
TEXAS TECH UNIVERSITY
Natural Science Research Laboratory

Occasional Papers

Museum of Texas Tech University Number 350 8 August 2017

Genetic Diversity and the Possible Origin of Contemporary Elk (*Cervus canadensis*) Populations in the Trans-Pecos Region of Texas

Christopher D. Dunn, Matthew R. Mauldin, Marisa E. Wagley, Jeremy E. Wilkinson, Caleb D. Phillips, and Robert D. Bradley

Abstract

Elk (*Cervus canadensis*) historically are among the most widely distributed members of the deer family, occupying much of the United States, Canada, and northern Mexico. The natural distribution of this species decreased substantially in the early 20th century, presumably resulting in the extirpation of populations in Texas. In the past 40 years, several herds of free-ranging elk have re-appeared in the Trans-Pecos region of Texas. For some herds, it is not known if the origin was: 1) the result of individuals that escaped from captive herds; 2) an expansion of previously transplanted individuals from South Dakota and Oregon into Texas; or 3) the result of natural emigrants from southeastern New Mexico into the Trans-Pecos region. The objective of this study was to use DNA sequences from the mitochondrial cytochrome-*b* gene and D-Loop region, in combination with nine microsatellite loci, to assess genetic divergence, relationships, and origin(s) of the contemporary elk herds in Texas. Findings of the mitochondrial sequence data depicted a high degree of relatedness among individuals throughout the sampling area; whereas, microsatellite data revealed differences in frequencies of alleles in the Glass Mountain populations of Texas compared to samples from South Dakota, New Mexico, and the Davis Mountains. Further, computer simulations of population genetic parameters based on the microsatellite data supported a scenario depicting the origin of contemporary elk in Texas likely was the result of natural emigrants from New Mexico or descendants of previously introduced individuals from New Mexico. In addition, simulations did not detect evidence of a genetic bottleneck during the past 350 generations, indicating a long, shared history between Texas and New Mexico populations.

Key words: *Cervus canadensis*, elk, Trans-Pecos

Introduction

Prior to European settlement, wapiti or elk (*Cervus canadensis*) were among the most widely distributed members of the deer family on the North American continent, numbering over 10 million individuals (Davis 1940). The distribution historically was partitioned into eight recognized subspecies distributed

across northern portions of the North American grasslands (modern day Canada and Alaska) southward to the southern edge of the Great Plains grasslands into modern day Mexico (Davis 1940; Hall 1981; O'Gara and Dundas 2002). Extensive sightings of elk were documented from 1600 to 1877 followed by drastic drop off in the population following in the 1880s (Davis 1940). Due to anthropogenic events in the late 1800s, current distribution ranges and population sizes across all subspecies of elk were reduced to approximately 10% of their historical North America geographic range and total population numbers were reduced to approximately 41,000 individuals in the late 1880s (Nowak 1999; Bryant and Maser 1982). By the 1900s, market hunting, sport hunting, habitat modification, and general agriculture practices further reduced overall population numbers and in some cases, subspecies such as *C. c. merriami* and *C. c. canadensis* were driven to extinction (Davis 1940; McCullough et al. 1996; Toweill and Thomas 2002). Bailey (1905) reported that elk had not been observed in the Trans-Pecos region for 20 years suggesting that elk, presumably, were extripated in Texas.

Isolated populations currently are known from the Trans-Pecos and Panhandle regions of Texas. They include herds in the Davis, Glass, Guadalupe, Sierra Diablo, and Wylie Mountains, and in the northern Panhandle (Witt 2008; Gill 2013; Schmidly and Bradley 2016). The Trans-Pecos area elk population reached an estimated 400 individuals by the late 1930s and has gradually increased in recent years (Wright and Thompson 1935; Davis 1940; Genoways et al. 1979; Witt 2008; Coykendall 1990). In 2016, the population of elk in the Trans-Pecos region was estimated at approximately 3,500 individuals (Schmidly and Bradley 2016). The origin of these current populations has been highly debated for the past 30 years due to incomplete or uncertain records pertaining to historic introduction, importation for game ranches, and a possible influx of individuals into Texas from neighboring populations in New Mexico—which themselves probably are products of introductions from Colorado (Ligon 1927; New Mexico Department of Game and Fish 1967; Findley 1975).

At least three possible scenarios can explain the origin of contemporary populations of elk in the Trans-Pecos region of Texas. First, it is well-documented that individuals were introduced in the Trans-Pecos region several times by private parties (Davis 1940; Swepston 1985). Further, many of the high-fence hunting facilities, located throughout the Trans-Pecos region, have introduced and bred captive-reared individuals to generate populations for hunting revenue. Several of these herds are routinely observed in the lower elevations surrounding Alpine, Fort Davis, Fort Stockton, Marathon Basin, Stockton Plateau, and other foothill and grassland regions in the Trans-Pecos region. Under this scenario, few records are available and few follow up studies have been conducted to evaluate the success of said introductions. It is possible that at least some current populations of elk in the Trans-Pecos are the result of escapees that later founded local populations; although many of these cases are difficult to document.

The second scenario involves situations where previous introductions were conducted to establish free-ranging populations instead of captive populations. We have treated this scenario separate from introductions listed under the first scenario because of the timing (some nearly one hundred years ago), these introductions are accompanied by reliable sources of documentation and historical data, and in some cases, they are re-occurring studies to monitor the success of the introductions. One of the best-documented introductions occurred in 1928, when Judge J. C. Hunter and his associates released 44 elk, collected from the Black Hills of South Dakota, into McKittrick Canyon located in the Guadalupe Mountains of Texas (Davis 1940; Schmidly 2002). This herd steadily increased in number and reached a size of approximately 350 individuals by the mid 1960s. Although this herd declined in the 1980s (McAlpine 1990), a viable population remains in this area. Additional reintroductions in the Davis, Eagle, Glass, Sierra Diablo, and Wylie Mountains occurred on several occasions with varying success and levels of documentation (Swepston 1985; Coykendall 1990; Gill 2013). For example, a large-scale introduction in 1988 involved the transplant of 48 elk into the Davis Mountains and 51 elk into the Wylie Mountains from an eastern Oregon source population (Coykendall 1990). It is possible that descendants of these introductions in the Davis and Wylie Mountains spawned the populations now inhabiting the Trans-Pecos region (Eagle, Glass, and Guadalupe Mountains, as well as the intervening lowlands).

A third scenario that could explain the repopulation of elk in the Trans-Pecos region is from natural emigration from nearby New Mexico populations. The Trans-Pecos elk populations are connected to current populations of elk in New Mexico by several potential routes including the Sacramento, Delaware, and Guadalupe Mountains; or alternatively lowland crossings across foothills and plains regions. These natural corridors could be used for immigration of elk into Texas from established herds occupying New Mexico. It is possible that populations throughout the current distribution are conforming to the resident species theory, which promotes the presence of population-specific allele frequencies, and would indicate that populations between the two states have been separate and are pursuing their own evolutionary trajectories (Pohler et al. 2014). However, if populations are freely traveling between the border of the states and breeding, admixture would result in an inability to detect any type of genetic structure between the two states. It is worth noting that elk were extripated in New Mexico around 1908 and modern-day populations are a result of reintroduction efforts involving Rocky Mountain Elk (*C. e. nelsoni*) from Colorado (Ligon 1927; New Mexico Department of Game and Fish 1967; Findley 1975).

The goal of this study was to determine if any of these three possible scenarios (or combination of scenarios) reflects the most appropriate explanation for the elk located in the Trans-Pecos region. Therefore, DNA sequences from the mitochondrial cytochrome-*b* (*Cyt*-b) and D-loop control region, and nine microsatellite markers were examined due to their ability to detect differences at the species, subspecies, and population level (Meredith et al. 2005, 2007; Ludt et al. 2004). To elucidate among these three scenarios, samples from the Davis and Glass mountain ranges were compared to samples collected throughout the range of *C. c. nelsoni*. Further, samples from South Dakota (*C. c. canadensis*) were included to serve as representatives of historic individuals that were transplanted into the Trans-Pecos region.

MATERIALS AND METHODS

Sampling.—Samples were collected from free-ranging populations in the Glass (January 2008 and September/October 2009) and Davis (August 2014) Mountains (see Appendix). Ear clips and hair samples were obtained from 13 different individuals that were captured during a three-year ecological and movement study conducted on elk in this region (Pohler et al. 2014). Six samples from the Davis Mountains were acquired opportunistically from road kill and velvet sheds by Texas Parks and Wildlife Department (TPWD). The New Mexico Department of Game and Fish (NMDGF) and TPWD provided tissues (muscle, hair) and ear clip samples (2014/2015 hunting season) from neighboring populations potentially connected through possible emigration routes. Eight opportunistic samples (i.e., road kill/chronic wasting studies) were collected by NMDGF in 2013-2014 and added to the sampling scheme. South Dakota Game Fish and Parks provided four samples from the Black Hills in the fall of 2013. The Natural Science Research Laboratory (NSRL) Museum of Texas Tech University, provided two samples collected in southern New Mexico. In total, 38 samples (19 from Texas, 15 from New Mexico, and four from South Dakota) from 10 localities were examined in this study (Fig. 1).

Specific data were recorded including locality and sex, and each individual was assigned a TK number, a unique identification number of the NSRL. No voucher specimens were collected due to the game species status, however, all possible tissues (muscle, hair, ear clips, and liver) were sampled and stored at -80° C.

DNA sequencing.—Genomic DNA (nuclear and mitochondrial) was extracted following the protocols of the manufacture for a Qiagen DNeasy blood and tissue kit (Qiagen Inc., Valencia, California). Polymerase chain reaction (PCR) was used to amplify the entire *Cyt*-b gene (1,140 base pairs). Amplification followed methods from Ludt et al. (2004) using primers LGL766/765 (Bickham et al. 1995; Bickham et al. 2004). D-loop primers (L0, D1, E1, and S0; Douzery and Randi, 1997) were used to amplify a 1,296 base pair region. PCR methods followed the standard HotStar Taq (Qiagen Inc., Valencia, California) amplification protocol. PCR amplifications for both mitochondrial

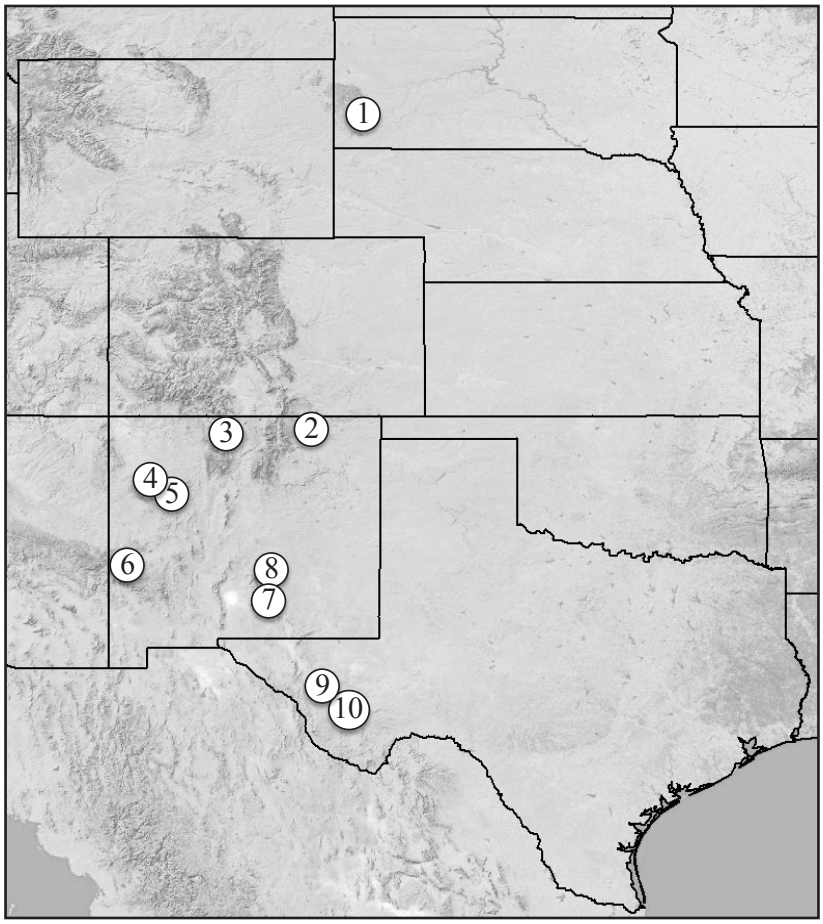


Figure 1. Map depicting the location of sampling sites. White circles denote collection localities of individuals examined in this study and numbers refer to the localities provided in the Appendix.

regions were conducted in 25-µL reactions containing 25 ng of DNA, 5 units of Taq, 100 µM of each primer, 25 µM of $MgCl_2$, 10 µM of Bovine serum albumin, 10 µM of a nucleotide mixture and 5.8 µL of double distilled water (ddH_2O). The thermal profile for both *Cyt*-b and D-loop was as follows: an initial denaturation of 94° C for 3 min, followed by 28 cycles of 94° C for 30 sec, 45° C for 30 sec, and 72° C for 40 sec, and a final extension at 72° C for 20 min. PCR products were purified with ExoSAP-IT (Affymetrix, Santa Clara, California).

PCR Primers and ABI Prism Big Dye version 3.1 terminator technology (Applied Biosystems, Foster City, California) were used to cycle sequence the amplified products. Cycle sequencing reactions were purified using Sephadex G50 (Amersham Pharmacia Biotech, Piscataway, New Jersey) cleanup protocols and were analyzed with an ABI 3100-Avant automated sequencer. Resulting sequences were aligned and proofed using Sequencher 4.0 or 4.1.2 software (Gene Codes, Ann Arbor, Michigan); chromatograms were examined to verify all base calls. All Cytb sequences obtained in this study were deposited in GenBank and are listed in the Appendix.

Microsatellite amplification.—Nine microsatellites were selected from previous studies based on their effectiveness in detecting population-level allelic frequency differences in elk. Primers t-28, t-108, t-158, t-193, t-501, t-107, t-273, t-115, and t-507 (Meredith et al. 2005, 2007; Table 1) were amplified in 33 individu-

Table 1. The primers and dye tags used in this study are indicated below. Standard allelic and genotypic information is included as follows: number of alleles, size range, observed heterozygosity (H_o), expected heterozygosity (H_e), and polymorphic informative content (PIC).

Primer	5' dye Label	No. Alleles	Size Range (base pairs)	H_o	H_e	PIC
T26	6FAM	10	328–398	0.625	0.802	0.765
T107	6FAM	15	235–259	0.441	0.719	0.695
T108	6FAM	11	136–181	0.611	0.879	0.854
T115	6FAM	12	180–192	0.742	0.807	0.775
T156	VIC	22	143–249	0.813	0.932	0.911
T193	6FAM	5	184–220	0.733	0.727	0.667
T501	NED	15	252–290	0.531	0.767	0.729
T507	NED	13	148–202	0.647	0.862	0.837

als using PCR methods similar to Meredith (2007). The thermal profile includes a heating phase at 80° C, followed by 95° C for 10 minutes, then 40 cycles each of 45 second denaturation at 95° C, a 90 second annealing step from 48° C to 64° C (based on melting point for each primer) and 60 second elongation at 72° C with a final extension for 10 minutes at 72° C. Allele separation included 0.5 µl of 400 or 500HD RX size standard (Applied Biosystems Inc., Waltham, MA), 8.5 µl of Hi-Di Formamide (Applied Biosystems Inc., Waltham, MA), and 1 µl of PCR product. GeneMapper software (version 4.0; Applied Biosystems Inc., Foster City, California) was used to determine fragment size, and ultimately allelic composition. Alleles were binned (averaged) to account for marginal error in determining fragment size.

Data analyses.—Gene and nucleotide diversity were estimated using MEGA v6 (Tamura et al. 2013) for all individuals included in the study. The program MUSCLE (Edgar 2004) within the MEGA v6 software package (Tamura et al. 2013) was used to align 30 sequences of both *Cyt*-b gene and D-loop region to outgroup and reference sequences of *C. elaphus*, *Odocoileus virginianus*, and *Odocoileus hemionus*.

All full-length *Cyt*-b sequences were uploaded into Popart (Leigh and Bryant 2015). A parsimony network was constructed to determine the number of haplotypes throughout the distribution (Clement et al. 2000). Results were then mapped over the distribution/sample locality map of Texas, New Mexico, and South Dakota.

To infer the evolutionary relationships of individuals, Bayesian phylogenetic analyses were conducted on the *Cyt*-b, D-loop, and combined dataset of both mitochondrial genes in MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). *Cyt*-b gene and D-loop sequences were aligned and partitioned by gene and *Cyt*-b sequences were further partitioned by codon position. Model selection using MrModeltest 2.3 (Naylander et al. 2004) selected GTR + I + Γ model of nucleotide substitution for both genes; values for model parameters were not defined *a priori* but were treated as unknown variables with uniform priors. Bayesian analyses were performed using 4 simultaneous Markov chains at a "temperature" of 0.02, with random, unconstrained starting trees. Each chain ran for 20,000,000 generations with trees sampled every 1,000 generations. Three independent MrBayes analyses were conducted to ensure that each run produced similar stationary likelihood values. The first 1,000,000 trees of each run were discarded as burn-in and the remaining trees were used to calculate the posterior probabilities and 50% majority rule consensus tree. Clade probabilities of ≥ 0.95 were used to indicate nodal support.

Microsatellite analysis.— Structure version 2.3.4 (Pritchard et al. 2000) was used to estimate genetic population structure within the three sampling areas using the multi-locus genotype data. Initially, K (hypothesized population number) was set to 3 to avoid underestimation of the number populations represented in the study; assuming that K would equal either: all individuals belong to a single population (K = 1), that the individuals belong to two populations (K = 2), and that the individuals belong to three or more populations (K = 3). These tests were then replicated two times using Admixture and no Admixture. Structure parameters: burn-in length = 1,000,000 Monte Carlo Markov chain repetitions = 10,000,000, K = 3, with ten iterations at each K value. The allelic dataset was uploaded into Structure, analyzed and the output data were compiled and compressed to be loaded into Structure Harvester to determine the most appropriate value of K (Earl et al. 2012). Highest value obtained for ΔK was used to determine the estimated populations of both runs, and the appropriate file of population assignment.

The program GenAlEx version 6.501 (Peakall and Smouse 2006) was used to estimate allele frequencies as well as observed and expected heterozygosity (H_o and H_e). The theoretical populations defined by the harvester analysis were used as *a priori* groupings for Analysis of Molecular Variance (AMOVA), and Principal Coordinate Analysis (PCoA). Probability of Identity analyses (probability of encountering 2 individuals with identical genotypes) was calculated within GenAlEx. Polymorphic information content (PIC) was estimated using CERVUS version 2.0 (Marshall et al. 1998).

Six DIY Approximate Bayesian Computation simulations (DIYABC, Cornuet et al. 2014) were designed to examine the three scenarios used to explain the possible origin of contemporary elk in Texas. Two simulations per scenario (one inferring a population bottleneck and one without a bottleneck) were run to more thoroughly assess potential variables associated with the three possible scenarios. The DIYABC simulations were as follows: 1) fragmentation of Texas populations from the historic panmictic distribution (*sensu* early 1800 North America population) with a genetic bottleneck; 2) successful establishment of South Dakota elk into the Guadalupe Mountains of Texas (in approximately 1928) with a genetic bottleneck; 3) natural immigration of elk into Texas (approximately 1930's) from naturally occurring herds in New Mexico with a genetic bottleneck; 4) fragmentation of Texas populations from the historic panmictic distribution without a bottleneck (*sensu* 1800 North America population); 5) successful dispersal of elk into Texas from the South Dakota individuals that were introduced into the Guadalupe Mountains without a bottleneck (approximately 1928); and 6) natural immigration of elk into Texas (approximately 1930's) from established herds in New Mexico without a bottleneck.

Parameters of the DIYABC simulations (n = population size; t = time in generations) were based on historical data from Gill (2013) with a generation time of two years. The effective population size used in the simulation (n = 378) was estimated from the LDNE program (Waples and Do 2008). For simulations, parameters incorporated included: current population size, population size during inferred bottleneck, duration of bottleneck, pre-bottleneck population size, historic population size, and estimates of generations. Bayesian posterior probabilities were then used to compare the simulated and observed data.

RESULTS

Thirty *Cyt*-b sequences were examined and possessed the following nucleotide composition: T = 29.1%, C = 27%, A = 30.8%, and G = 13.1%. Thirty-one sites were determined to be variable and six sites were phylogenetically informative. The D-loop dataset consisted of 33 sequences and the nucleotide sequence composition as follows: T = 30.3%, C = 22.5%, A = 31.5%, and G = 15.8%. Eighty-four sites were determined as being variable, 74 of these sites were the result of insertions or deletions, and eight where identified as standard phylogenetically informative sites.

Cyt-b and D-loop sequences were analyzed individually and then concatenated into a single dataset (n = 30). All three Bayesian analyses produced similar topologies: consequently, only the results of

the concatenated analysis are discussed herein (Fig. 2). Nodal support was recovered for the separation of the European red deer (*C. elaphus*) and elk (*C. canadensis*); however all samples of elk were contained with a single clade (albeit no differentiation of populations was supported). Genetic divergence values (Kimura-2 parameter; Kimura 1980) indicated that individuals that comprised clades I and II differed by 0.38% and all individuals differed from European red deer by 5.73%.

The microsatellite dataset (33 individuals) possessed allelic richness values ranging from 5 (locus T-195) to 22 alleles (locus T-156) with an average of 12.88 (Table 1). Observed heterozygosity values (Table 1) ranged from 0.441 (locus T-107) to 0.813 (locus T-156). Probability of Identity was 4.0 X 10^{-8} (1 in 25,000,000 individuals). In general, the results of the Structure Harvester analysis indicated that samples could be separated into two populations (Fig. 3). Structure results indicate that Population 1 contained 13 individuals from the Glass Mountains (n = 11), Davis Mountains (n = 1), and New Mexico (n = 1) with a posterior probability ≥ 0.50; and that Population 2 was comprised of 14 individuals from the Glass Mountains

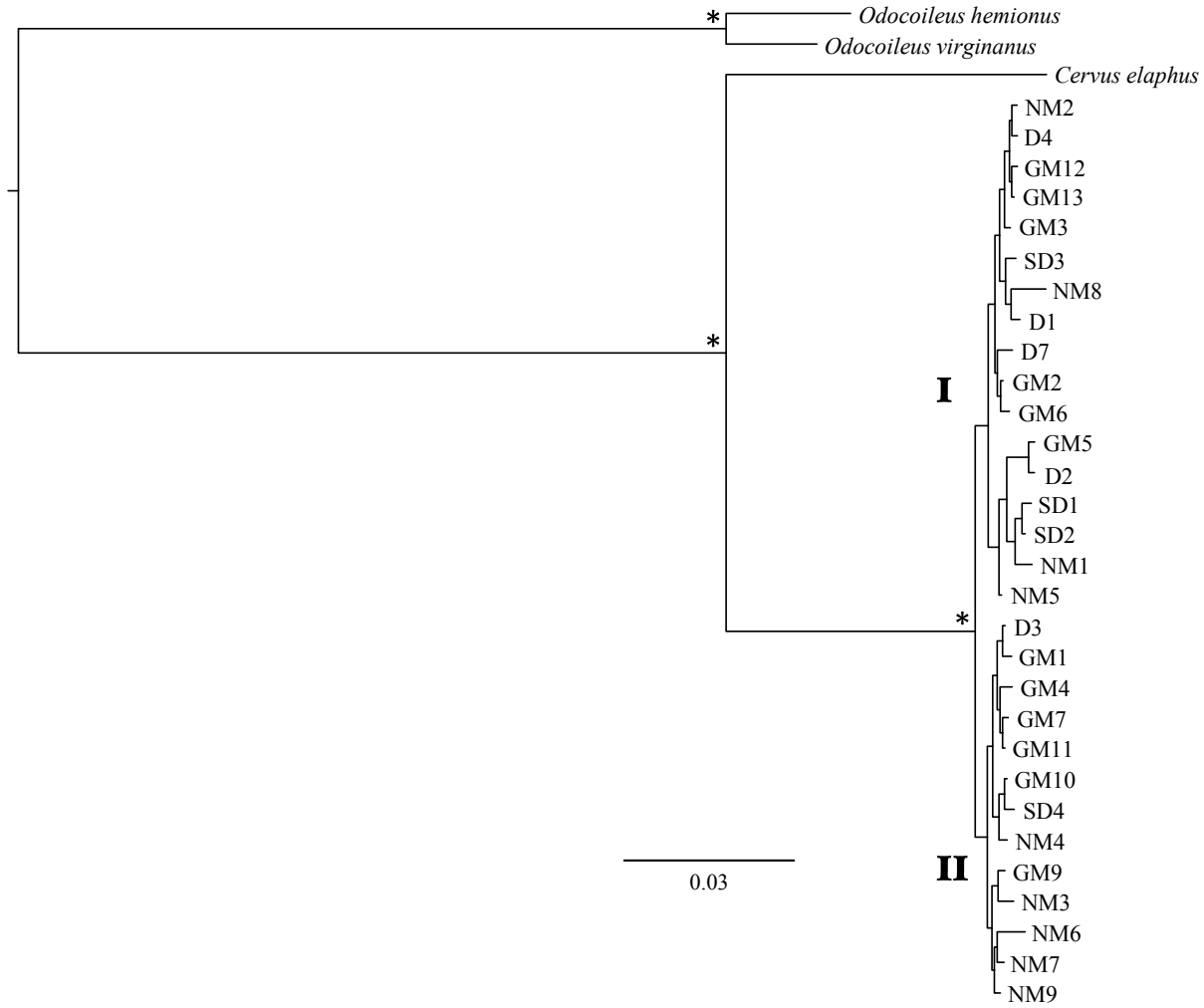


Figure 2. Bayesian tree generated from the concatenated sequence dataset (*Cyt*-b and D-loop sequences). Roman numerals indicate clades as referred to in the text. Asterisks (*) indicate a posterior clade probability of 95% or greater. Individual labels are Glass Mountains (GM), Davis Mountains (D), New Mexico (NM), and South Dakota (SD). Locality and specimen data are provided in the Appendix.

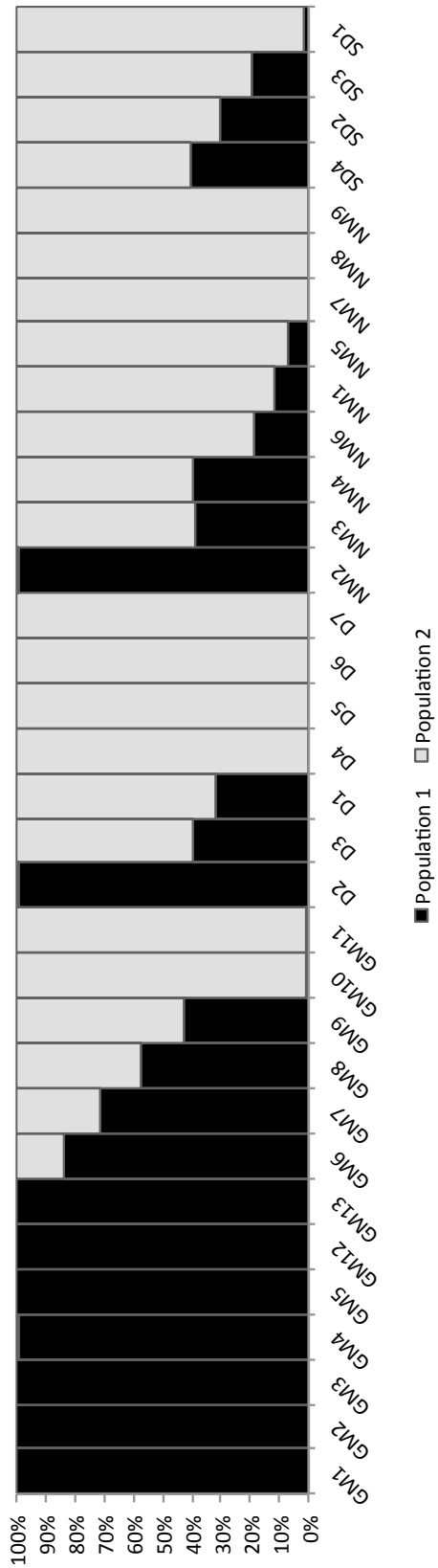


Figure 3. Assignment of microsatellite populations as determined by the software program Structure. The specimen identification, for each sample, is indicated below each histogram. Shading signifies the proportion of alleles assigned to either Population 1 (black bars) or Population 2 (gray bars).

(n =2), New Mexico (n = 8), and South Dakota (n = 4) with a posterior probability ≥ 0.50.

Structure population designations were used for the subsequent AMOVA and PCoA analyses. The AMOVA partitioned the genetic variation into 4% among populations and 96% within populations. The overall fixation index ($F_{st;}$ population differentiation due to genetic structure) between Populations 1 and 2 was 0.046 implying a panmictic population. Principal coordinate analysis (PCoA) was used to visualize relationships of inferred populations across the first two axes of variation (PCA 1 - 21.3% and PCA 2 - 12.2%; Fig. 4). Individuals from inferred Populations 1 and 2 generally segregated primarily based on the second axis of variation.

Microsatellite data processed in DIYABC showed support for one of the six simulations (natural immigration of elk into Texas from naturalized and native herds in New Mexico - without a bottleneck) was supported (posterior probability value = 0.99) (Table 2). All other simulations resulted in posterior probability values ≤ 0.45 leading to the dismissal of those simulation conditions as possible explanations for the origin of contemporary elk in Texas. Further, simulations did not detect evidence of a genetic bottleneck during the past 350 generations indicating a long, shared history between Texas and New Mexico populations.

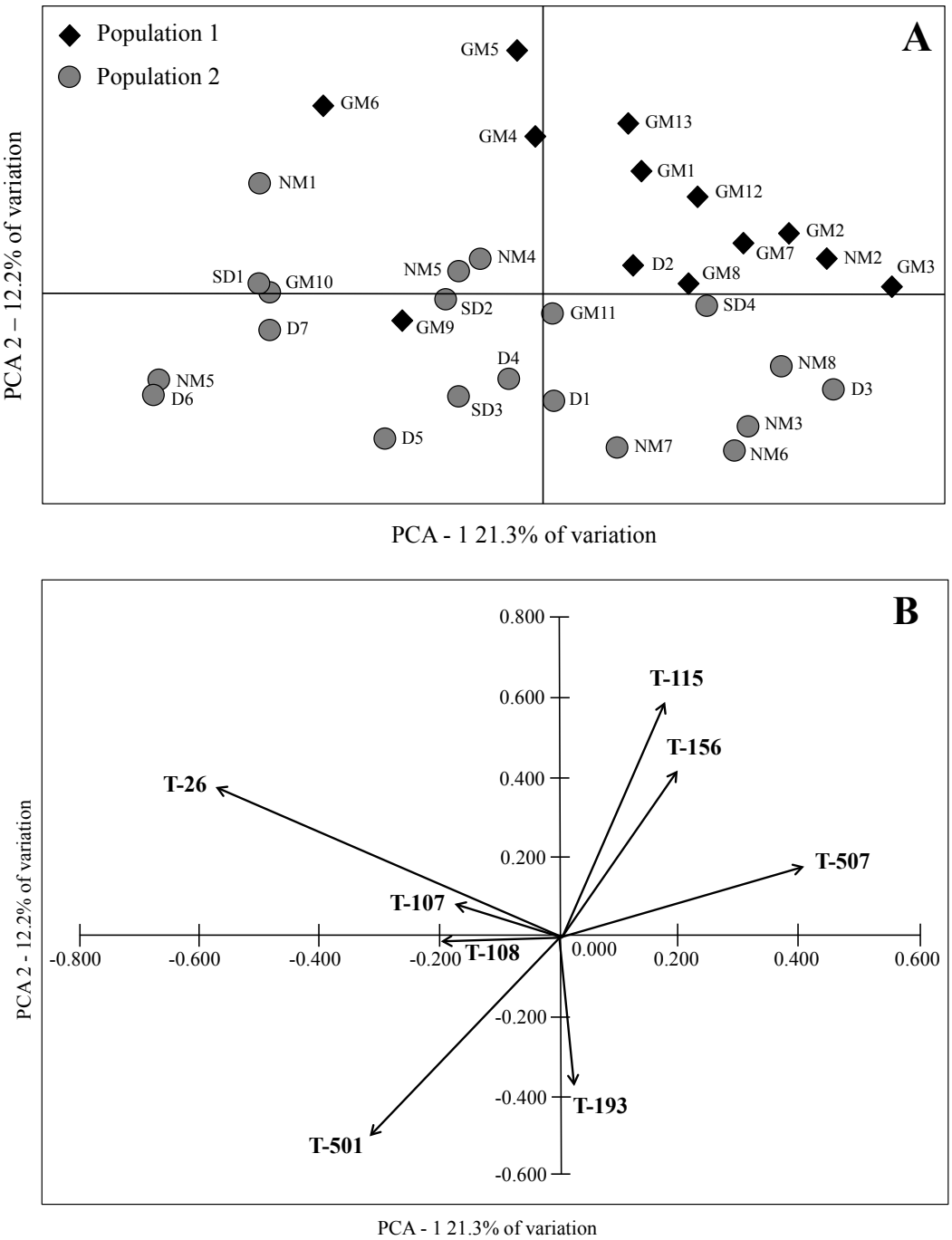


Figure 4. A) Assignment of populations as determined from the principal coordinate analysis. Coordinates 1 and 2 explain 21.3% and 12.2% of the variation of the dataset, respectively. Groups are assigned based on designation by the Structure analysis. Individual labels are Glass Mountains (GM), Davis Mountains (D), New Mexico (NM), and South Dakota (SD). Black diamonds represent Population 1 and grey circles indicate samples assigned to Population 2 by Structure Harvester. B) Locus effect on the principle component analysis (PC1 to PC2) depicted in Figure 4A. Direction and distance away from the origin (0.000) indicates the magnitude of variation contributed by each locus effecting the group association in Panel A.

Table 2. Information used to conduct the six simulation analyses as outlined in the text, with simulated demographic histories 1–3 representing population bottlenecks and simulated demographic histories 4–6 a constant population size. Simulated demographic histories 1 and 4 modeled a shared ancestry for all contemporary populations, simulated demographic histories 2 and 5 represent Texas samples sharing a most recent common ancestor with South Dakota samples, and simulated demographic histories 3 and 6 represent Texas populations sharing a most recent common ancestor with New Mexico samples. Abbreviations are as follows: S = simulated demographic history; N1–N5 = population size, ranging from lowest to upper set limit; N2b = population size ranging from lowest to upper set limit within a bottle neck; T1-db, generation size, during a bottle neck; T1–T4 = generation size, ranging from lowest to upper set limit; PV = posterior probability values; and CI = confidence interval values. 0.95 PV or greater indicates a supported simulated demographic history.

S	N1–N3	N2b	T1-db	T1	T2	N4	T3	N5	T4	PV	CI
1	(500, 2,000)	(1, 100)	(1, 10)	(60, 120)	(75, 160)	(1,000, 10,000)	(120, 190)	(10,000, 1,000,000)	(250, 350)	0.01	(0.00, 0.02)
2	(500, 2,000)	(1, 100)	(1, 10)	(60, 120)	(75, 160)	(1,000, 10,000)	(120, 190)	(10,000, 1,000,000)	(250, 350)	0.04	(0.03, 0.06)
3	(500, 2,000)	(1, 100)	(1, 10)	(60, 120)	(75, 160)	(1,000, 10,000)	(120, 190)	(10,000, 1,000,000)	(250, 350)	0.01	(0.00, 0.02)
4	(500, 2,000)	-	-	(60, 120)	(75, 160)	(1,000, 10,000)	(120, 190)	(10,000, 1,000,000)	(250, 350)	0.02	(0.01, 0.03)
5	(500, 2,000)	-	-	(60, 120)	(75, 160)	(1,000, 10,000)	(120, 190)	(10,000, 1,000,000)	(250, 350)	0.45	(0.43, 0.46)
6	(500, 2,000)	-	-	(60, 120)	(75, 160)	(1,000, 10,000)	(120, 190)	(10,000, 1,000,000)	(250, 350)	0.99	(0.99, 0.99)

DISCUSSION

Analyses of the concatenated dataset containing the *Cyt*-b and D-loop sequences did not separate samples based on a recognizable geographic pattern (Figure 2); instead individuals from all major sampling regions (South Dakota, New Mexico, Davis Mountains, and Glass Mountains) were contained within both clades I and II. Strong support was obtained at the base of clades I and II (posterior probability value of 1.00); however, the genetic divergence between the two clades was small (0.35%). The lack of topological resolution from mitochondrial sequences is similar to that reported in other genetic studies of intraspecific relationships among elk (Ludt et al. 2004). It appears that these genetic markers are not evolving at a sufficient rate to distinguish among populations.

Analysis of microsatellite data detected allele frequency differences among individuals sampled from the Trans-Pecos populations. Allelic richness values ranged from 5 (locus T-195) to 22 alleles (locus T-156) with an average of 12.88 (Table 1). Meredith et al. (2007) reported reduced microsatellite variation in Tule elk given the severe bottleneck effects in the late 1800s. Many of the microsatellite markers reported in their study possessed fewer than five alleles (lowest reported in this study was five with average of 7.3). Low levels of genetic variability in the Tule elk was suggested to have occurred as a function of low numbers of founders rather than insufficient sampling (average number of alleles = 3.2). The genetic uniqueness of the Tule elk (Meredith et al. 2007) resulted from lack of genetic variation, not from novel genetic variability when compared against Rocky mountain elk (*C. c. nelsoni*) and Roosevelt elk (*C. c. roosevelti*). In contrast, the current study detected a different pattern in allelic richness (20 unique alleles in Population 1) throughout the distribution of *C. c. nelsoni* with high levels of genetic diversity.

Results of the Structure Harvester analysis indicated that the most appropriate partitioning of genetic differences is best explained by recognizing two populations. The first population contained a total of 13 individuals from the Glass Mountains (n = 11), Davis Mountains (n = 1), and New Mexico (n = 1), whereas the second population contained 14 individuals from the Glass Mountains (n =2), New Mexico (n = 8), and South Dakota (n = 4). When comparing the Glass Mountains (Locality 10) to the Davis Mountains (Locality 9), the two populations shared 73% of alleles with 27% unique to the Glass Mountains (20 alleles). The Davis Mountains individuals (Locality 9) were then compared to the individuals sampled closest in New Mexico (Locality 8), which shared 88% of their alleles with 12% being unique to Locality 8. The results indicated the contemporary Texas herds could have originated from a New Mexico population dispersing into the Davis, Guadalupe, and Glass Mountains. Alternatively, the introduction of elk into the Glass Mountains in 1944 (one bull and two cows, Swepston 1985) followed by gene flow from this "founder population" to populations in the Davis Mountains and New Mexico populations may explain the shared genotypes of these populations and the presence of unique alleles in the Glass Mountains. This indicates that either elk in the Davis Mountains genetically are closer to elk in New Mexico, or alternatively there recently has been recent gene flow between the two populations. Further, the individuals from South Dakota did not form an independent group from either the Texas or New Mexico samples.

The combined genetic data (*Cyt*-b, D-loop, and microsatellites) and computer simulations were used to evaluate the three major scenarios concerning the reappearance of elk in Texas. Both population Structure analysis and simulations indicated that the scenarios involving reintroduced or escapees from high fence ranches (Scenario 1), and the reintroduction of elk into Texas from South Dakota (Scenario 2) were not supported. However, these results supported simulation 6 (without an inferred bottleneck, posterior probability = 0.99), which modeled the immigration of elk into Texas from New Mexico without a bottleneck. The fact that simulations did not detect evidence of a genetic bottleneck during the past 350 generations depicts a long, shared history between Texas and New Mexico populations.

Differences in the allelic abundance in elk populations in Texas support the recent and natural immigration of individuals from New Mexico or the survival of genotypes from past introductions involv-

ing individuals from New Mexico populations. It is possible that elk emigrated from either the Guadalupe Mountains or surrounding foothills into the northern regions of the Trans-Pecos via the Davis, Sacramento, and Delaware Mountain ranges. If descendants of the introduced individuals transplanted into the Davis Mountain region (from eastern Oregon) contributed significantly to the gene pool, then the contemporary samples from the Davis Mountain should not have shown a genetic affinity to the Glass Mountain population or to the individuals sampled from New Mexico; instead they should genetically be different. Unfortunately, we were not able to included samples from eastern Oregon into this study; consequently that scenario cannot be rejected until such samples are examined. Consequently, the scenario that Texas elk are a natural population and a product of emigration from herds in southern New Mexico and subsequent establishment of viable populations remains a viable scenario. More thorough sampling of elk populations throughout the Trans-Pecos area and southeastern New Mexico is needed to determine genetic boundaries and possible population subdivsion. Further, samples from Colorado and Oregon are needed to establish genotypes for comparison to Texas populations.

ACKNOWLEDGMENTS

We thank Laramie L. Lindsey, Taylor J. Soniat, James Q. Francis, Nicté Ordoñez-Garza, Emily A. Wright, Emma K. Roberts, and Whitney N. Watson for assistance in laboratory work and reviewing previous versions of this manuscript. John Karges provided valuable historical insights concerning the history of Trans-Pecos elk. Partial funding was provided by the Texas Academy of Science, Texas Society of Mammalogists, Texas Tech University Association of Biologists, and Aaron Games of the Rocky Mountain Elk Foundation. We would also like to thank Nicole Quintana (New Mexico Department of Game and Fish), Steven Griffin (South Dakota Game, Fish, and Parks), Austin Stolte (Texas Parks and Wildlife Department), and Louis Harveson (Sul Ross State University) for assistance with sample collection. Thanks also are extended to Heath Garner and Kathy MacDonald for assistance in depositing tissues into the NSRL at the Museum of Texas Tech University.

LITERATURE CITED

Bailey, V. 1905. Biological survey of Texas. North American Fauna 25:1–222.

Bickham, J. W., C. C. Wood, and J. C. Patton. 1995. Biogeographic implications of cytochrome b sequences and allozymes in sockeye (*Oncorhynchus nerka*). Journal of Heredity 86:140–144.

Bickham, J. W., J. C. Patton, D. A. Schlitter, I. L. Rautenbach, and R. L. Honeycutt. 2004. Molecular phylogenetics, karyotypic diversity, and partition of the genus *Myotis* (Chiroptera: Vespertilionidae). Molecular Phylogenetics and Evolution 33:333–338.

Bryant, L. D., and C. Maser. 1982. Classification and distribution. Pp. 1–59 in Elk of North America: Ecology and Management (J. W. Thomas and D. E. Toweill, eds.). Stackpole Books, Harrisburg, Pennsylvania.

Clement, M., D. Posada, and K. A. Crandall. 2000. TCS: a computer program to estimate gene genealogies. Molecular Ecology 9:1657–1659.

Cornuet, J.M., P. Pudlo, J. Veyssier, A. Dehne-Garcia, M. Gautier, R. Leblois, J.M. Marin, and A. Estoup. 2014. DIYABC v2.0: A software to make approximate Bayesian computation inferences about population history using single nucleotide polymorphism, DNA sequence, and microsatellite data. Bioinformatics 30:1187–1189.

Coykendall, A. 1990. 1988 Elk transplants in the Davis Mountains, Jeff Davis County, and the Wylie Mountains, Culberson County, Texas. M.S. Thesis, Sul Ross State University, Alpine.

Davis, W. B. 1940. Mammals of the Guadalupe Mountains of western Texas. Occasional Papers of the Museum of Zoology, Louisiana State University 7:69–84.

Douzery, E., and E. Randi. 1997. The mitochondrial control region of Cervidae: Evolutionary patterns and phylogenetic content. Molecular Biology and Evolution 14:1154–1166.

Earl, D. A. 2012. Structure Harvester: A website and program for visualizing Structure output and implementing the Evanno method. Conservation Genetics Resources 4:359–361.

Edgar, R. C. 2004. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32:1792–1797.

Findley, J. S., A. H. Harris, D. E. Wilson, and C. Jones. 1975. Mammals of New Mexico. University of New Mexico Press, Albuquerque.

Genoways, H. H., R. J. Baker, and J. E. Cornely. 1979. Mammals of the Guadalupe Mountains National Park. Pp. 259–270 in Biological Investigations in the Guadalupe Mountains National Park, Texas: Proceedings of a Symposium Held at Texas Tech University, Lubbock, Texas, April 4–5, 1975 (H. H. Genoways and R. J. Baker, eds.). National Park Service, Proceedings and Transactions Series No. 4.

Gill, R. B., C. Gill, R. Peel, and J. Vasquez. 2013. Are elk native to Texas? Self-published, Richardson B. Gill, San Antonio, Texas. Accessed online, http://circleranchtx.com/are-elk-native-to-texas-yes/.

Hall, E. R. 1981. The Mammals of North America. 2nd ed. John Wiley & Sons, Inc., New York.

Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16:111–120.

Langvatn, R., and A. Loison. 1999. Consequences of harvesting on age structure, sex ratio and population dynamics of red deer *Cervus elaphus* in central Norway. Wildlife Biology 5:213-223.

Leigh, J. W., and D. Bryant. 2015. Popart: Full-feature software for haplotype network construction. Methods in Ecology and Evolution 6:1110–1116.

Ligon, J. S. 1927. Wild Life of New Mexico: Its Conservation and Management. State Game Commission, Dept. of Game and Fish, Santa Fe, New Mexico.

Ludt, C. J., W. Schroeder, O. Rottmann, and R. Kuehn. 2004. Mitochondrial DNA phylogeography of red deer (*Cervus elaphus*). Molecular Phylogenetics and Evolution 31:1064–1083.

Mahmut, Halik, R. Masuda, M. Onuma, M. Takahashi, J. Nagata, M. Sazuki, and N. Ohtaishi. 2002. Molecular phylogeography of the red deer (*Cervus elaphus*) populations in Xinjiang of China: Comparison with other Asian, European, and North American populations. Zoological Science 19:485–495.

Marshall, T. C., J. Slate, L. E. B. Kruuk, and J. M. Pemberton. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. Molecular Ecology 7:639–655.

McAlpine, S. 1990. Continued decline of elk populations within Guadalupe Mountains National Park, Texas. The Southwestern Naturalist 35:362–363.

McCullough, D. R., J. K. Fischer, and J. D. Ballou. 1996. From bottleneck to metapopulation: Recovery of the tule elk in California. Pp. 375–404 in Metapopulations and Wildlife Conservation (D. R. McCullough, ed.). Island Press, Washington, D.C.

Meredith, E. P J. A. Rodzen, K. F. Levine, and J. D. Banks. 2005. Characterization of an additional 14 microsatellite loci in California Elk (*Cervus elaphus*) for use in forensic and population applications. Conservation Genetics 6:151–153.

Meredith, E. P., J. A. Rodzen, J. D. Banks, R. Schaefer, H. B. Ernest, T. R. Famula, and B. P. May. 2007. Microsatellite analysis of three subspecies of elk (*Cervus elaphus*) in California. Journal of Mammalogy 88:801–808.

Naylander, J. A., F. Ronquist, J. P. Huelsenbeck, and J. L. Nieves-Aldrey. 2004. Bayesian phylogenetic analysis of combined data. Systematic Biology 53:47–67.

New Mexico Department of Game and Fish. 1967. New Mexico Wildlife Management. New Mexico Game and Fish Commission, Santa Fe.

Nowak, R. M. 1999. Walker's Mammals of the World, 6th ed. Johns Hopkins University Press, Baltimore, Maryland.

O'Gara, B. W., and R. G. Dundas. 2002. Distribution: Past and present. Pp. 67–119 in North American Elk: Ecology and Management (D. E. Toweill and J. W. Thomas, eds,). Smithsonian Institution Press, Washington, D.C.

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

Pohler, P. S., L. A. Harveson, and P. M. Harveson. 2014. Demographic characteristics of elk in the Glass Mountains, Texas. Wildlife Society Bulletin 38:466–472.

Polziehn, R. O., J. Hamr, F. F. Mallory, and C. Stobeck. 2000. Phylogenetic status of North American wapiti (*Cervus elaphus*) populations. Molecular Ecology 9:1561–1576.

Polziehn, R. O., and C. Strobeck. 2002. A phylogenetic comparison of red deer and wapiti using mitochondrial DNA. Molecular Phylogenetics and Evolution 22:342–356.

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

Randi, E., N. Mucci, F. Claro-Hergueta, A. Bonnet, and E. J. P. Douzery. 2001. A mitochondrial DNA control region phylogeny of the Cervinae: Speciation in *Cervus* and implications for conservation. Animal Conservation 4:1–11.

Ronquist, F., and J. P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.

Schmidly, D. J. 2002. Texas Natural History: A Century of Change. University of Texas Press, Austin.

Schmidly, D. J., and R. D. Bradley. 2016. Mammals of Texas. Texas Tech University Press, Lubbock.

Sweptson, D. A. 1985. Big game investigations—elk introductions. Federal Aid in Wildlife Restoration Performance Report, Project W-109 R-7, Job 52, Texas Parks and Wildlife Department, Austin.

Tamura, K., G. Stecher, D. Peterson, A. Filipski, and S. Kumar. 2013. MEGA6: Molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution 30:2725–2729.

Toweill, D. E., and J. W. Thomas. 2002. North AmericanElk: Ecology and Management. Smithsonian Institution Press, Washington, D.C.

Waples, R. S., and C. Do. 2008. LDNE: A program for estimating effective population size from data on linkage disequilibrium. Molecular Ecology Resources 8:753–756.

Williams, E. S. 2005. Chronic wasting disease. Veterinary Pathology Online 42:530–549.

Witt, B. R. 2008. Range size and habitat use of elk in the Glass Mountains, Texas. M.S. Thesis, Sul Ross State University, Alpine.

Wright, G. M., and B. H. Thompson. 1935. Fauna of the national parks of the United States: Wildlife management in the national parks. USDI National Park Service, Fauna Series No. 2. U.S. Government Printing Office, Washington, D.C.

Addresses of authors:

CHRISTOPHER D. DUNN

Department of Biological Sciences
Texas Tech University
Lubbock, TX 79406-3131 USA
christopher.dunn@ttu.edu

MATTHEW R. MAULDIN

Department of Biological Sciences
Texas Tech University
Lubbock, TX 79406-3131 USA
mmauldin@cdc.gov

MARISA E. WAGLEY

Department of Biological Sciences
Texas Tech University
Lubbock, TX 79406-3131 USA
marisa.wagley@ttu.edu

JEREMY E. WILKINSON

RTL Genomics
Lubbock, TX 79407
jeremy.wilkinson@ttu.edu

CALEB D. PHILLIPS

Department of Biological Sciences and the Museum
Texas Tech University
Lubbock, Texas 79409-3131
caleb.phillips@ttu.edu

ROBERT D. BRADLEY

Department of Biological Sciences and the Museum
Texas Tech University
Lubbock, Texas 79409-3131
robert.bradley@ttu.edu

Editor for this manuscript was David Ray

APPENDIX

Specimens examined in the DNA sequencing and microsateliite portions of this study. For each specimen, the collection locality, specimen identification number (SD = South Dakota, NM = New Mexico, D = Davis Mountains, and GM = Glass Mountains), museum identification number (TK), and GenBank accession number are provided in parentheses. GenBank accession numbers for cytochrome-*b* sequences are to the left of the slash and D-loop accession numbers are to the right. For three samples (TK254263, TK254264, and TK174564) only microsatellite data were obtained. Localities refer to those shown on Figure 1.

Cervus canadensis.—Locality 1. South Dakota: Pennington County; Custer State Park (SD1: TK254268, KY751417/KY751473 and SD2: TK254269, KY751416/KY751474), Deerfield (SD3: TK254266, KY751419/KY751471), and Jewel Cave (SD4: TK254267, KY751418/KY751472).

Locality 2. New Mexico: Coflax County; 13 mi SE of Raton (NM9: TK254258, KY751420/KY751470).

Locality 3. New Mexico: Rio Arriba County; 3 miles south of Valle Grando Peak (NM4:TK254254, KY751425/KY751466).

Locality 4. New Mexico: McKinley County; Marquez Wildlife Management Area (NM2: TK254252, KY751427/KY751464).

Locality 5. New Mexico: McKinley County (NM3: TK254253, KY751426/KY751465).

Locality 6. New Mexico: Catron County; Pelona Mountain (NM5: TK254255, KY751424/KY751467; NM6: TK254256, KY751423/KY751468; and NM7: TK254257, KY751422/KY751469).

Locality 7. New Mexico: Otero County; 8 miles W. Sacramento (NM8: TK123174, KY751421/KY751450).

Locality 8. New Mexico: Chaves County; Benito canyon in the White Mountain Wilderness (NM1: TK254251, KY751428/KY751463).

Locality 9. Texas: Jeff Davis County; Davis Mountains (D1: TK254259, KY751433/KY751459; D2: TK254260, KY751432/KY751460; D3: TK254261, KY751431/KY751461; D4: TK254262, KY751430/KY751462; D5: TK254263, NA/NA; D6: TK254264, NA/NA; and D7: TK254265, KY751429/KY751475).

Locality 10. Texas: Brewster County; Glass Mountains (GM1: TK254270, KY751438/KY751451; GM2: TK254271, KY751437/KY751455; GM3: TK254272, KY751435/KY751456; GM4: TK254273, KY751436/KY751458; GM5: TK254274, KY751439/KY751449; GM6: TK254275, KY751440/KY751454; GM7: TK254276, KY751434/KY751448; GM8: TK174564, NA/NA; GM9: TK254277, KY751443/KY751452; GM10: TK254278, KY751442/KY751447; GM11: TK254279, KY751444/KY751446; GM12: TK254280, KY751441/KY751453; and GM13: TK254281, KY751445/KY751476).

Cervus elaphus.—AB021099

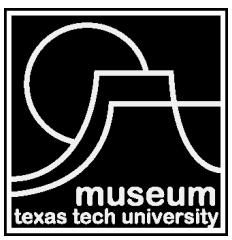
Odocoileus hemionus.—HM222707

Odocoileus virginianus.—DQ379370

Publications of the Museum of Texas Tech University

Institutional subscriptions to Occasional Papers are available through the Museum of Texas Tech University, attn: NSRL Publications Secretary, Box 43191, Lubbock, TX 79409-3191. Individuals may also purchase separate numbers of the Occasional Papers directly from the Museum of Texas Tech University.

Series Editor: Robert D. Bradley
Production Editor: Lisa Bradley



ISSN 0149-175X

Museum of Texas Tech University, Lubbock, TX 79409-3191