha Carvajal. I also thank the genetics crew for 1) keeping the lab fun and lively around the clock, 2) their support and criticism, and 3) many discussions regarding the application of genetic tools to ecological questions. I particularly acknowledge Kelly Corman, Amber Dunn, Carlos Lopez, Karla Logan, Damon Williford, Renee Keleher, Aaron Foley, and Johanna Delgado Acevedo. Furthermore, Dr. Fred Bryant and the Caesar Kleberg Wildlife Research Institute staff deserve large thanks for their valuable and much appreciated support. Yolanda Ballard, Jere Sepulveda, DeAnna Greer, Chris Reopelle, Sara Barrera, and Becky Trant offered essential insight that helped me successfully complete my degree.

Finally, I thank the collaborators and funding sources that were imperative to the success of this project. Thanks to Bill Applegate, Louis Harveson, The Museum of

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CHAPTER I

MOUNTAIN LIONS IN THE UNITED STATES AND TEXAS

OVERVIEW

The mountain lion (*Puma concolor*) is a large, cryptic carnivore that occupies a variety of habitats from northern Canada to the southern extent of South America (Hornocker 1970, Hall 1981, Nowak 1991, Culver et al. 2000). Mountain lions are mostly solitary (Hornocker 1969, 1970, Seidensticker et al.1973), exhibit a polygynous mating system (Murphy 1998, Logan and Sweanor 2001), and occupy large territories (10s–100s of km^2; Seidensticker et al.1973, Ross and Jalkotzy 1992, Pierce et al. 1999, Logan and Sweanor 2001). These large predators are highly mobile (Beier 1995, Ruth et al. 1998), as revealed by dispersal distances up to 1,067 km (Thompson and Jenks 2005). The behavioral and ecological characteristics of mountain lions have facilitated contradictory social perceptions among humans. Many idealize mountain lions for their charismatic qualities, whereas others are fearful and view them as direct competitors for resources. Indeed, mountain lion-human conflicts have persisted for many years (Wade et al. 1984).

Mountain lions have experienced persecution for centuries throughout much of their range in the contiguous U.S. through recreational and bounty programs (i.e., trapping and hunting). The primary goal of these removal efforts was to minimize depredation of livestock and big-game species (Doughty 1983, Wade et al. 1984, Logan and Sweanor 2001). However, by the 1960s and 1970s most western U.S. states regulated the harvest of mountain lions classifying them as game animals (Beausoleil et

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Chapter 1 of this thesis follows the format of the *Journal of Wildlife Management*.

al. 2008). This classification followed accomplishments in mountain lion research identifying the biological roles they fulfill in ecosystems (Noss et al. 1996). The social acceptance regarding unlimited take of a charismatic predator was also rapidly declining by the 1970s (Russ 1996). The regulation of mountain lion harvest has contributed to population stability throughout much of the western U.S. (e.g., Logan and Sweanor 2001), and created opportunity for re-expansion into their historical range.

However, mountain lion populations in Texas have sustained a restricted distribution due to predator removal programs, bounties, and loss of habitat throughout the 19th and early 20th century (Doughty 1983, Wade et al. 1984). Currently, Texas is the only state in the U.S. where mountain lions are not classified as a game species, allowing unregulated take with no mandatory inspection (Doughty 1983, Wade et al. 1984, Russ 1996). Young (2008) summarized harvest data spanning 1919–2006 from the U.S. Department of Agriculture, Texas Division of Wildlife Services, which indicated a mean of ~21 individuals taken per year and 2 substantial peaks in harvest during 1930–1938 and 1979–2004.

The unlimited harvest of mountain lions in Texas has led many to question the viability of populations in the state. In response, the Texas Parks and Wildlife Department (TPWD) began recording sightings and voluntary mortality reports from various sources in 1982. The goal of this effort was to document relative changes in mountain lion populations as well as distributional shifts in Texas. Recent (1980s–2000s) mortality reports suggest that mountain lion populations are expanding (Figure 1.1; Sullins 2002). However, since 1970 (officially classified as non-game) there

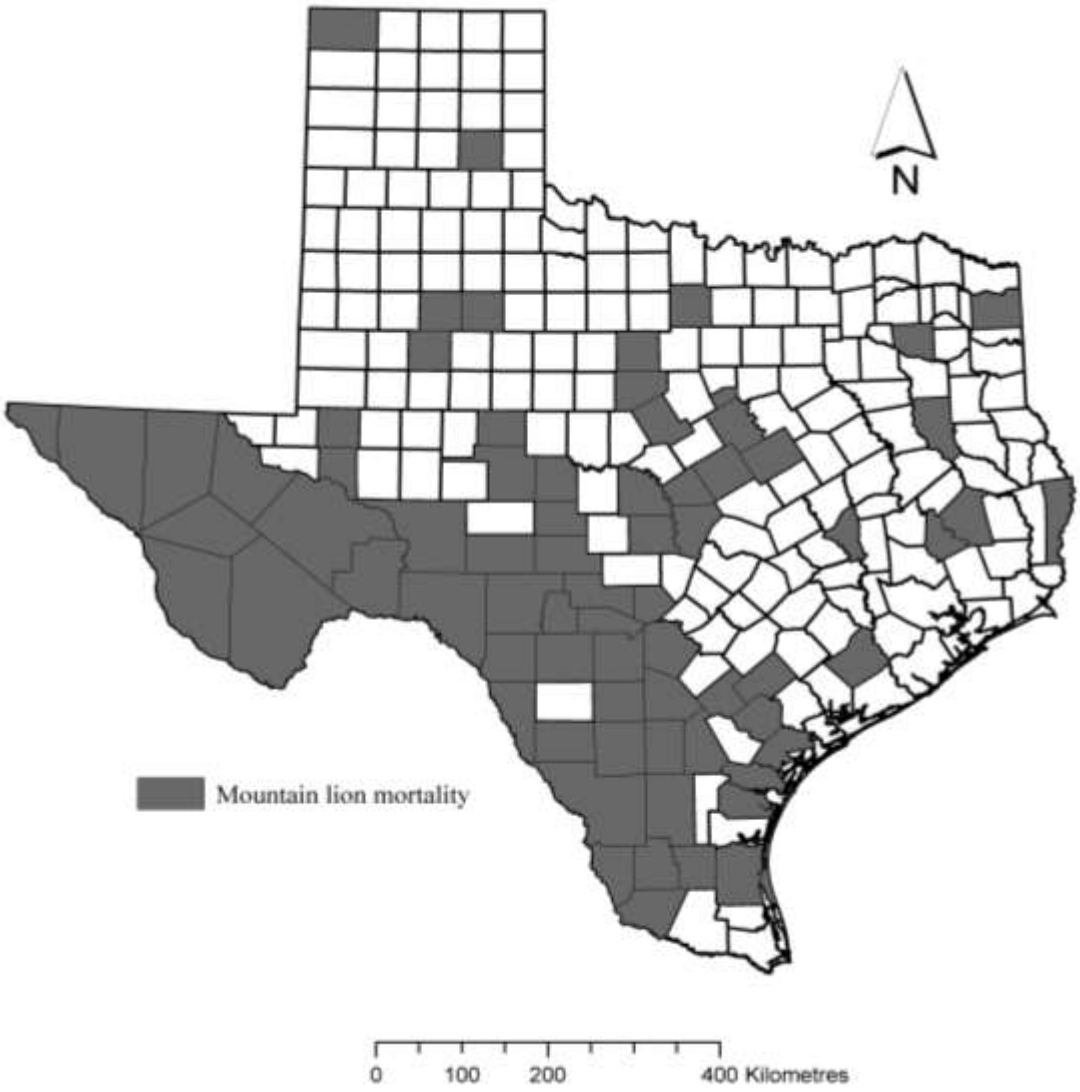

Figure 1.1. The distribution of mountain lion mortalities reported in Texas, USA, during 1983–2005. Reports were documented by the Texas Parks and Wildlife Department.

has been no change in the status of mountain lions or harvest regulations and concerns of over-harvest remain (Russ 1996). TPWD has sponsored several studies since 1982 to better evaluate the status of mountain lions in Texas.

ECOLOGY AND DEMOGRAPHICS IN TEXAS

Mountain lions in Texas primarily select large prey species, such as mule deer (*Odocoileus hemionus*) and white-tailed deer (*Odocoileus virginianus*), and supplement their diet with a variety of other species (e.g., collared peccary [*Tayassu tajacu*], livestock, porcupine [*Erethizon dorsatum*], nine-banded armadillo [*Dasypus novemcinctus*], striped skunk [*Mephitis mephitis*]; McBride 1976, Leopold and Krausman 1986, Smith et al. 1986, Waid 1990). The age structure of mountain lions in Texas was estimated from radio-telemetry studies, and is indicative of an exploited population where a high proportion of individuals are in young (e.g., 24–48 months) age classes (McBride 1976, Smith et al. 1986, McBride and Ruth 1988, Waid 1990, Harveson et al. 1996). Harveson et al. (1996) extrapolated density and home range estimates from previous studies assuming an effective area size 1.5 times greater than the documented study area; estimates ranged from 1.39–15 mountain lions/1,000 km^2. Home range estimates ranged from 59 km^2–1,032 km^2 for females (Andersen 1983, Smith et al. 1986), and 207 km^2–1,032 km^2 for males (Andersen 1983, Smith et al. 1986).

Relatively recent mountain lion research has continued to address fundamental ecological questions as well as explore the genetic properties of the southern and western Texas populations. Harveson (1997) evaluated the ecology of a mountain lion population in southern Texas and found that mean annual ranges were smaller for females (131.76 km^2) than for males (503.48 km^2), and annual male-male and female-male range overlap

was extensive. Riparian habitats were preferred, while chaparral-dominant habitats were avoided or used in proportion to available by both sexes. Subadult dispersal distances for males (n = 4) ranged from 11.0–95.5 km and 6.3–23.1 km for females (n = 6; Harveson 1997). Mountain lions in southern Texas preferred preying on white-tailed deer, exhibited a density of 0.59–0.74 individuals/100 km^2, and survival of 0.81 (males) and 0.59 (females), respectively (Harveson 1997). Conclusions of this research suggested high mortality coupled with low productivity of females may limit mountain lion populations in southern Texas (Harveson 1997).

In another recent ecological study of mountain lions, Pittman et al. (2000) examined a population occurring in western Texas. Male mountain lions exhibited larger home ranges (348.6 km^2) than that of females (205.9 km^2), and ~25% home range overlap was documented among and between sexes (Pittman et al. 2000). Similar to previous studies, fecal analyses indicated that mule deer and collared peccary were preferred prey. Pittman et al. (2000) identified mountain lion density ranging from 0.26–0.59 individuals/100 km^2, and suggested that populations were limited by high male and female mortality rates.

Lastly, 2 genetic studies were conducted in Texas addressing population genetic structure of mountain lions (Walker et al. 2000, J. E. Janecka, Texas A&M University-Kingsville, unpublished data). Walker et al. (2000) identified low levels of heterozygosity within southern Texas (southern TX = 0.294) relative to the mountain lion mean in North America (mean = 0.42, SE = 0.16; Culver et al. 2000). Populations in southern and western Texas also exhibited reduced gene flow (Walker et al. 2000). More localities were sampled in the most recent mountain lion study, however, results

generally supported Walker et al. (2000) conclusions; a genetic subdivision between southern and western Texas and low levels of variation in southern Texas (J. E. Janecka, Texas A&M University-Kingsville, unpublished data).

Previous research on mountain lions in Texas has provided an important foundation of knowledge. However, essentially all studies have suffered from small sample sizes, limited geographic extent, and short duration leaving many questions unexplored. I employed genetic methods using harvested individuals (recently and historically) to expand the knowledge of mountain lions in Texas, both spatially and temporally. I addressed questions related to population continuity, genetic diversity, and movements of mountain lions in Texas and New Mexico (Chapter II), and evaluated temporal changes of mountain lion populations in Texas (Chapter III).

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CHAPTER II

GENETIC DIVERSITY, POPULATION GENETIC STRUCTURE, AND MOVEMENTS OF MOUNTAIN LIONS (*PUMA CONCOLOR*) IN TEXAS

Delineating population boundaries and identifying long-distance movements are important for successful wildlife conservation and management. I used genetic tools to investigate genetic diversity, population structure, and long-distance movements of mountain lions (*Puma concolor*) in Texas. I amplified 11 microsatellite loci for 245 individuals sampled from Texas and New Mexico during 1985–2010. Analyses indicated New Mexico and western Texas exhibited moderate levels of genetic diversity (H_E = 0.61 and 0.58), whereas diversity in southern Texas was lower (H_E = 0.47). Bayesian clustering and F_{ST} suggested my sample is comprised of 3 genetically differentiated groups; New Mexico, western Texas, and southern Texas. Levels of differentiation associated with southern Texas were high (F_{ST} = 0.10–0.15), while differentiation between New Mexico and western Texas was moderate (F_{ST} = 0.06). I documented long-distance movement among these groups, as well as dispersal eastward from New Mexico and western Texas. Results suggest populations in New Mexico and Texas exhibit a metapopulation structure, and that western and southern Texas should be treated as 2 management units. Southern Texas displayed characteristics of a fragmented population, and further investigation is warranted to examine the current population status. Dispersal results indicate mountain lion populations in New Mexico and western Texas may be important for future recolonization in the southern U.S.

Chapter 2 of this thesis follows the format of the *Journal of Mammalogy*.

Key words: Bayesian clustering, genetic diversity, genetic structure, long-distance movement, mountain lion, *Puma concolor*, Texas

INTRODUCTION

The distribution of mountain lions (*Puma concolor*) in North America has declined over the last 200 years due to habitat loss and persecution (Anderson et al. 2010; Logan and Sweanor 2001). However, many populations are currently expanding (Pierce and Bleich 2003). In the United States (U.S.) mountain lions are increasingly being observed far from known populations (Beier 2010; Thompson and Jenks 2010, 2005). As populations expand in some areas, but remain tenuous in others, knowledge of movements and dispersal is important for mountain lion conservation.

At the regional level, mountain lions in Texas are on the periphery of the range in the U.S. Populations were historically distributed throughout the state, but over time have declined in census size and geographic distribution. Today, breeding populations are known to persist only in western and southern Texas (Schmidly 2004).

Harvest and habitat loss are likely responsible for reducing population sizes and distribution in Texas. The livestock industry was ubiquitous in Texas during the late 1800s–mid 1900s, and as a result predator removal efforts were extensive (Lehmann 1969). Removal reduced mountain lion populations in western Texas and along the Rio Grande River (Lehmann 1969; Wade et al. 1984). Mountain lion habitat has also been reduced and fragmented due to agriculture, urbanization, and energy developments. Furthermore, mountain lions in Texas have been designated as a nongame species from 1970–present, which has allowed unlimited take (Harveson et al. 1996; Russ 1996). It is

probable that excessive harvest has negatively influenced census size and geographic distribution.

Only a few studies have been conducted on mountain lions in Texas. Results suggest that populations in both western and southern Texas are limited by low survival (Harveson 1997; Young et al. 2010) and reproductive rates (Harveson 1997; Pittman et al. 2000). Individuals in southern Texas also exhibit lower levels of genetic diversity, and appear to be isolated from western Texas (Walker et al. 2000). However, additional data are needed to examine mountain lion ecology in Texas because all previous studies were limited by small sample sizes.

Mountain lions are financially and logistically difficult to survey, particularly when using traditional methods (e.g., marking). For example, they generally occur at low densities, exhibit large home ranges and elusive behavior, inhabit rough terrain, and display cryptic coloration (Logan and Sweanor 2001). Furthermore, under the nongame designation in Texas hunters and trappers are not required to report mountain lion harvest. This prevents managers from using harvest as a demographic index to monitor population trends (e.g., Anderson and Lindzey 2005). Alternatives tools such as genetic methods are useful to circumvent the challenges with studying mountain lions, and can inform questions related to wildlife management (DeYoung and Honeycutt 2005).

Genetic data are increasingly being used in carnivore research (e.g., Haag et al. 2010; Spong et al. 2000). For highly mobile species such as mountain lions, genetic data have been used to delineate population boundaries and management units (e.g., Anderson et al. 2004; Ernest et al. 2003; McRae et al. 2005). Managers are interested in characterizing units because demographic goals or objectives can then be formulated.

Additionally, genetic data have been used to identify long-distance movement and populations of origin (Frantz et al. 2006; Wasser et al. 2008), which can inform interpopulation connectivity and prioritize habitats or populations important for conservation (Beier 2010; LaRue and Nielsen 2008). Assigning origins could also aid mediation of mountain lion-human conflicts by revealing areas with higher probabilities of interactions (Thompson and Jenks 2010). In the U.S. predicting conflicts is a priority as mountain lions are increasingly being observed in more human dominated landscapes (Beier 2010).

Historical persecution, habitat loss, and unregulated harvest have provoked questions regarding the viability of mountain lion populations in Texas (Russ 1996). The overall goal of this study was to examine genetic characteristics and dispersal within Texas and adjacent populations. I sampled mountain lions from New Mexico for comparison and to assess dispersal in the greater region. My objectives were to 1) estimate genetic diversity, 2) characterize population genetic structure, and 3) assign origin to long-distance dispersers. Information from my study will expand on previous work in Texas, inform the current status of mountain lions in Texas, and perhaps identify populations that are providing dispersers eastward.

MATERIALS AND METHODS

Study area.—I conducted this study throughout New Mexico, western Texas, and southern Texas (Fig. 2.1), but my main focus was on Texas. Western Texas is primarily a desert environment dominated by shrubs, cacti (*Cactus spp.*), and grasses with a few isolated mountain ranges where trees such as oak (*Quercus spp.*), juniper

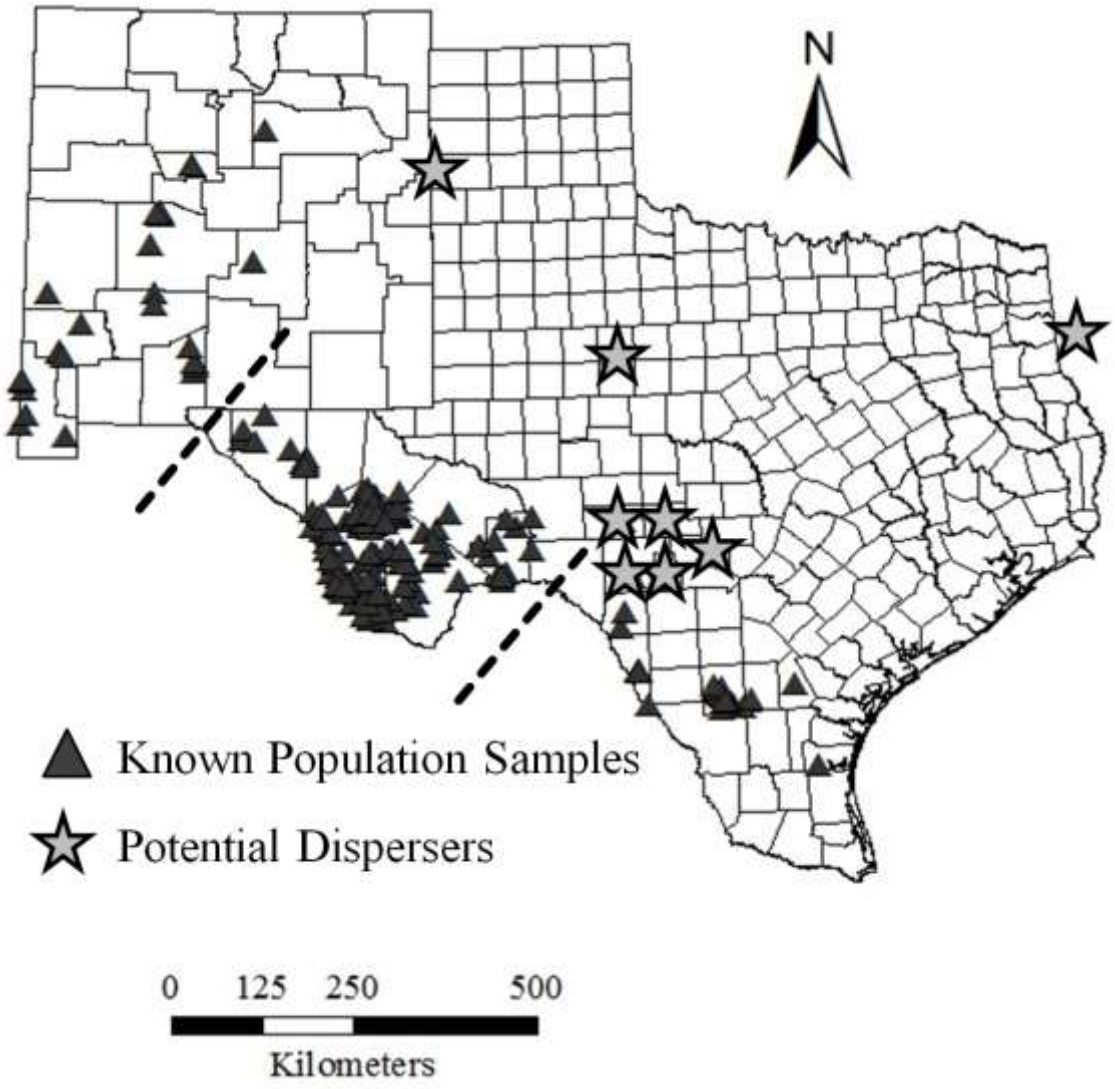

FIG. 2.1—Mountain lion sampling distribution (*n* = 245) throughout Texas and New Mexico during 1985–2010. Triangles represent individuals sampled from known populations (*n* = 237) and stars indicate potential dispersers sampled east of known populations (*n* = 8); 1 each from Kerr, Fisher, Deaf Smith, Edwards, Kimble, Sutton, and Real county, Texas, and Bossier City, Louisiana. I grouped individuals based on spatial proximity and ecoregion for analyses; New Mexico (*n* = 31), western Texas (*n* = 178), and southern Texas (*n* = 28).

(*Juniperus spp.*), and pine (*Pinus spp.*) are abundant (Bailey 1980). Southern Texas is characterized as an arid environment exhibiting low elevations, mild topography, and dense brush interspersed with grasses and trees [e.g., honey mesquite (*Prosopis glandulosa*)].

Sample collection and DNA analysis.—I obtained mountain lion tissue samples from Texas and New Mexico during 1985–2010. Samples from Texas were donated by hunters and trappers, sampled from road-kills, or collected when marking individuals during previous research. New Mexico samples were provided by the Museum of Southwestern Biology, Division of Genomic Resources (MSB #58960–58963, 92685, 142863, 142867–142871, 142873, 142878, 142882, 142884–142887, 142890–142891, 142893, 142896, 142901–142902, 142909–142911, 142913, 142923, 142928, 145874, and 157080). Tissue was frozen, dried, or placed in lysis buffer (Longmire et al. 1997) until DNA extraction.

I extracted DNA from all tissue samples using a commercial kit (Qiagen DNeasy tissue kit, Valencia, California). I used the polymerase chain (PCR) reaction to amplify 11 microsatellite loci (FCA008, FCA035, FCA043, FCA077, FCA082, FCA090, FCA096, FCA132, FCA133, FCA176, FCA205) described by Menotti-Raymond et al. (1999). I amplified all loci individually in 10 µL reaction volumes, which contained 5 µL AmpliTaq Gold® PCR Master Mix (Applied Biosystems, Foster City, California), 0.24 µM of each primer, and 10–50 ng of DNA. I used a touchdown PCR profile with thermal conditions consisting of an initial denaturation at 94°C for 10 min, 20 cycles of 94°C for 30 s, 62°C for 30 s, 61°C for 30 s, 60°C for 30 s, and 72°C for 60 s, followed by 30 cycles of 94°C for 30 s, 55°C for 90 s, and 72°C for 60 s, with a final extension of

60°C for 10 min. After PCR, I mixed 3 µL of PCR product for each individual. I then applied 1.5–2 µL of the PCR product mix to a separate denaturing formamide and size standard mixture (Hi-Di Formamide, GeneScan ROX 500; Applied Biosystems). I loaded the resulting mixtures onto an ABI 3130*xl* DNA analyzer (Applied Biosystems) for fragment separation. I visually inspected each microsatellite locus and sized fragments using GeneMapper® version 4.0 (Applied Biosystems). All runs on the DNA analyzer had a positive and negative PCR control. I re-ran 10% of individuals to calculate a genotyping error rate.

Genetic diversity and Hardy-Weinberg equilibrium.—I computed genetic diversity overall, and for the 3 regions (Fig. 2.1). I estimated mean (over 11 loci) observed heterozygosity (H_O), expected heterzygosity (H_E—Nei 1987), and number of alleles/locus (A) using the computer program ARLEQUIN version 3.5 (Excoffier and Lischer 2010). I estimated mean allelic richness (a_r) using HP-RARE version 1.0 (Kalinowski 2005). I tested Hardy-Weinberg expectations using F_{IS} (Weir and Cockerham 1984), and assessed statistical significance (2-sided) by comparing the observed value against a null value derived from 1,023 permutations of alleles among individuals. I computed and tested F_{IS} using ARLEQUIN version 3.5 (Excoffier and Lischer 2010).

Genetic associations with distance.—I characterized mountain lion genetic associations with geographic (Euclidean) distance using 2 approaches. First, I grouped individuals by county, or combined proximate counties (i.e., rough county) to maintain $n \geq 5$ (New Mexico—Bernalillo–San Miguel, Dona Ana, Grant–Catron, Hidalgo, and Socorro–Sierra–Lincoln; Texas—LaSalle–McMullen–Kleberg–Live Oak, Maverick–

Kinney–Webb, Brewster–Pecos, Culberson–Hudspeth, Jeff Davis–Reeves, Presidio, and Terrell–Val Verde). I then explored the relationship between genetic [$F_{ST}/(1-F_{ST})$—Weir and Cockerham 1984] and Euclidean distance (Rousset 1997). F_{ST} is the proportion of genetic diversity explained by allele frequency differences among groupings (Holsinger and Weir 2009). I used linear regression to test for a relationship between pairwise estimates of $F_{ST}/(1-F_{ST})$ and Euclidean distance. I computed the standard error of the slope by jackknifing over loci.

Second, I implemented spatial autocorrelation to explore the spatial extent of population structure. At the individual level, autocorrelation analyses describe the correlation between average gene frequencies of a pair of individuals (Hardy and Vekemans 1999; Scribner et al. 2005). I used Moran's *I* (Hardy and Vekemans 1999) as the measure of autocorrelation because of its extensive use and robust performance (Epperson 2004). I computed mean Moran's *I* values for all pairs of individuals within 15 Euclidean distance classes. I used 15 classes with an approximately equal number of pairs to ensure large sample sizes and low coefficient of variation within each class (Hardy and Vekemans 2009). I tested the statistical significance (2-sided) of Moran's *I* means for each distance class by comparing observed values to a randomized value computed using 1,000 permutations of individual locations. I calculated the standard error of Moran's *I* by jackknifing over loci. I used the program SPAGeDi version 1.3 (Hardy and Vekemans 2002) to perform regression and spatial autocorrelation analyses.

Genetic structure.—To further evaluate population genetic structure I implemented traditional genetic differentiation methods as well as Bayesian clustering. I used the county groupings (mentioned above) and 3 regions to compute pairwise and

overall F_{ST} (Weir and Cockerham 1984) using the computer program ARLEQUIN version 3.5 (Excoffier and Lischer 2010). I tested statistical significance (2-sided) by comparing the observed value to a null value derived from 1,023 permutations of genotypes among groups (i.e., counties or regions).

Next, I applied 2 Bayesian clustering algorithms that incorporate spatial locations. I employed the algorithm implemented in GENELAND (Guillot et al. 2005a, 2005b) version 3.2.4 using program R version 2.11.1 (R Development Core Team 2011). This model uses a Markov chain Monte Carlo (MCMC) approach to infer genetic discontinuities among geo-referenced genotypes. I evaluated 1–8 possible genetic clusters (*K*) with 8 independent runs for each *K*. I implemented the spatial model with 10 km of uncertainty to account for inexact sample coordinates. I assumed allele frequencies to be correlated and used 100,000 MCMC iterations while recording 1,000 (thinning = 100). I selected *K* using the mode of the maximized posterior probability. Additionally, I applied the Bayesian algorithm described by Corander et al. (2003) using BAPS version 5. This approach uses stochastic optimization to infer the posterior mode of genetic structure in the data. I implemented the spatial clustering of individuals (Corander et al. 2008) and explored $K = 1–8$ with 8 independent runs. I selected *K* based on the partitioning of individuals that maximized the log marginal likelihood. The optimal partition of individuals in GENELAND and BAPS should minimize Hardy-Weinberg and linkage disequilibrium within clusters.

Lastly, I employed a nonspatial Bayesian clustering algorithm using the computer program STRUCTURE version 2.2. This model uses a MCMC to infer genetic clusters and assign individuals to ancestral populations while minimizing Hardy-Weinberg and

linkage disequilibrium within clusters (Prichard et al. 2000). The algorithm also estimates ancestry proportions (*q*-values) to each genetic cluster for each individual. I selected the admixture model and assumed allele frequencies were correlated (Faulsh et al. 2003). I performed 100,000 MCMC burn-in repetitions to reduce initial configuration effects, followed by 500,000 MCMC repetitions of data collection. I explored 1–8 genetic clusters, with 8 independent runs to evaluate consistency. I calculated the arithmetic mean and standard deviation of the log probability of the data [Ln P(D)] across runs for each *K* to identify the plateau and determine the optimal number of clusters (Prichard et al. 2007). I also calculated the ΔK statistic (Evanno et al. 2005) and used *q*-values as an index (Prichard et al. 2007) to inform the selection of *K*.

Assigning origin to dispersers.—I determined origin for dispersers among known populations and for potential migrants sampled outside of known populations using Bayesian clustering and assignment tests. I used mean *q*-values (over 8 runs) from the STRUCTURE analyses mentioned above and geographic locations to identify long-distance dispersal among known populations. I defined long-distance as movement from western Texas to southern Texas or vice versa, and southern Texas to New Mexico or vice versa (i.e., $\geq$ 200 km). Similar to previous studies (e.g., Latch et al. 2006, 2008), I considered individuals residents of a cluster if $q > 0.75$ and admixed if q was 0.25–0.75.

Next, I used 3 Bayesian assignment methods to assign origin to the potential dispersers sampled east of known populations. Here, I used assignment methods because I had determined which known populations exhibited genetic differentiation, and I could use that information to define reference populations. In other words, assignment methods are a more explicit way to discern genetic origins when reference populations are known

a priori. In all analyses I considered the 3 regions mentioned previously as reference populations, and the potential dispersers as unknowns. First, I used the modified assignment approach of Rannala and Mountain (1997) in GeneClass version 2 (Piry et al. 2004). This approach provides likelihood ratio scores for each unknown individual to each reference population (Piry et al. 2004), and I used scores > 85% to indicate assignment. Second, I employed the assignment methods implemented in STRUCTURE version 2.2 (Falush et al. 2003) and BAPS version 5 (Corander et al. 2003). For both analyses I assumed that $K = 3$, corresponding to the regional reference populations. I employed the USEPOPINFO option in STRUCTURE (Faulsh et al. 2003), and executed 100,000 MCMC burn-in and 500,000 data collecting repetitions. I assumed allele frequencies were correlated, no admixture, and updated frequencies with only reference individuals. Because results of this analysis can be sensitive to the *a priori* assigned migration rate (MIGPRIOR), I analyzed the data using a range of values (i.e., 0.001–0.10) as suggested by Prichard et al. (2007). The choice of MIGPRIOR did not substantially influence results, thus I only present results using MIGPRIOR = 0.05 (default value). Because I incorporated prior population information (i.e., USEPOPINFO) and assumed no admixture more certainty is associated with assignments compared to the admixture analysis mentioned above. Thus, I used a more stringent *q*-value ($q > 0.85$) to indicate genetic assignment (Frantz et al. 2006). Finally, I employed the trained clustering methodology (Corander et al. 2006, 2008b) in BAPS (Corander et al. 2003). I explored the assignment of each unknown individual to regional reference populations, one-by-one. To evaluate the strength of assignment to each cluster I multiplied by 2 the absolute value of change in the log marginal likelihood (i.e., Bayes

factor—Corander et al. 2009) of individual *i* being assigned to the alternative cluster *j*. Values of 0 indicate the assigned reference population, and movement to another population with a change ≥ 6 suggests substantial support for assignment (Kass and Raftery 1995). A change ≤ 2 indicates poor assignment support.

RESULTS

Genetic data.—I successfully genotyped 245 mountain lions (57% males, 39% females, and 4% had no sex information) at 11 microsatellite loci (Fig. 2.1). Of the total, 237 genotypes were sampled from known populations in Texas and New Mexico, and 8 were from presumed long-distance dispersers; 1 each from Kerr, Fisher, Deaf Smith, Edwards, Kimble, Sutton, and Real County, Texas, and Bossier City, Louisiana. Positive and negative PCR controls were consistent and did not exhibit any contamination. My genotyping error rate was < 1%.

Estimates of mean heterozygosity, number of alleles/locus, and allelic richness were moderate for New Mexico and western Texas as well as the total sample (Table 2.1). However, estimates of genetic diversity for southern Texas were 10%–25% lower than western Texas and New Mexico. Hardy-Weinberg equilibrium tests using F_{IS} indicated that western Texas, southern Texas, and the total sample did not significantly deviate from expectations (Table 2.1). New Mexico, however, exhibited a statistically significant excess of homozygotes.

Genetic associations with distance.—I observed a positive linear relationship (slope = 0.0002, *SE* = 0.00007) between genetic and Euclidean distance (Fig. 2.2), which

TABLE 2.1.—Mean estimates (over 11 loci) of observed (H_O) and expected heterozygosity (H_E—Nei 1987), number of alleles/locus (A), allelic richness (a_r—Kalinowski 2005) and Hardy-Weinberg equilibrium departure (F_{IS}—Weir and Cockerham 1984) for known mountain lion populations in Texas and New Mexico sampled during 1985–2010. Standard deviations (*SD*) are in parentheses, and *n* indicates sample size.

Region	*n*	H_O	H_E	A	a_r	F_{IS}
New Mexico	31	0.57 (0.21)	0.61 (0.22)	4.55 (1.64)	4.43 (1.59)	0.07*
Western Texas	178	0.56 (0.22)	0.58 (0.23)	5.09 (1.81)	4.23 (1.39)	0.02
Southern Texas	28	0.45 (0.25)	0.47 (0.25)	3.91 (1.64)	3.85 (1.60)	0.02
Total	237	0.55 (0.21)	0.59 (0.23)	5.55 (1.92)	5.53 (1.90)	0.02

*Significantly different ($P < 0.05$) than null value derived from 1,023 permutations of alleles among individuals.

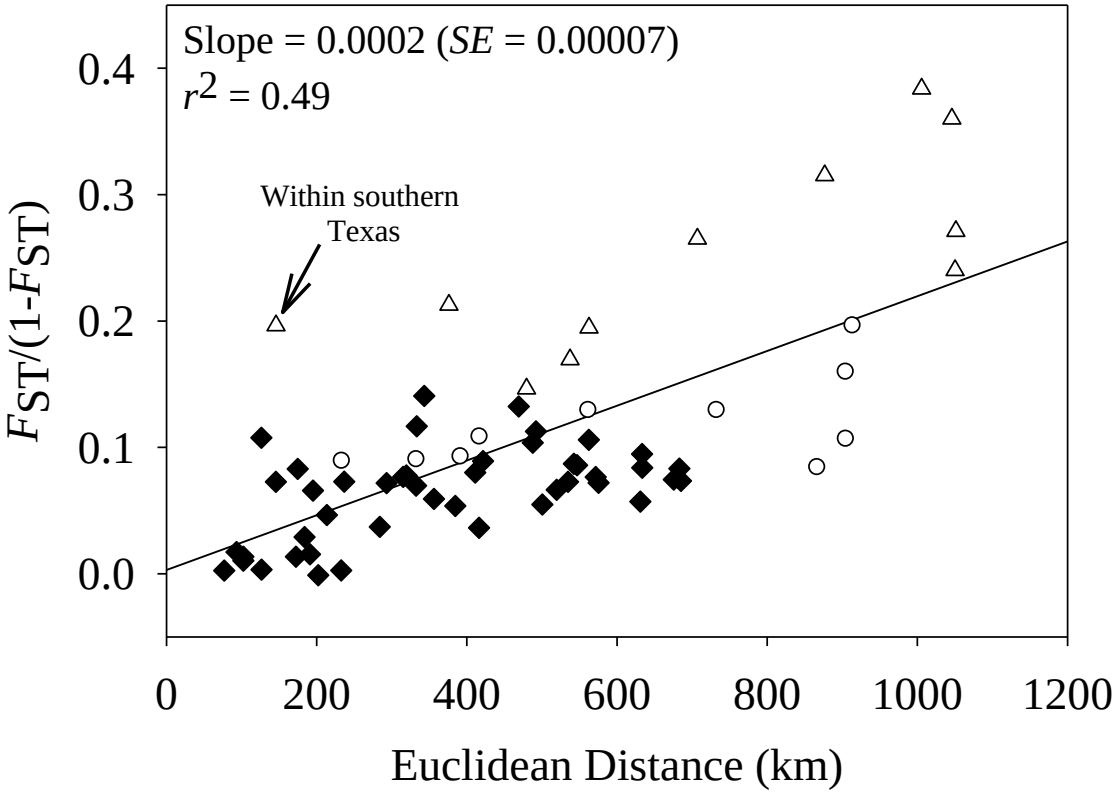

FIG. 2.2—Genetic distance [$F_{ST}/(1-F_{ST})$—Weir and Cockerham 1984] over Euclidean distance (km) for mountain lions sampled from known populations (n = 237) during 1985–2010. Samples were grouped by rough county in Texas and New Mexico while maintaining $n \geq 5$. Dark diamonds represent comparisons within western Texas and New Mexico. Open circles indicate comparisons of the western county grouping in southern Texas (i.e., Maverick–Kinney–Webb) to other regional groups. Open triangles signify comparisons of the southeastern county group in southern Texas (i.e., LaSalle–McMullen–Kleberg–Live Oak) to other regional groups; the comparison within southern Texas is also included (see arrow). Estimates of slope and r^2 were computed using linear regression over all comparisons. The estimate of *SE* was derived by jackknifing over loci.

indicated a significant pattern of isolation-by-distance (IBD). Euclidian distance accounted for approximately half of the variation in genetic distance ($r^2 = 0.49$). Within the southern Texas region, most pairwise comparisons involving the western counties (Maverick–Kinney–Webb) followed the predicted IBD pattern. However, all comparisons including the eastern counties (LaSalle–McMullen–Kleberg–Live Oak) were greater than the predicted relationship. This indicated that the observed IBD cline was a poor predictor of genetic distance for the eastern county grouping in southern Texas.

Spatial autocorrelation analyses (Fig. 2.3) indicated a positive statistical difference between observed and permuted values for the first 9 distance classes (~20–250 km), except class 5 (~105 km). Moran's *I* values in the first (~20 km) and second (~40 km) distance class were 2 times greater and equal to second-cousins expectations, respectively. These higher values indicated high levels of genetic association among proximate individuals. I observed negative autocorrelation between distance classes 10–15 (~370–820 km), substantiating the presence of an IBD pattern. Together, linear regression and spatial autocorrelation provided evidence for an IBD cline, regional level genetic structure, and genetic association among individuals at distances < 50 km.

Genetic structure.—I observed significant ($P < 0.05$) overall genetic differentiation among county groupings ($F_{ST} = 0.067$) and the 3 regions ($F_{ST} = 0.080$) indicating moderate levels of genetic structure. The regional division appeared to be more appropriate given F_{ST} was higher, accounting for more genetic variation than the county groupings. Fifty-six of 66 pairwise comparisons among samples grouped by county were statistically > 0.0 (Table 2.2). Estimates of F_{ST} between Texas and New

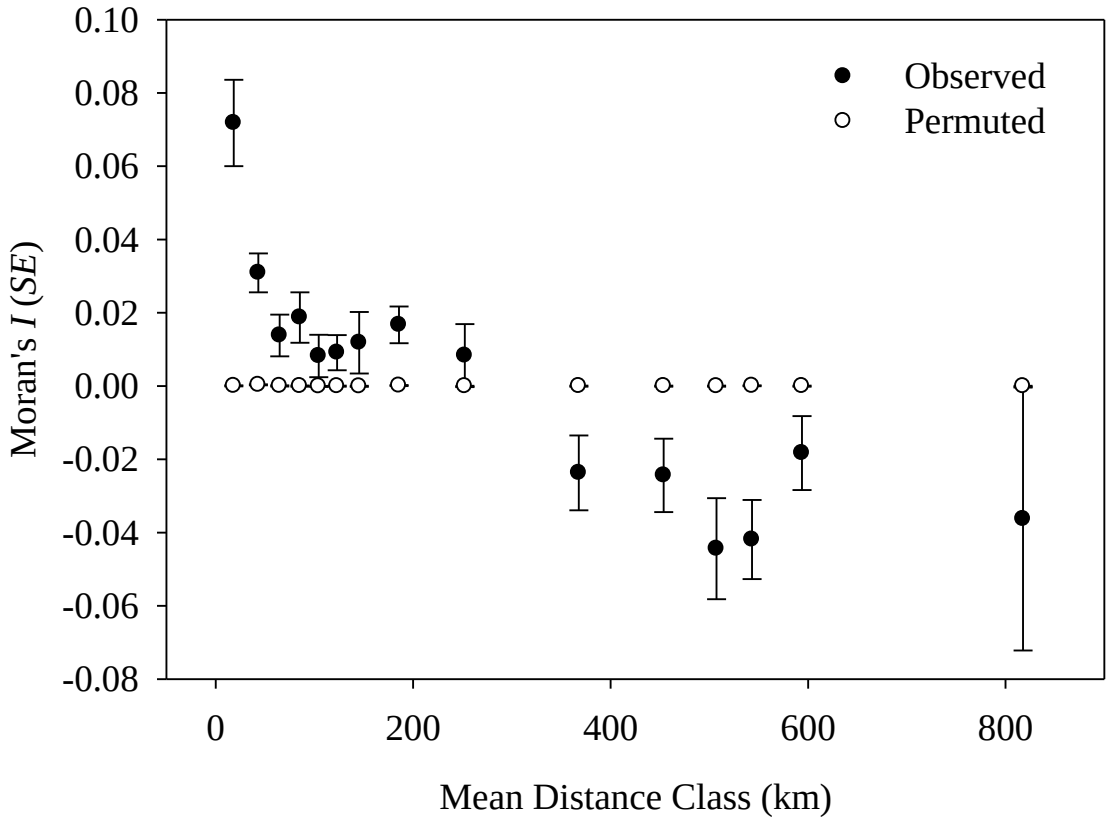

FIG. 2.3—Mean autocorrelation coefficients (Moran's *I*—Hardy and Vekemans 1999) and Euclidean distance (km) among all pairs of individuals using 15 distance classes for known mountain lion populations (n = 237) in Texas and New Mexico sampled during 1985–2010. Dark circles represent observed Moran's *I* values, and light circles represent null values based on 1,023 permutations of individual locations. Error bars indicate ± 1 *SE*, and were computed by jackknifing over loci.

TABLE 2.2.—Pairwise estimates of F_{ST} (Weir and Cockerham 1984) for mountain lions in Texas and New Mexico sampled during 1985–2010. Samples were grouped by county or proximate counties to maintain $n \geq 5$. Black circles and NS represent statistically significant ($P < 0.05$) and not significant estimates, respectively, based on 1,023 permutations of genotypes among groups.

	New Mexico					Texas						
County groupings (sample size)	Bernalillo–San Miguel	Dona Ana	Grant–Catron	Hidalgo	Socorro–Sierra–Lincoln	LaSalle–McMullen–Kleberg–Live Oak	Maverick–Kinney–Webb	Brewster–Pecos	Culberson–Hudspeth	Jeff Davis–Reeves	Presidio	Terrell–Val Verde
Bernalillo–San Miguel (n = 5)	–	0.040	0.074	0.031	0.088	0.267	0.147	0.079	0.077	0.080	0.060	0.068
Dona Ana (n = 5)	NS	–	0.053	0.014	0.016	0.247	0.117	0.080	0.082	0.074	0.057	0.059
Grant–Catron (n = 5)	NS	NS	–	0.023	0.064	0.200	0.097	0.072	0.128	0.096	0.074	0.075
Hidalgo (n = 7)	NS	NS	NS	–	0.065	0.217	0.133	0.074	0.056	0.102	0.066	0.079
Socorro–Sierra–Lincoln (n = 9)	●	NS	●	●	–	0.279	0.069	0.098	0.102	0.118	0.083	0.088
LaSalle–McMullen–Kleberg–Live Oak (n = 21)	●	●	●	●	●	–	0.159	0.124	0.212	0.158	0.144	0.173
Maverick–Kinney–Webb (n = 7)	●	●	●	●	●	●	–	0.078	0.103	0.089	0.083	0.077
Brewster–Pecos (n = 30)	●	●	●	●	●	●	●	–	0.070	0.016	0.002	0.004
Culberson–Hudspeth (n = 11)	●	●	●	●	●	●	●	●	–	0.069	0.049	0.067
Jeff Davis–Reeves (n = 52)	●	●	●	●	●	●	●	●	●	–	0.011	0.015
Presidio (n = 71)	●	●	●	●	●	●	●	NS	●	●	–	0.000
Terrell–Val Verde (n = 14)	●	●	●	●	●	●	●	NS	●	●	NS	–

Mexico were moderate–high (0.056–0.279), generally increasing with Euclidean distance as expected under IBD. All estimates including the LaSalle–McMullen–Kleberg–Live Oak group were notably high (F_{ST} = 0.124–0.279). The 3 pairwise comparisons among regions were statistically positive ($P < 0.05$), and further indicated considerable differentiation associated with southern Texas (New Mexico–southern Texas F_{ST} = 0.15, western Texas–southern Texas F_{ST} = 0.10, and New Mexico–western Texas F_{ST} = 0.06).

Of the 8 runs using GENELAND, the maximized posterior probability of K occurred at 3 for 6 runs, and at 5 for 2 runs. However, the maximized probability for all 6 runs at K = 3 was higher than at K = 5. Therefore, I inferred the optimal number of clusters to be 3 (Fig. 2.4). The clusters of individuals and membership probability suggested by GENELAND corresponded exactly to the 3 regions; New Mexico, western Texas, and southern Texas. Similarly, BAPS results indicated that the log marginal likelihoods for the 10 best visited partitions were maximized at K = 3, providing a posterior probability of 1 for K = 3. The clustering of individuals from BAPS (Fig. 2.5) approximately corresponded to New Mexico, western Texas, and southern Texas corroborating the results from GENELAND.

The STRUCTURE results are less clear than those from GENELAND and BAPS. The mean Ln P(D) appeared to reach a plateau at K = 2 or 3, peaked at K = 4, and declined and became more variable at $K > 4$ (Fig. 2.6). The ΔK statistic of Evanno et al. (2005) provided moderate support for K = 2 and 3, but high support for K = 4 (Fig. 2.6). Ancestry proportions (q-values) for most individuals at K = 2, 3, and 4 maintained high

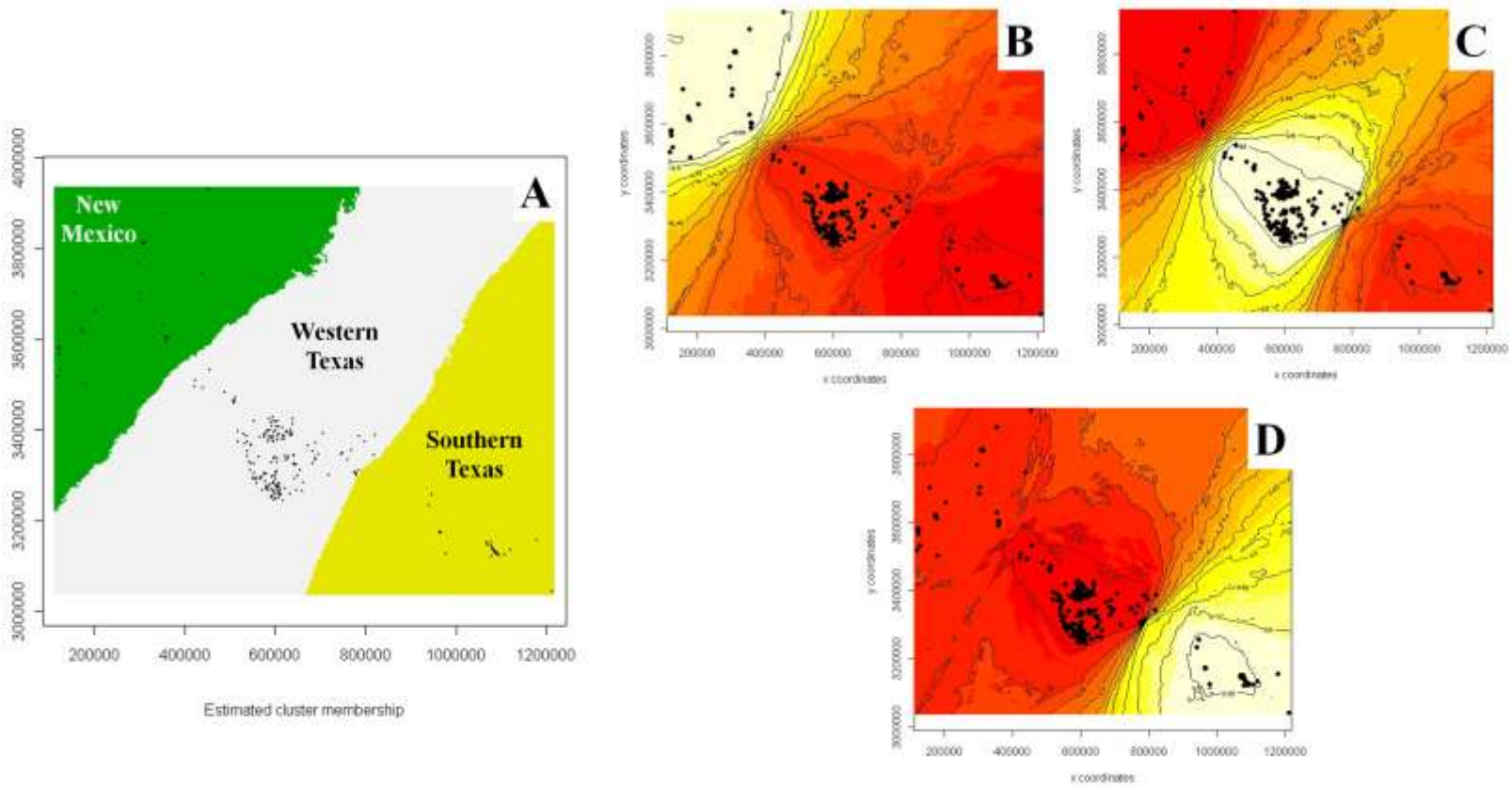

FIG. 2.4—Genetic clustering results from GENELAND for known mountain lion populations (n = 237) in Texas and New Mexico sampled during 1985–2010. A) Map of the estimated genetic membership, which corresponds to the 3 regional groups; New Mexico, western Texas, and southern Texas. B) Probability surface indicating which samples belong to New Mexico, C) western Texas, and D) southern Texas. Dark–light colors indicate low–high probabilities of membership.

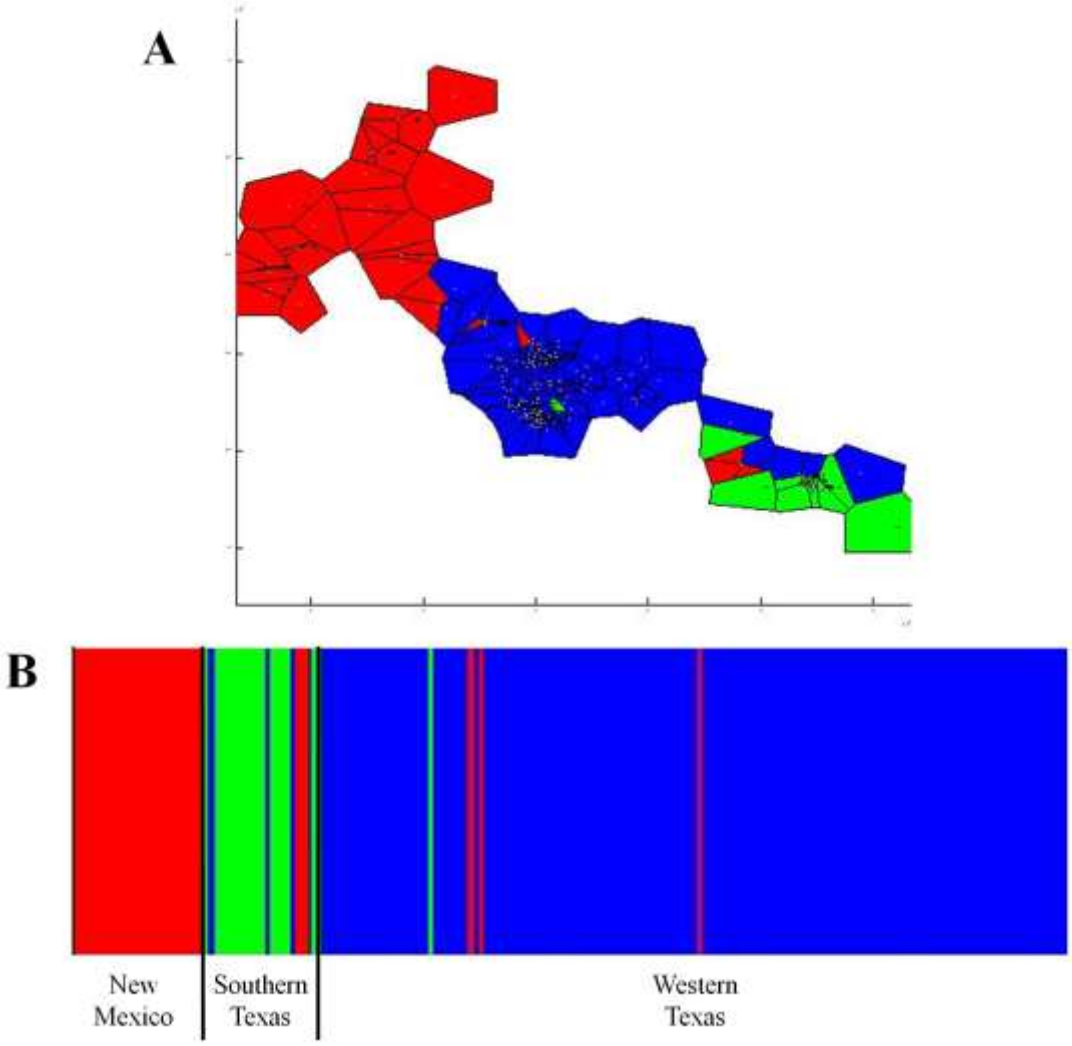

FIG. 2.5—Genetic clustering results from BAPS for known mountain lion populations (n = 237) in Texas and New Mexico sampled during 1985–2010. A) Colored Voronoi tessellations around each sample location for all individuals. Colors correspond to each genetic cluster (K = 3). B) Individual assignments to each genetic cluster with geographic sampling locations labeled below; New Mexico, southern Texas, and western Texas. Each column represents 1 individual.

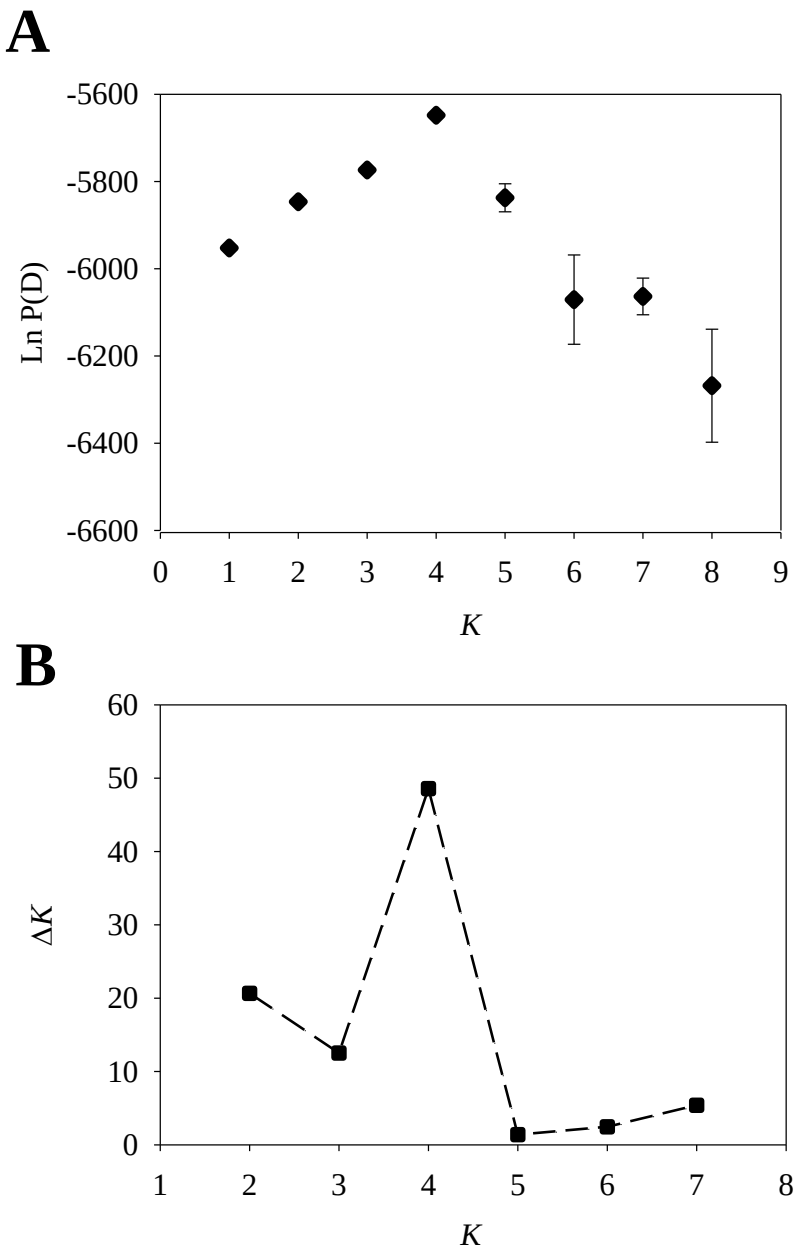

FIG. 2.6—The log probability of the data [Ln P(D)] and Δ*K* (Evanno et al. 2005) from STRUCTURE for known mountain lion populations (*n* = 237) in Texas and New Mexico sampled during 1985–2010. A) Mean Ln P(D) over *K* for 8 independent runs. Error bars indicate ± 1 *SD*, and *K* is the assumed number of genetic clusters. B) Estimate of Δ*K* for *K* = 2–7 using estimates of Ln P(D) from STRUCTURE.

values indicating support for all 3 scenarios (Fig. 2.7). However, at K = 2–4 western Texas displayed more admixture (q = 25–75%) than the other regions: K = 2—New Mexico (29%), southern Texas (18%), western Texas (34%); K = 3—New Mexico (32%), southern Texas (21%), western Texas (66%); K = 4—New Mexico (23%), southern Texas (18%), western Texas (51%). To examine if there was a biological feature or association responsible for the additional cluster in western Texas I mapped individuals assuming K = 4. I included individuals in a cluster only if q was > 0.65. There was no clear biological interpretation of the additional cluster in western Texas. Incoherent clustering has been documented in clumped and opportunistic sampling designs (McRae et al. 2005; Schwartz and McKelvey 2009), as well as in data exhibiting IBD (Frantz et al. 2009); both of which are characteristic of my data. However, I conducted exploratory analyses by separating males and females to determine if dispersal differences were responsible for the additional cluster in western Texas. For both males (n = 98) and females (n = 73) results indicated K = 1, but when combined Ln P(D) and ΔK (Evanno et al. 2005) suggested K = 2. Accordingly, I explored genetic differentiation between sexes in western Texas, which proved to be low (F_{ST} = 0.005, P > 0.05). I was unable to identify biological support for the additional cluster in western Texas. Therefore, I concluded that my sample was composed of only 3 genetic clusters, a solution unequivocally supported by F_{ST} analyses and 2 of 3 clustering algorithms. The partition of individuals from GENELAND, BAPS, and STRUCTURE suggested the clusters generally corresponded to the 3 regions of New Mexico, western Texas, and southern Texas.

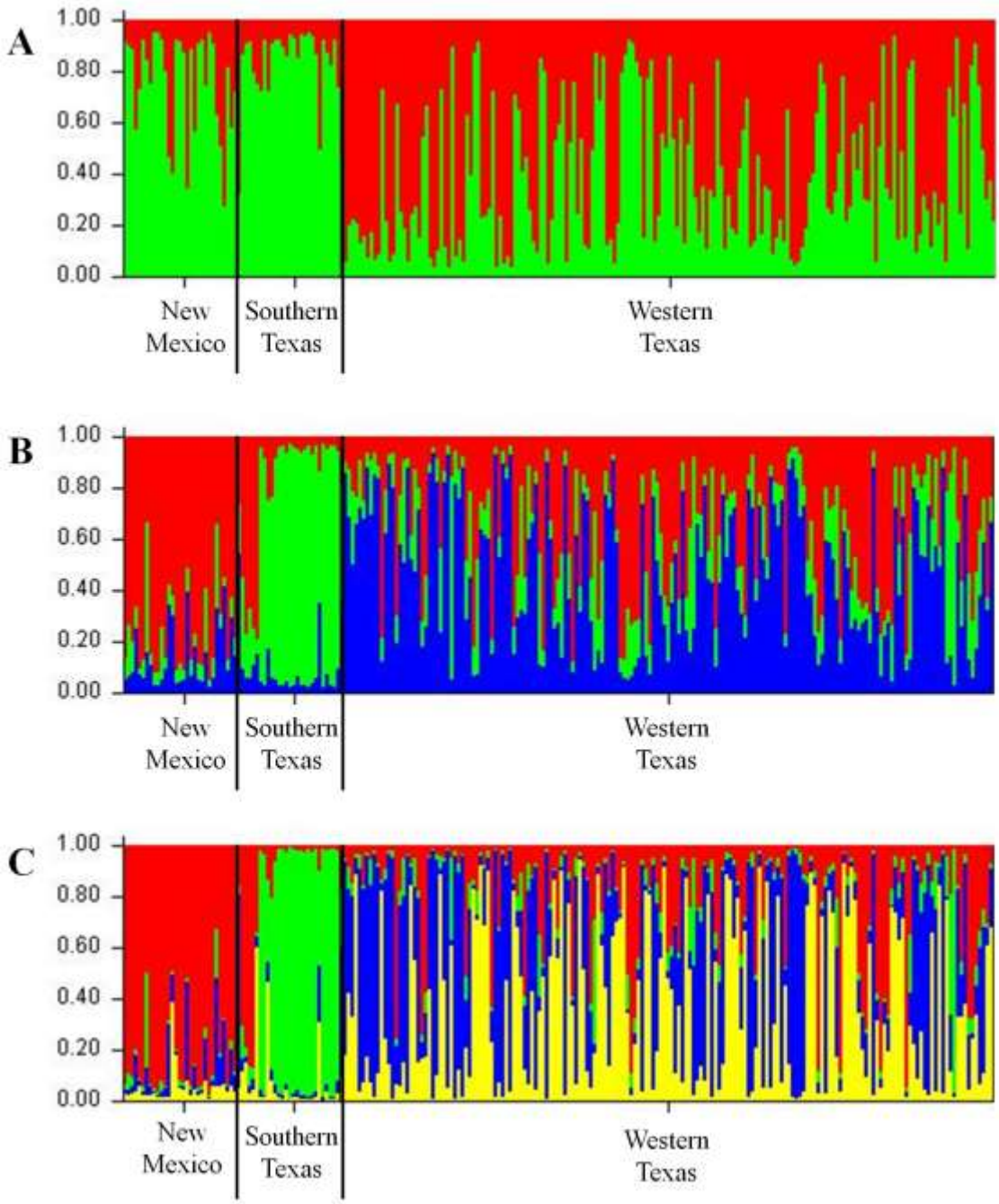

FIG. 2.7—Estimated ancestry proportions (*q*-values) in columns from STRUCTURE for known mountain lion populations (n = 237) in Texas and New Mexico sampled during 1985–2010. A) *q*-values for all individuals assuming K = 2, B) K = 3, and C) K = 4. Each colored column represents 1 individual, and geographic sampling locations are labeled below.

Assigning origin to dispersers.—I identified long-distance movements among known populations using mean *q*-values from STRUCTURE and assuming $K = 3$. Two adult males sampled in Jeff Davis and Brewster county, western Texas, exhibited ancestry to southern Texas (PC040—$q = 0.793$, $SD = 0.010$; MLA19—$q = 0.933$, $SD = 0.003$). In addition, 2 males and 1 adult female sampled in Maverick, LaSalle, and Kinney county, southern Texas, exhibited ancestry to New Mexico (PC007—$q = 0.780$, $SD = 0.005$; PC189—$q = 0.794$, $SD = 0.005$; and PC121—$q = 0.753$, $SD = 0.008$).

Before implementing assignment tests it is important to ensure that a sufficient number of loci and individuals have been sampled from reference populations (Manel et al. 2002). Reasonable levels of genetic diversity and differentiation are also required. My reference populations were composed of 28–178 individuals genotyped at 11 loci with reasonable levels of H_E and F_{ST}, which provided adequate power to assign origins (Latch et al. 2006; Manel et al. 2002). Results from GeneClass, STRUCTURE, and BAPS were consistent and implied strong genetic assignments for 6 of the 8 potential dispersers (Table 2.3). PC001 and PC042 were strongly assigned to New Mexico. This is particularly interesting because PC001 was a male sampled in Bossier City, Louisiana, > 800 km from New Mexico. The assignment for PC0042 is not surprising because this male was sampled < 10 km from New Mexico. PC004, PC123, PC163, and PC165 all exhibited strong assignments to western Texas. These assignments are reasonable because PC004 was a male sampled in north-central Texas, and PC123 (male), PC163 (female), and PC165 (male) were all sampled in central Texas.

Unfortunately, I was unable to assign 2 dispersers. PC003 was moderately–weakly assigned to all reference populations by GeneClass and STRUCTURE suggesting

TABLE 2.3.—Genetic assignments from GeneClass (Rannala and Mountain 1997), STRUCTURE (Prichard et al. 2000), and BAPS (Corander et al. 2006, 2008b) for 8 potential dispersers sampled east of known mountain lion populations during 2005–2009. Reference populations were individuals sampled in Texas and New Mexico during 1985–2010; New Mexico (n = 31), southern Texas (n = 28), western Texas (n = 178). All dispersers are adults except PC004 and PC123, which ages are unknown. All samples are males except PC123 (female). Assignment values from GeneClass and STRUCTURE indicate likelihood ratio scores and estimated ancestry proportions (q-values) to each population, respectively. Likelihood ratios > 85% and q-values > 0.85 indicate substantial support for assignment.

		GeneClass			STRUCTURE			BAPS*		
Sample	Sample Location	New Mexico	Southern Texas	Western Texas	New Mexico	Southern Texas	Western Texas	New Mexico	Southern Texas	Western Texas
PC001	Bossier City, LA	99.09	0.91	0.00	0.89	0.02	0.10	0.00	9.80	25.20
PC003	Kerr County, TX	30.67	12.91	56.42	0.33	0.04	0.63	1.60	2.80	0.00
PC004	Fisher County, TX	0.10	0.03	99.88	0.02	0.00	0.98	13.80	16.60	0.00
PC042	Deaf Smith County, TX	99.86	0.00	0.14	0.99	0.00	0.01	0.00	22.60	13.40
PC123	Edwards County, TX	0.00	0.00	99.99	0.00	0.00	0.99	21.00	22.20	0.00
PC163	Real County, TX	0.01	1.81	98.19	0.01	0.01	0.98	19.60	8.20	0.00
PC164	Kimble County, TX	65.55	0.00	34.45	0.54	0.00	0.46	0.00	24.00	1.00
PC165	Sutton County, TX	0.17	0.11	99.72	0.07	0.00	0.93	13.20	13.80	0.00

* BAPS values represent 2 times the absolute of change in the log marginal likelihood of individual i being alternatively assigned to cluster j. Values of 0 are the assigned reference population, and movement to another population with a change ≥ 6 indicates significant support for assignment (Kass and Raftery 1995).

admixed ancestry. However, BAPS provided essentially no support to any reference population (i.e., a change < 3 in 2 times the log marginal likelihood) indicating that PC003 could be from an unsampled source. Further, by all methodologies PC164 was moderately and weakly assigned to western Texas and New Mexico with essentially no support for southern Texas. PC164 appeared to be a product of combined ancestry from New Mexico and western Texas. Overall, assignments from GeneClass, STRUCTURE, and BAPS indicated long-distance movements have occurred across my sampling area.

DISCUSSION

Disparate patterns of genetic structure have been observed in mountain lions, ranging from continuous (Anderson et al. 2004; Culver et al. 2000; Sinclair et al. 2001)–highly structured (Ernest et al. 2003). Genetic and geographic distance associations in my sample indicated that genetic structure was present from the local–regional scale. At the local scale (< 50 km), autocorrelation analyses suggested that sampled mountain lions exhibited genetic associations similar to other large carnivores (Fabbri et al. 2007). However, family members in southern Texas may have been sampled (Harveson 1997), inflating these associations. Female philopatry (Logan and Sweanor 2001, 2010; Sweanor et al. 2000), high sampling effort (e.g., hunting, trapping, etc.) at local scales (Schwartz and McKelvey 2009), or a combination could further contribute to the non-independence among proximate individuals. At the regional scale, autocorrelation and regression analyses identified a significant IBD pattern indicating a decrease in gene flow with increasing geographic distance. This pattern is consistent with other continuous (Anderson et al. 2004; Sinclair et al. 2001) as well as structured mountain lion populations (Ernest et al. 2003; McRae et al. 2005).

Traditional F_{ST} and Bayesian clustering analyses mostly agreed providing a consensus for 3 differentiated groups at the regional level (New Mexico, western Texas, and southern Texas. However, the nonspatial algorithm in STRUCTURE rendered support for 4 genetic clusters (split western Texas into 2 clusters). Discrepant results from the clustering algorithms may be due to sampling constraints or ecological processes. Convenient and clumped sampling schemes (McRae et al. 2005; Schwartz and McKelvey 2009) as well as patterns of IBD (Frantz et al. 2009), have created spurious genetic discontinuities in clustering analyses. My sampling scheme was necessarily clumped and opportunistic, and the data exhibited patterns of IBD producing an amenable environment for spurious clustering. Alternatively, high harvest of mountain lions can promote immigration from adjacent populations (Cooley et al. 2009). Mountain lion harvest is unabated in Texas, and as a result my sample from western Texas may have had a high proportion of immigrants from surrounding unsampled populations (e.g., Mexico). Analyses from STRUCTURE indicated higher levels of admixture within western Texas, which might provide support for the harvest-immigration hypothesis. Overall, however, the data provided the most support for $K = 3$, corresponding to the regions of New Mexico, western Texas, and southern Texas.

The southern Texas region exhibited high levels of genetic differentiation (F_{ST} = 0.10–0.15) when compared to the remaining regions, substantiating the findings from Walker et al. (2000). Within southern Texas, notable differentiation was displayed by the county grouping of LaSalle–McMullen–Kleberg–Live Oak (F_{ST} = 0.12–0.28). In fact, these levels were essentially identical to highly fragmented or isolated populations in southwestern California (Ernest et al. 2003). Additionally, all comparisons including the

LaSalle–McMullen–Kleberg–Live Oak group in regression analyses fell above the predicted IBD relationship, indicating factors other than geographic distance are likely influencing genetic differentiation.

First, other vagile carnivores (e.g., Lynx—*Lynx canadensis*) have exhibited higher differentiation in peripheral populations compared to interior populations (Schwartz et al. 2003). Peripheral populations can suffer from smaller census sizes, fewer opportunities for gene flow, and are generally more sensitive to distributional shifts over time (Schwartz et al. 2003). Southern Texas represents the most peripheral population in the southwestern U.S., and may have experienced 1 or more of these mechanisms. Second, the urban development and sprawl throughout central Texas and along the Mexico-U.S. border has presumably restricted mountain lion movements and gene flow into southern Texas. Connectivity from adjacent populations to southern Texas may have also been reduced as a result of predator removal during the 19th and 20th century (Wade et al. 1984). This is plausible because removal was targeted around domestic sheep and goats, which were abundant in most habitats linking western Texas and Mexico to southern Texas (Lehmann 1969). It may be fruitful to sample museum specimens to determine if historical differentiation between southern and western Texas are similar to present levels.

The differentiation I documented between western Texas and New Mexico was moderate and consistent with other carnivores occupying desert habitats (Onorato et al. 2007). A noteworthy exception was the Culberson–Hudspeth county grouping in western Texas, which was differentiated from other groupings within the region as well as New Mexico (F_{ST} = 0.05–0.13). The patchy landscape across New Mexico and western Texas

could be influencing these levels of differentiation. The landscape matrix is generally composed of moderate–high quality mountain lion habitat, intervened with low quality habitat (Young 2009) that presumably impedes movements (Sweanor et al. 2000). Alternatively, the Culberson–Hudspeth area may be a corridor for individuals moving from southeastern New Mexico into western Texas. I did not sample southeastern New Mexico, but previous research suggests mountain lions in that region are differentiated from western Texas (Gilad et al., in press). Lastly, the clumped sampling in my data could have contributed to the differentiation associated with the Culberson–Hudspeth group. Additional research is needed to determine if convenience sampling or natural process are driving differentiation in this area.

Although many mountain lions populations exhibit genetic differentiation, long-distance dispersal has been documented (Thompson and Jenks 2010; 2005). My analyses revealed long-distance movements have occurred across my sampling area, and that dispersal appeared to be male-biased (11 males, 2 females). Among the known populations, I documented movement into and out of southern Texas. However, the high levels of differentiation and lower genetic diversity associated with southern Texas implies immigrants are not surviving to reproduce. Further investigation is warranted to determine the reproductive success of dispersers.

For 6 of the 8 potential dispersers sampled eastward New Mexico and western Texas were the assigned origin. The adult male sampled in Louisiana was > 800 km from its assigned origin in New Mexico, implying extensive movement. However, an important note is that our New Mexico reference population may represent a genetic pool greater than the state boundaries (e.g., southern Rocky Mountains). Dispersers sampled

in northern and central Texas were mostly assigned to western Texas, which offers some support to predicted paths of eastward movement (LaRue and Nielsen 2008). Of the 2 remaining dispersers, 1 exhibited mixed ancestry and the other I could not conclusively assign. My inability to discern an origin for PC003 suggests that I have not sampled all sources. Mountain lion movement from Mexico into Texas is probably occurring, and may explain why I was unable to assign PC003 (a male sampled < 200 km from the Mexico border). Additional samples from different genetic stocks are needed to determine origin for this individual, as well as other dispersers throughout the U.S.

I have shown that mountain lions in Texas and New Mexico represent 3 genetic groups at the regional level with differing levels of connectivity and genetic diversity. Further, populations in New Mexico, western Texas, and perhaps other unsampled populations are facilitating eastward mountain lion movements. These finding have clear implications for management and conservation. First, genetic diversity in New Mexico and western Texas is at seemingly healthy levels compared to other mountain lion populations (Culver et al. 2000), and will be maintained if effective population size remains large (Allendorf and Luikart 2007). Management strategies should aim at maintaining large effective sizes in these regions to perpetuate diversity and healthy peripheral populations in the U.S. Southern Texas, however, displayed moderate levels of genetic diversity along with high levels of differentiation; values comparable to fragmented or isolated populations in California (Ernest et al. 2003). I did document natural movements into southern Texas, but reproduction may be negated due to high mortality as suggested by previous work (Harveson 1997). Natural dispersal into southern Texas is promising because it has potential to increase diversity and reduce

differentiation if reproduction occurs. Strategies should be implemented to increase fitness of these emigrants during movement and after establishment. For instance, lowering harvest pressure in potential movement corridors into southern Texas could be 1 alternative. Furthermore, additional research on mountain lions in southern Texas is needed. It is essential to characterize population productivity and survival because it will inform the current status and future persistence of mountain lions in this region.

Second, my data suggests that mountain lions in the southwestern U.S. are not contiguous (Logan and Sweanor 2001; Sweanor et al. 2000). The levels of differentiation I documented between southern and western Texas is high, and similar to previous work despite my larger sample size and sampling area. Therefore, I echo the suggestion by Walker et al. (2000) that western and southern Texas be treated as 2 management units. This information informs the status of mountain lion connectivity in Texas, and should be considered when implementing management prescriptions that impact fitness. In addition, my data indicates New Mexico and western Texas should be considered separate units connected through moderate levels of genetic exchange. Habitat conservation for mountain lions in New Mexico, Texas, as well as Mexico will likely sustain large effective population sizes (Allendorf and Luikart 2007) with high probabilities of persistence.

Finally, mountain lions from New Mexico and western Texas are moving east. Mountain lions in New Mexico and Texas will be important to conserve if future recolonization in the southern U.S. is desired. Additionally, as landscapes continue to change more research is needed predicting mountain lion movement paths out of known populations (Sweanor et al. 2000). These efforts would help prioritize important

movement corridors and identify locations of high mountain lion-human conflict; both of which are imperative for the future of mountain lion conservation (Hornocker 2010).

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CHAPTER III

THE DEMOGRAPHIC HISTORY OF AN ELUSIVE CARNIVORE: USING MUSEUMS TO INFORM MANAGEMENT

Summary

1. Characterizing population trends is a priority for wildlife managers. Unfortunately, surveying elusive and inconspicuous carnivores is extremely difficult. I applied a retrospective genetic approach to examine how historical and recent conditions influenced the demographic history of mountain lions *Puma concolor* in Texas.

2. I sampled archived and recent genetic material and amplified 10 microsatellite loci. I indexed population trends by estimating historical and recent genetic diversity, spatial and temporal genetic differentiation and effective population size.

3. Mountain lions in southern Texas exhibited a 10–20% decline in genetic diversity over time, whereas all estimates were similar for western Texas. From historical–current conditions, genetic differentiation between western and southern Texas increased by 2.5 times. Temporal genetic differentiation in southern Texas was 7 times that of western Texas, which appeared to be responsible the increase in genetic differentiation between western and southern Texas.

4. Estimates of effective population size revealed a much lower average population size in southern Texas relative to western Texas. Southern Texas also exhibited a > 50% decline in effective size over time, whereas estimates for western Texas indicated population stability.

Chapter 3 of this thesis follows the format of the *Journal of Applied Ecology*.

5. *Synthesis and applications.* All evidence suggests mountain lions in western Texas have remained at high and relatively stable levels over time. In contrast, my results show dramatic temporal declines and changes have occurred in southern Texas. Lower genetic diversity in southern Texas, and high genetic structure between southern and western Texas is a recent phenomenon. Furthermore, additional research examining population productivity and survival in southern Texas is essential. Management actions such as population monitoring and harvest reduction may be needed to ensure the persistence of mountain lions in this region. This study emphasizes the importance and utility of genetically sampling museum and recent specimens to assist wildlife management and conservation.

Key-words: Effective size, genetic differentiation, genetic diversity, microsatellite loci, museum specimens, population trends, *Puma concolor*, Texas

Introduction

An important component of natural resource conservation and management is monitoring population trends (Marsh & Trenham 2007). Changes in census size and demographic parameters can inform harvest prescriptions, population augmentation, introductions and overall conservation status (Witmer 2005). However, implementing a monitoring program using traditional techniques such as marking individuals can be logistically and financially demanding (Barea-Azcon *et al*. 2007). This is particularly true for territorial, inconspicuous and elusive species that inhabit dense or rugged habitats (Witmer 2005); many large carnivores exemplify these characteristics.

Genetic tools have been employed in many ways to assist the monitoring of carnivore populations (e.g., De Barbra *et al.* 2010). Genetic data can discriminate

individuals or species and thus provide estimates of abundance, vital rates and characterize changes in geographic distribution (Schwartz, Luikart & Waples 2006). The use of genetic data has become relatively common to investigate the abundance and distribution of many carnivore species (Boulanger, Himmer & Swan 2004; McKelvey *et al.* 2006). Furthermore, genetic techniques can be used to assess demographic trends through time (Wandeler, Hoeck & Keller 2007). Comparison of genetic data from museum specimens to contemporary samples can elucidate the effects of historical and recent events on genetic diversity and structure. The genetic analysis of historical samples has informed the conservation and management of brown bears *Ursus arctos* (L.) (Miller & Waits 2003), Florida panthers *Puma concolor coryi* (B.) (Culver *et al.* 2008) and gray wolves *Canis lupus* (L.) (Flagstad *et al.* 2003).

Throughout North America mountain lions *Puma concolor* (L.) have experienced severe declines in census size and geographic distribution because of habitat loss and predator management policies (Logan & Sweanor 2001; Anderson *et al*. 2010). Currently, populations mainly inhabit the western half of the continent. During the mid–late 1900s most western states in the United States modified policies and regulated the harvest of mountain lions (Anderson *et al*. 2010). Regulation allowed populations in some areas to recover to high levels (Logan & Sweanor 2001). Today, large populations generally exhibit moderate levels of genetic diversity and low genetic differentiation (Culver *et al*. 2000; Anderson, Lindzey & McDonald 2004). Small and peripheral populations exhibit lower diversity and high differentiation (Walker *et al*. 2000; Ernest *et al*. 2003).

In Texas, USA, mountain lions are peripheral to the greater distribution and breeding populations occur only in the western and southern portions of the state (Fig. 3.1; Schmidly 2004). The harvest of mountain lions is not regulated in Texas and mandatory inspection is not required (Harveson *et al*. 1996; Russ 1996; Anderson *et al*. 2010). Therefore, harvest cannot be used by managers to inform demographic indices of population trends (e.g., Anderson & Lindzey 2005). Furthermore, little information is available to assist mountain lion management in Texas. Previous studies indicate all Texas populations have young age structures (Harveson *et al*. 1996), and exhibit low survival (Harveson 1997; Young *et al.* 2010) and reproduction (Harveson 1997; Pittman, Guzman & McKinney 2000). Genetic data suggest low diversity in southern Texas and genetic structure between southern and western Texas, implying southern Texas may be isolated (Walker *et al.* 2000). Together, unlimited harvest and sparse information warrant management concern, and additional research is clearly needed.

My overall goal was to assess the demographic history of mountain lions in Texas over the past century using microsatellite DNA data. Microsatellite loci are highly variable genetic markers and consist of tandem repeats of a short sequence motif (Allendorf & Luikart 2007). I used historical and contemporary samples from western and southern Texas to estimate (i) genetic diversity, (ii) spatial genetic differentiation, (iii) temporal genetic differentiation and (iv) effective population size. My genetic approach also allowed me to determine if high differentiation and low diversity in southern Texas (Walker *et al*. 2000) was present historically.

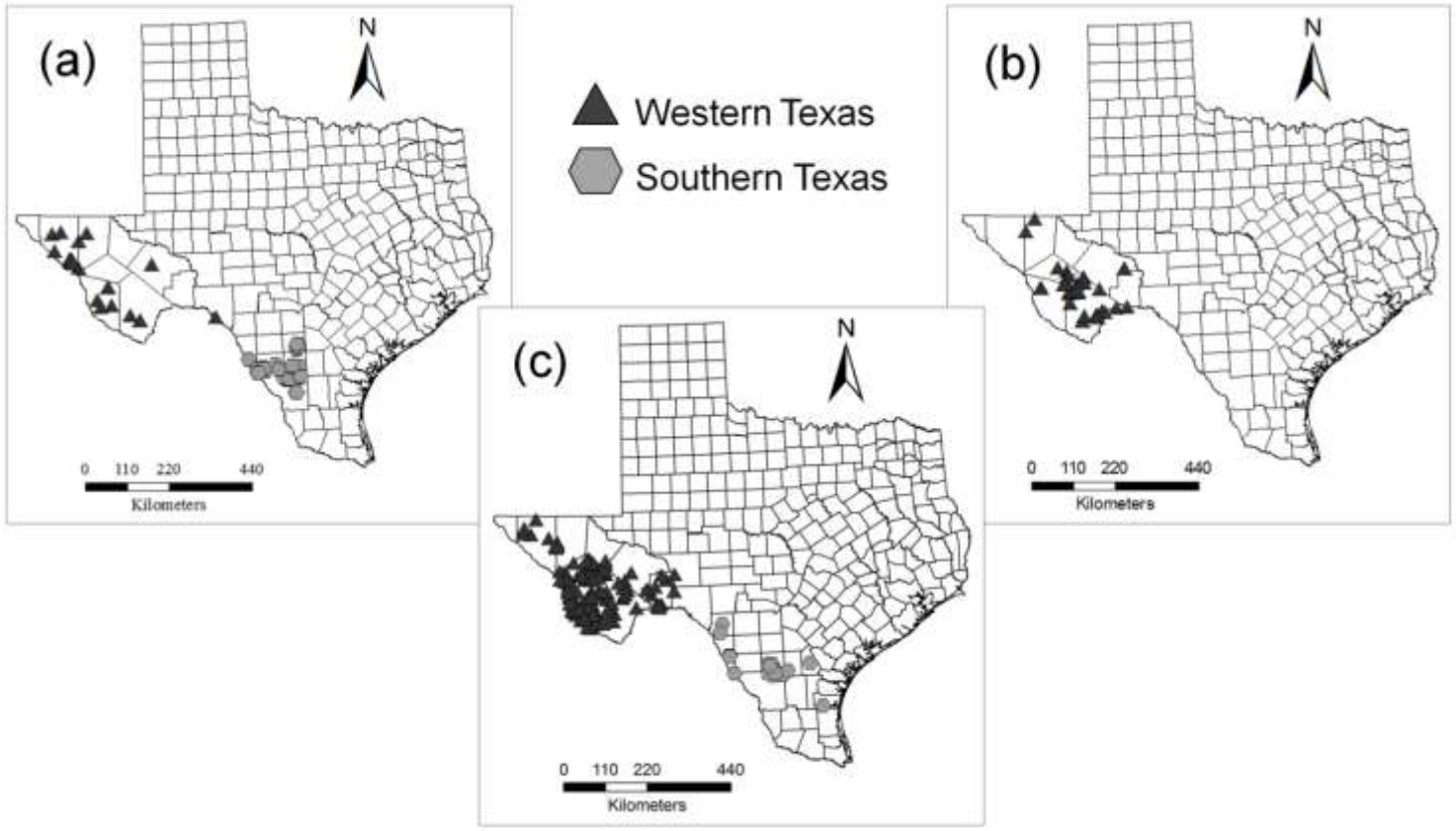

Fig. 3.1. Mountain lion sampling distribution throughout western and southern Texas, USA. (a) Samples from western Texas during 1935–1955 (median = 1938; *n* = 27) and southern Texas during 1934–1942 (median = 1937; *n* = 34). (b) Samples from western Texas during 1979–1989 (median = 1983; *n* = 42). (c) Samples from western Texas during 2000–2010 (median = 2006; *n* = 168) and southern Texas during 1985–2009 (median = 1996; *n* = 28).

Materials and methods

I obtained tissue samples from mountain lions collected in western and southern Texas spanning the temporal period 1905–2010. For the historical samples, I collected *c*. 100–200 mg of bone material taken from the maxilloturbinates of mountain lion skulls housed in museum collections, following Wisely, Maldonado & Fleischer (2004). The contemporary samples consisted of muscle tissues donated by hunters and trappers, collected from road-kills, or taken from live-trapped individuals during previous research. Muscle tissue was frozen, dried or placed in lysis buffer (Longmire, Maltbie & Baker 1997) and stored at -20 °C prior to DNA extraction.

I used separate protocols to extract DNA from muscle tissue and maxilloturbinate samples. For muscle tissue, I extracted DNA using the DNeasy Tissue Kit and a commercial protocol (Qiagen). For maxilloturbinates, I ground samples using a mortar and pestle and placed them in lysis buffer (0·5 M EDTA pH 8·0, 0·5% SDS and 0·5 mg/ml proteinase K; Wang, Woiderski & Driver 2005). I handled a maximum of nine samples (eight maxilloturbinate samples and one extraction negative) during each extraction day to reduce the potential for cross-contamination. I incubated samples for ≥ 24 h at 50 °C and extracted DNA using a QIAquick PCR Purification Kit (Qiagen, California, USA) and a modified extraction protocol developed by Wang *et al.* (1998) for ancient DNA samples.

Maxilloturbinates from museum specimens generally exhibit lower DNA quality and quantity than modern tissue resulting in a higher probability of contamination during extraction and PCR-setup (Wandeler, Hoeck & Keller 2007). Therefore, I extracted DNA and prepared PCRs for all maxilloturbinate samples in an isolated laboratory where

no mammalian DNA had previously been extracted or amplified. All materials used for DNA extraction and PCR were designated only for that purpose and were cleaned with RNAse Away® (Molecular BioProducts, California, USA) or 50% bleach before and after use.

For each individual I used the polymerase chain reaction (PCR) to amplify 10 microsatellite loci (FCA008, FCA035, FCA043, FCA077, FCA082, FCA090, FCA096, FCA133, FCA176, FCA205) described by Menotti-Raymond *et al.* (1999). I amplified loci individually in 10 µL reaction volumes that contained 5 µL AmpliTaq Gold® PCR Master Mix (Applied Biosystems), 0·24 µM of each primer, and 1–1·5 µL of extracted DNA. However, for maxilloturbinate reactions I increased primer concentration to 0·50 µM, added 0·2 mg/µL of bovine serum albumin, and increased the quantity of extracted DNA to 1·5–2·5 µL. I used a touchdown PCR profile with an initial denaturation at 94 °C for 10 min, 20 cycles of 94 °C for 30 s, 62 °C for 30 s, 61 °C for 30 s, 60 °C for 30 s, and 72 °C for 60 s, followed by 30 cycles of 94 °C for 30 s, 55 °C for 90 s, and 72 °C for 60 s, with a final extension of 60 °C for 10 min. For maxilloturbinate reactions I reduced the first set of temperature cycles to 10 and increased the second set to 50. I combined 3 µL of PCR products for each individual and applied 1·5–2 µL of the PCR product mix to a denaturing formamide (Hi-Di Formamide; Applied Biosystems) and size standard mixture (GeneScan ROX 500; Applied Biosystems). I loaded the resulting mixtures onto a 3130*xl* genetic analyzer (Applied Biosystems) for fragment separation. I included a positive and negative PCR control with each run through the analyzer to identify contamination and run consistency. I inspected all loci and sized alleles using

GeneMapper® software v4.0 (Applied Biosystems). I re-ran 10% of muscle tissue samples used in analyses to calculate a genotyping error rate.

Additional measures are required to ensure genotypes are correct for museum samples because extracted DNA is at relatively low concentrations and quality (Wandeler, Hoeck & Keller 2007; Casas-Marce, Revilla & Godoy 2009). Errors can occur from contamination, allelic dropout and false alleles when using museum specimens (Miller & Waits 2003; Wandeler, Hoeck & Keller 2007). Therefore, in addition to the positive and negative PCR controls aforementioned, I attempted to amplify all extraction negatives several times to detect potential cross-contamination during DNA extraction. Additionally, I performed 2–5 separate reactions for each individual at each locus and only called alleles I observed ≥ 2 times.

DATA ANALYSIS

The historical and recent samples represented three temporal periods for western Texas (1935–1955, 1979–1989 and 2000–2010) and two periods for southern Texas (1934–1942 and 1985–2009); all temporal samples had $n \geq 27$ individuals. The median years for the western Texas samples were 1938, 1983 and 2006, and the southern Texas medians were 1937 and 1996 (see Appendix S1 for museum specimens used).

I created input files for data analyses using the computer program CONVERT (Glaubitz 2004). I tested Hardy-Weinberg expectations (HWE) using F_{IS} (Weir & Cockerham 1984) for two pooled statewide samples spanning the temporal periods of 1934–1955 and 1985–2010. I also assessed HWE for each temporal sample (i.e., southern Texas: 1937, 1996; western Texas: 1938, 1983 and 2006). I evaluated statistical significance (2-sided) by comparing the observed F_{IS} value against a null value computed

from 1023 permutations of alleles among individuals in the computer program ARLEQUIN 3.5 (Excoffier & Lischer 2010).

I performed several analyses to characterize changes in genetic diversity over time. I estimated observed heterozygosity (H_O), expected heterozygosity (H_E; Nei 1987), number of alleles (A) and allelic richness (a_r) per locus for each temporal sample. I calculated H_O, H_E and A using the computer program ARLEQUIN 3.5 (Excoffier & Lischer 2010) and a_r using HP-RARE 1.0 (Kalinowski 2004, 2005). I tested for temporal changes in a_r within southern and western Texas using a Wilcoxon signed rank test (one-sided). During demographic declines such as population bottlenecks alleles are lost before heterozygosity changes (Allendorf 1986; Leberg 2002; Schwartz, Luikart & Waples 2006). The estimates of a_r use a rarefaction method (Hurlbert 1971; Kalinowski 2004) to enable comparisons among unequal sample sizes.

Previous research indicated that mountain lions in western and southern Texas may be genetically differentiated ($F_{ST} > 0.10$), and that H_O in southern Texas was 40% lower than in western Texas (Walker *et al.* 2000). I estimated F_{ST} (Weir & Cockerham 1984) between southern and western Texas for the temporal period of 1934–1955 and 1985–2010 to determine if structure was present historically. F_{ST} is the proportion of genetic diversity explained by allele frequency differences among groups (Holsinger & Weir 2009). Next, I evaluated the magnitude of genetic change over time within each geographic region by calculating F_{ST} (Weir & Cockerham 1984) among the temporal samples. This analysis produced one estimate for southern Texas (1937–1996) and three estimates for western Texas (1938–1983, 1983–2006 and 1938–2006). I calculated F_{ST} using the computer program ARLEQUIN 3.5 (Excoffier & Lischer 2010) and determined

statistical significance (2-sided) by comparing the observed value to a null value based on 1023 permutations of genotypes among groups (i.e., regions or temporal periods).

I estimated variance (N_{eV}) and inbreeding (N_{eI}) effective population size for southern and western Texas to explicitly test for changes in population size over time. Effective population size is the size of an idealized population exhibiting the same rate of genetic change as the sampled population (Wright 1931). I used temporal changes in allele frequencies (Krimbas & Tsakas 1971; Waples 1989) and linkage disequilibrium (LD) among loci (Hill 1981) to derive estimates of N_{eV} and N_{eI}, respectively. Both methodologies make simplifying assumptions (e.g., Waples 1989; Leberg 2005; Waples 2006; Luikart *et al.* 2010) including population closure and no substructure.

The temporal method requires two or more temporally spaced samples of a species with non-overlapping generations to estimate N_{eV}. When applying temporal estimators to age structured species such as mountain lions it is important to describe how samples are pooled over time, define a generation, and identify the number of generations separating samples (Waples & Yokota 2007). For each discrete temporal sample from southern and western Texas I used the median year as the pooled year (described previously). I considered six years as a mountain lion generation because it was the mean age of adults in a neighboring population exposed to hunting (Logan & Sweanor 2001). My temporal samples from western and southern Texas covered a range of *c*. 4–11 mountain lion generations, which should ensure relatively unbiased and precise estimates of N_{eV} (Waples & Yokota 2007).

I estimated N_{eV} using a moment-based (Krimbas & Tsakas 1971; Nei & Tajima 1981; Pollock 1983; Waples 1989), Bayesian (Berthier *et al.* 2002) and pseudo-likelihood

(Wang 2001) method in the computer program NeEstimator 1.3 (Peel, Overden & Peel 2004) and MLNE 1.0 (Wang & Whitlock 2003). I employed 1000 updates in the Bayesian framework, and assumed a maximum N_{eV} = 500 for western and southern Texas using the Bayesian and likelihood methods (Wang 2001; Berthier *et al.* 2002).

Estimates of N_{eI} do not require temporally spaced samples (Luikart *et al*. 2010). I explored temporal changes in N_{eI} using the LD approach of Waples (2006) for each temporal sample from southern and western Texas. This produced five estimates separated by 4–11 generations, which should allow me to identify trends in population size (Tallmon *et al*. 2010). However, in age-structured samples estimates of N_{eI} based on the LD method reflect the effective number of breeders (N_b) that produced the cohorts present in each sample (Waples & Do 2010). Therefore, I used the computer program LDNE 1.31 (Waples & Do 2008) to compute N_b estimates and calculate 95% CIs following a jackknifing procedure. I employed the random mating model rather than the monogamy model because mountain lions exhibit a polygynous mating system (Murphy 1998). To reduce potential bias in the N_b estimates I only used alleles that were present at frequencies > 0·02 in analyses (Waples & Do 2010).

Results

I genotyped 10 microsatellite loci for 299 mountain lions (2% missing data) collected from Texas (50% males, 46% females, and 4% unknown). The sample sizes for the median year groups from western Texas included n = 27 (1938), n = 42 (1983) and n = 168 (2006), and n = 34 (1937) and n = 28 (1996) from southern Texas (Fig. 3.1). All positive PCR controls were consistent, and extraction and PCR negatives exhibited no contamination. My genotyping error rate for muscle tissues was < 1%.

GENETIC DIVERSITY AND DIFFERENTIATION

I observed a statistically positive F_{IS} for the recent (1985–2010) statewide sample indicating HWE were not satisfied (F_{IS} = 0·04, P = 0·02). F_{IS} for the historical statewide sample (1934–1955) was also positive and approached statistical significance suggesting deviations from HWE (F_{IS} = 0·05, P = 0·06). In contrast, both temporal groups from southern Texas (1937: F_{IS} = 0·04, P > 0·05; 1996: F_{IS} = 0·04, P > 0·05) and all groups from western Texas (1938: F_{IS} = 0·02, P > 0·05; 1983: F_{IS} = -0·05, P > 0·05; 2006: F_{IS} = 0·00, P > 0·05) satisfied HWE. Departures from HWE in statewide samples suggested a Wahlund effect (Allendorf & Luikart 2007).

Estimates of H_O, H_E and A for each temporal period in western Texas indicated only minor changes over time (Table 3.1) with mean H_E ranging from 0·59–0·56 during 1938–2006. I detected no difference in a_r (Table 3.2) for any comparisons conducted within western Texas (1938–1983, 1983–2006 and 1938–2006: Wilcoxon T = -0·36, P = 0·36). Levels of diversity in the historical sample from southern Texas are similar to diversity in western Texas. However, over time I documented a 20% decline in H_O and H_E, and a 12% decline in A within southern Texas (Table 3.1). I also observed a temporal reduction in a_r (Table 3.2) that approached statistical significance (1937–1996: Wilcoxon T = -1·58, P = 0·06).

Genetic differentiation between southern and western Texas for the historical sample was moderate (1934–1955: F_{ST} = 0·04, P < 0·01), but more than doubled in the recent sample (1985–2010: F_{ST} = 0·10, P < 0·01). I observed low yet significant temporal genetic differentiation within western Texas (1938–1983: F_{ST} = 0·03, P < 0·01; 1983–2006: F_{ST} = 0·01, P < 0·01; 1938–2006: F_{ST} = 0·02, P < 0·01). Southern Texas,

Table 3.1. Genetic diversity estimates (H_O, H_E, A) per locus for geographic and temporal samples (listed by median) of Texas mountain lions

	Western Texas									Southern Texas					
	1938 (n = 27)			1983 (n = 42)			2006 (n = 168)			1937 (n = 34)			1996 (n = 28)		
Locus	H_O	H_E	A	H_O	H_E	A	H_O	H_E	A	H_O	H_E	A	H_O	H_E	A
FCA008	0·07	0·07	3	0·05	0·05	2	0.00	0.00	1	0·09	0·11	2	0.00	0.00	1
FCA082	0·70	0·77	6	0·75	0·63	5	0·52	0·62	6	0·47	0·51	5	0·58	0·53	4
FCA090	0·70	0·77	6	0·76	0·77	6	0·77	0·76	7	0·65	0·75	7	0·71	0·78	6
FCA133	0·63	0·51	5	0·57	0·55	5	0·53	0·55	6	0·71	0·57	4	0·64	0·61	5
FCA176	0·44	0·57	4	0·33	0·39	4	0·41	0·40	5	0·43	0·49	3	0·11	0·10	3
FCA035	0·59	0·60	3	0·73	0·69	4	0·66	0·63	6	0·52	0·50	4	0·42	0·48	3
FCA043	0·81	0·80	5	0·64	0·67	5	0·70	0·75	6	0·67	0·75	6	0·56	0·58	6
FCA077	0·48	0·48	2	0·55	0·54	3	0·54	0·52	3	0·41	0·46	3	0·14	0·25	2
FCA096	0·43	0·67	4	0·78	0·78	5	0·76	0·78	5	0·56	0·70	5	0·56	0·56	5
FCA205	0·67	0·65	4	0·66	0·61	4	0·67	0·63	4	0·59	0·68	4	0·46	0·52	3
Mean (*SD**)	0·55 (0·21)	0·59 (0·21)	4·20 (1·32)	0·58 (0·23)	0·57 (0·22)	4·30 (1·16)	0·56 (0·23)	0·56 (0·23)	4·90 (1·79)	0·51 (0·18)	0·55 (0·19)	4·30 (1·49)	0·42 (0·25)	0·44 (0·24)	3·80 (1·69)

*Represents the standard deviations of estimate across loci.

Table 3.2. Allelic richness estimates per locus for temporal samples (listed by median) of mountain lions from western and southern Texas

Locus	Western Texas			Southern Texas	
	1938 (*n* = 27)	1983 (*n* = 42)	2006 (*n* = 168)	1937 (*n* = 34)	1996 (*n* = 28)
FCA008	2.56	1.75	1.00	1.98	1.00
FCA082	6.00	4.89	4.51	4.50	3.96
FCA090	5.94	5.49	5.21	6.33	5.94
FCA133	4.56	4.39	4.80	3.62	4.68
FCA176	4.00	3.94	4.14	3.00	2.69
FCA035	3.00	3.94	4.21	3.58	2.99
FCA043	5.00	4.50	4.57	5.94	5.47
FCA077	2.00	2.94	2.55	2.62	2.00
FCA096	4.00	5.00	5.00	4.58	4.89
FCA205	4.00	3.51	3.83	3.95	2.94
Mean	4.11	4.03	3.98	4.01	3.66
*SD**	1.34	1.10	1.28	1.38	1.59

*Represents the standard deviations of estimate across loci.

however, displayed temporal genetic differentiation seven times greater (1937–1996: F_{ST} = 0·13, $P < 0·01$) than western Texas.

EFFECTIVE POPULATION SIZE

Estimates of N_{eV} using the temporal approach produced statistically similar means within temporal periods for western and southern Texas (Table 3.3). There was weak support for an increase in N_{eV} within western Texas, as the 95% CIs for historical (1938–1983) and recent (1983–2006) N_{eV} did not overlap the means. The arithmetic mean across methods for each interval in western Texas were $N_{eV\ (1938\text{-}1983)}$ = 54, $N_{eV\ (1983\text{-}2006)}$ = 166, and $N_{eV\ (1938\text{-}2006)}$ = 109. Estimates based on the likelihood approach of Wang (2001) were consistently yet qualitatively higher than the moments (Pollock 1983; Waples 1989) or Bayesian (Berthier *et al.* 2002) estimates. The temporal interval of 1983–2006 produced the most variable estimates of N_{eV} in western Texas, but 1983–2006 was the shortest temporal span with only 4 generations separating samples. The historical (1938-1983) and overall estimates (1938-2006) capturing 7 and 11 generations were much more precise, reflected by narrower 95% CIs (Table 3.3). In southern Texas all temporal estimates were precise with an arithmetic mean of $N_{eV\ (1937\text{-}1996)}$ = 44. The mean estimate of N_{eV} for southern Texas was 60% lower than N_{eV} in western Texas.

The LD estimates of N_b for western Texas exhibited no statistical differences among temporal samples suggesting the population has remained stable over time (Table 3.4). The N_b estimates for the 1938 and 1983 temporal period were variable, but the 2006 estimate was comparatively precise. The disparity in precision may have reflected differing samples sizes, with larger samples tending to be more precise (Tallmon *et al.* 2010). In southern Texas there was weak support for a decline in N_b over time as the

Table 3.3. Estimates of variance effective population size (N_{eV}) over three temporal periods for mountain lions sampled (listed by median) from western and southern Texas. A moments (Waples 1989), Bayesian (Berthier *et al.* 2002) and likelihood (Wang 2001) method were used to derive estimates and 95% confidence intervals

Geographic region	Temporal interval	*n*	Waples 1989	95% CI	Berthier *et al.* 2002	95% CI	Wang 2001	95% CI
Western Texas	1938–1983	27–42	48	24–94	47	30–76	67	40–125
	1983–2006	42–168	146	62–467	125	73–204	228	113–500
	1938–2006	27–168	96	52–174	90	65–124	142	91–234
Southern Texas	1937–1996	34–28	36	20–63	53	29–65	41	28–63

Table 3.4. Linkage disequilibrium (Waples 2006) estimates of the effective number of breeders (N_b) for temporal samples (listed by median) of mountain lions from western and southern Texas

Geographic region	Temporal sample	*n*	N_b	95% CI*
Western Texas	1938	27	63	22–∞
	1983	42	68	32–544
	2006	168	91	65–134
Southern Texas	1937	34	21	12–42
	1996	28	9	4–18

*Confidence intervals were computed using a jackknifing procedure.

95% CIs for 1937 and 1996 did not overlap means (Table 3.4). Similar to N_{eV} results, mean N_b for southern Texas was 67–90% lower than western Texas over similar temporal periods.

Discussion

The demographic history of many species is poorly documented. Thus, a major challenge in conservation genetic studies is to determine if contemporary levels of genetic diversity and population structure are the result of recent or historical events. The initial genetic analyses of Texas mountain lions presented a similar challenge (Walker *et al*. 2000). The authors observed low genetic diversity in southern Texas, high structure between southern and western Texas, but were unable to evaluate alternative hypotheses without historical samples. Furthermore, the small number of samples (n = 16 and 9 for southern and western Texas, respectively) limited the inferential power of the analyses.

My diversity estimates in recent western and southern Texas are higher than reported by Walker *et al*. (2000), but generally supported their findings in that southern Texas displayed less diversity. My differing values were likely due to the additional samples and different loci used in my study. The inclusion of historical samples revealed a 10–20% temporal decline in diversity for southern Texas. Clearly, the lower diversity in contemporary mountain lions from southern Texas is a recent phenomenon.

The overall levels of diversity I documented were comparable to other populations. Estimates for western Texas and historical southern Texas were less than observed in mountain lions from South America (Culver *et al*. 2000), but were equivalent to large and presumably healthy populations in North America (Sinclair *et al*. 2001; Anderson, Lindzey & McDonald 2004; McRae *et al*. 2005). Recent estimates for

southern Texas were similar to severely fragmented populations in California, USA (Ernest *et al*. 2003).

The genetic differentiation between historical western and southern Texas was comparable to contiguous populations of mountain lions in western North America (Sinclair *et al*. 2001). However, contemporary genetic differentiation between western and southern Texas was similar to that reported by Walker *et al.* (2000) and isolated populations of mountain lions in California (Ernest *et al*. 2003). The increase in differentiation over the last 70 years appears to be the result of allele frequency changes (i.e., genetic drift) that have occurred in southern Texas (temporal $F_{ST} = 0{\cdot}13$) rather than western Texas (temporal $F_{ST} = 0{\cdot}01-0{\cdot}03$). Small levels of differentiation between historical southern Texas and the 1983 and 2006 samples from western Texas ($F_{ST} = 0{\cdot}04-0{\cdot}05$) provide further support for temporal changes occurring mainly in southern Texas. Indeed, the recent genetic structure observed between mountain lions in southern and western Texas (Walker *et al*. 2000) was not present historically.

Estimates of N_{eV} and N_b substantiated my genetic diversity and differentiation findings. The temporal approach (Waples 1989) revealed similar estimates of N_{eV} in western Texas over time, and point estimates were similar to other temporally stable populations of large carnivores (Miller & Waits 2003). Estimates of N_{eV} for southern Texas were much lower than western Texas providing evidence for a smaller average population size over time. Estimates of N_b produced results similar to N_{eV} indicating no population changes over time in western Texas, and a lower average population size in southern Texas. Estimates of N_b for southern Texas also suggested a decrease over time. The recent estimate of N_b for southern Texas was 80% lower than the average N_{eV}

estimate, and is similar to a reintroduced population of brown bears (De Barbra *et al*. 2010). The disparity between N_{eV} and N_b estimates is likely because N_{eV} reflects the harmonic mean over the sampled time period (Waples & Yokota 2007), while N_b represents the number of breeders producing the sampled cohorts (Waples & Do 2010). In the case of southern Texas, temporal N_{eV} could have been influenced by larger historical population sizes whereas, recent estimates of N_b may be indicative of a small effective size in the contemporary population. This hypothesis was supported by the temporal decline I observed in genetic diversity within southern Texas.

Historically, mountain lions in western and southern Texas displayed similar genetic diversity and low genetic structure. Over time, western Texas exhibited essentially no change in diversity and effective population size, and showed low levels of temporal genetic differentiation. However, genetic diversity and effective size decreased to low levels in southern Texas, and temporal genetic differentiation was extensive. Furthermore, genetic structure has doubled between western and southern Texas over time. My findings highlight that mountain lions in western Texas have remained relatively stable over time, but that obvious changes and declines have occurred in southern Texas.

The human footprint and geographic location may be responsible for the stability and changes of western and southern Texas. First, urban development and sprawl have increased dramatically in southern Texas along the Mexico-USA border and in central Texas. Furthermore, the Rio Grande Valley region of southern Texas supports vast areas of cropland on both sides of the border. Collectively, development and agriculture have reduced and fragmented habitat for mountain lions and increased the potential for auto

collisions and other mountain lion-humans conflicts in populated regions. Changes in habitat reducing connectivity could be responsible for the increase in genetic differentiation for the most peripheral population in southern Texas. In contrast, much of western Texas remains rangeland with little urban development. The large geographic area in western Texas, lack of urbanization, and proximity to adjacent mountain lion populations in New Mexico, USA, and Mexico may have maintained a large effective size. Movement occurs among western Texas, New Mexico and probably Mexico (Chapter II), and the population boundaries of western Texas could easily extend beyond state borders.

Second, during late 1800–mid 1900 livestock production was the dominant industry in Texas (Lehmann 1969). Predator control was widely practiced to support livestock production, and predator removals included mountain lions (Wade *et al*. 1984). I found no evidence of decline in effective population size for western Texas, but predator control may have reduced effective size and genetic diversity in southern Texas; particularly if predator control contributed to isolation from neighboring populations. Finally, the range of mountain lions contracted during the 1900s due to habitat alteration and predator control, leaving the southern Texas population isolated on the eastern periphery of the former range. Location on the landscape has been shown to influence population size and genetic diversity in species of carnivores (Schwartz *et al*. 2003). Peripheral populations may display smaller population sizes, fewer opportunities for gene flow and greater fluctuations in population size due to geographic range shifts (Schwartz *et al*. 2003). Compared to western Texas, southern Texas exhibited lower historical effective sizes indicating southern Texas may have exhibited peripheral characteristics by

the early 1900s. Thus, the temporal decline in diversity and effective size within southern Texas could be due to population isolation, range contraction, and mortality due to predator control and other interactions with humans.

CONSERVATION AND MANAGEMENT IMPLICATIONS

My results demonstrate the utility of applying a retrospective genetic approach (Schwartz, Luikart & Waples 2006) to evaluate the demographic history of an elusive carnivore. Although exposed to unlimited hunting and a history of land-use change and persecution mountain lions in western Texas appear to have remained at high and stable levels. The current level of harvest may not have a large negative effect on the population. However, my analyses offer no insight on the consequences of increasing harvest in western Texas, which could easily be realized under current regulations. Additionally, it is possible that genetic connectivity to adjacent populations is assisting the stability I observed in western Texas. Connectivity to proximate populations should be considered when applying habitat or population prescriptions. Future research examining mountain lion survival and movements in western Texas would inform questions regarding harvest mortality and interpopulation connectivity. Given the current information, implementing a monitoring program using indices such as harvest reports (Anderson & Lindzey 2005) with genetic sampling would be prudent for future mountain lion management and conservation in western Texas.

Declines have occurred in genetic connectivity, genetic diversity and effective population size for mountain lions in southern Texas. In fact the temporal decline in diversity and current effective size are outside of the ranges suggested for long-term population persistence (Soule *et al*. 1986). Furthermore, the decline in diversity within

southern Texas was 10–20% of the overall decline observed in Florida panthers (Culver *et al*. 2000); a population that has displayed physical symptoms of inbreeding depression (Roelke, Martenson & O'Brien 1993). Additional loss of diversity may occur through genetic drift if the high mortality and low productivity previously documented (Harveson 1997) are sustained.

Management actions may be needed if mountain lions are to be maintained in southern Texas. First, the current population size or trend in southern Texas is unknown. Population monitoring efforts are needed to estimate reproductive rates, survival and population viability without management intervention. Reporting mountain lion harvests in southern Texas would assist monitoring efforts. If current harvest is unsustainable, regulation of harvest may be needed (Young 2009). A harvest management plan would allow managers to focus harvest on areas of potential mountain lion-human conflict, while maintaining survival rates of residents and dispersers at sustainable levels. Unlike the Florida panther, southern Texas exchanges migrants with neighboring populations in western Texas, New Mexico (Chapter II) and perhaps Mexico. Successful reproduction by dispersers would increase genetic connectivity, genetic diversity and effective size; all of which are characteristic of healthy populations (e.g., Spong, Johansson & Björklund 2000). Overall, it is apparent that conservation programs are likely necessary to ensure the persistence of mountain lions in Texas. This work illustrates the utility of using museum collections and current genetic samples to examine population histories of wildlife that are data deficient and difficult to survey.

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Appendix S1. Mountain lion museum samples (maxilloturbinates) from Texas, USA, used in analyses. Samples are organized by Texas counties. All individuals were sampled during 1934–1989. Samples were attained from the National Museum of Natural History (USNM), Texas Tech University (TTU), Sul Ross State University (SRSU), Field Museum of Natural History (FMNH), or Carnegie Museum of Natural History (CM), USA.

Brewster County, Texas: FMNH83479– FMNH83480, SRSU 2212, TTU35131, TTU41009– TTU41010, TTU41648–TTU41659, TTU41667, TTU41740–TTU41742, TTU49620–TTU49623, USNM261685.

Culberson County, Texas: TTU41660–TTU41661, USNM251600, USNM262111.

Dimmit County, Texas: USNM251393, USNM261616, USNM262475, USNM262698–USNM262699, USNM263859, USNM264679–USNM264680, USNM264680, USNM271676.

Frio County, Texas: USNM261750, USNM262108–USNM262109, USNM262130–USNM262131, USNM262186.

Hudspeth County, Texas: USNM261686, USNM262110, USNM263413, USNM263523, USNM263769–USNM263770, USNM264177, USNM264458, USNM264682, USNM265342, USNM271857–USNM271858, USNM272085, USNM272311, USNM273167.

Jeff Davis County, Texas: TTU41662–TTU41665.

La Salle County, Texas: USNM263858, USNM264379–USNM264380.

Maverick County, Texas: USNM262185.

Pecos County, Texas: TTU41666, TTU41668–TTU41669, USNM251599.

Presidio County, *Texas*: CM21404, CM21406, TTU41670–TTU41677, USNM263772–USNM263773, USNM271675.

Terrell County, *Texas*: SRSU 2869.

Val Verde County, *Texas*: USNM261614.

Webb County, *Texas*: USNM251375, USNM251418, USNM251468–USNM251469, USNM261615, USNM263775–USNM263776, USNM263860, USNM264178–USNM264180, USNM264678, USNM272086, USNM272310, USNM272350.

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