Section 6 (Texas Traditional) Report Review

Form emailed to FWS S6 coordinator (mm/dd/yyyy): Click here to enter a date.

TPWD signature date on report: 8/25/2016

Project Title: Assessment of Genetic Diversity of the State Threatened Texas Kangaroo Rat, *Dipodomys elator*, Using Museum Specimens

Final or Interim Report? Final

Grant #: TX E-163-R

Reviewer Station: Arlington ESFO

Lead station concurs with the following comments: NA (reviewer from lead station)

Interim Report (check one):

- ☐ Acceptable (no comments)
- ☐ Needs revision prior to final report (see comments below)
- ☐ Incomplete (see comments below)

Final Report (check one):

- ☒ Acceptable (no comments)
- ☐ Needs revision (see comments below)
- ☐ Incomplete (see comments below)

Comments:

No comments. Accepted as is.

FINAL PERFORMANCE REPORT

As Required by

THE ENDANGERED SPECIES PROGRAM

TEXAS

Grant No. TX E-163-R

(F14AP00823)

Endangered and Threatened Species Conservation

Assessment of Genetic Diversity of the State Threatened Texas Kangaroo Rat, *Dipodomys elator*, Using Museum Specimens

Prepared by:

Dr. Russell Pfau

Carter Smith
Executive Director

Clayton Wolf
Director, Wildlife

25 August 2016

FINAL REPORT

STATE: Texas GRANT NUMBER: TX E-163-R-1

GRANT TITLE: Assessment of Genetic Diversity of the State Threatened Texas Kangaroo Rat, *Dipodomys elator*, Using Museum Specimens.

REPORTING PERIOD: 1 September 2014 to 30 September 2016

OBJECTIVE(S). To assess patterns of genetic diversity in the state threatened Texas kangaroo rat (*Dipodomys elator*) using museum specimens.

Segment Objectives:

Task 1 February 2015 Obtain skin snips from museum specimens.
Task 2 March 2015 Extract DNA from skin snips.
Task 3 April 2015 Conduct PCRs to amplify two sections of the control region of the mitochondrial DNA.
Task 4 May-June 2015 Perform DNA sequencing.
Task 5 July 2015 Analyze DNA sequences to determine patterns of genetic diversity.
Task 6 August 2015- June 2016 Draft report and submit to TPWD. Draft manuscript for submission to Journal of Mammalogy. Present findings at research conference.

Significant Deviations:

None.

Summary Of Progress:

Please see Attachment A.

Location: Department of Biological Sciences, Tarleton State University, Stephenville, Texas USA.

Cost: Costs were not available at time of this report, they will be available upon completion of the Final Report and conclusion of the project.

Prepared by: Craig Farquhar Date: 25 August 2016

Approved by:  Date: 25 August 2016
C. Craig Farquhar

ATTACHMENT A

Assessment of Genetic Diversity of the State Threatened Texas Kangaroo Rat, *Dipodomys elator*, Using Museum Specimens

Russell S. Pfau

Department of Biological Sciences

Tarleton State University

Stephenville, TX 76402

254-968-9761

pfau@tarleton.edu

Abstract

The Texas kangaroo rat (*Dipodomys elator*) is listed as a threatened species by the Texas Parks and Wildlife Department because of its scarcity and the small geographic range from which it is known. For species such as this, it is important to understand the genetic factors affecting extinction risk and management decisions. Low genetic diversity can result in inbreeding depression (reduced reproduction and survival) and reduce a species' ability to adapt to environmental change. Additionally, understanding genetic structure among populations is important in defining management units and developing management strategies. The overall objective of my study was to assess patterns of genetic diversity in the Texas kangaroo rat. Specific aims included: 1) documentation of historical levels of genetic diversity at multiple localities across the distribution of the Texas kangaroo rat, 2) documentation of degree and patterns of genetic divergence across the distribution of the Texas kangaroo rat, 3) comparison of levels of genetic diversity between two different time periods at the same locality to document potential loss of diversity within the past few decades. To address these aims, I sequenced portions of mitochondrial DNA (mtDNA) and examined nuclear microsatellites. I found a striking lack of mtDNA variation which could be explained by an historical, species-wide genetic bottleneck (likely prior to the arrival of modern humans). Because of the lack of mtDNA variation, this marker is not effective in fully addressing the aims of this study. In contrast, microsatellites exhibited sufficient variation, and analyses were conducted using data from 11 loci and four populations. Allelic diversity and heterozygosity were similar between populations and temporal samples. Estimates of effective population size (*Ne*) ranged from 5-856, depending on method and population, with Iowa Park showing consistently lower values than Quanah. All methods addressing population structure indicated that the Iowa Park population was divergent from the others, with Vernon and Harrold showing a somewhat intermediate relationship but with a closer affiliation with Quanah than Iowa Park, despite their closer proximity to Iowa Park. This pattern does not conform to isolation by distance, thus genetic drift appears to have played a greater role than gene flow in establishing genetic structure. There was much less divergence between temporal samples compared to geographic samples, indicating that genetic drift has had only minimal impacts in shifting allelic frequencies over the time periods examined here.

Introduction

The Texas kangaroo rat (*Dipodomys elator*) is listed as a threatened species by the Texas Parks and Wildlife Department because of its scarcity and the small geographic range from which it is known (Martin 2002, Stangl and Schafer 1990). Recently, WildEarth Guardians (WG) petitioned United States Fish and Wildlife Service (USFWS) to federally list the Texas kangaroo rat (WG 2010) and the USFWS responded that listing may be warranted due to habitat loss and genetic isolation of populations (USFWS 2011). Historically, *D. elator* is known only from 11 counties in north-central Texas and two in southern Oklahoma. However, from 1996 to 2000, Martin (2002) surveyed the entire historic range of this species and found it only in five Texas counties (Archer, Childress, Hardeman, Motley, and Wichita) and none in Oklahoma, suggesting an apparent decline.

Most species of kangaroo rats prefer sandy habitats; however, *D. elator* has been found primarily in soils with high clay (as opposed to deep, sandy soils primarily occupied by D. *ordii*) content which support short grasses (Dalquest and Collier 1964, Roberts and Packard 1973, Dalquest and Horner 1984, Stangl et al. 1992, Goetze et al. 2007; Stasey 2010). It has rarely been recorded in locations with dense vegetation. Changes in vegetation composition and conversion of pastureland to monoculture have resulted in fragmentation of *D. elator* habitat (Diamond and Shaw 1990; Nelson et al. 2009) and may have contributed to its apparent decline. Several studies have examined the distribution and ecology of this species (Dalquest and Collier 1964, Roberts and Packard 1973, Stangl and Schafer 1990, Jones et al. 1988, Diamond and Shaw 1990, Moss and Mehlhop-Cifelli 1990, Stangl et al. 1992, Martin 2002, Goetze et al. 2007), but only one has assessed genetic diversity and population structure (Hamilton et al. 1987).

Conservation genetics seeks to understand the genetic factors affecting extinction risk and management decisions (Frankham et al. 2002, Smith and Wayne 1996). Low genetic diversity can result in inbreeding depression (reduced reproduction and survival) and reduce a species' ability to adapt to environmental change. Additionally, understanding genetic structure among populations is important in defining management units and developing management strategies. However, documenting patterns of genetic diversity in *D. elator* is hampered by the challenge of obtaining specimens from all but one population (in Wichita County, near Iowa Park, TX) due to it being rare, extirpated, and/or on inaccessible private property in other parts of its distribution. Fortunately, numerous museum specimens (including skins, skulls, and skeletons) and toe clips collected over several decades have been preserved and are available to researchers. Genetic analysis from tissues of museum specimens have proven valuable in threatened and endangered species (Roy et al. 1994, Thomas et al. 1990, Wandeler et al. 2007), including another species of kangaroo rat (Matocq and Villablanca 2001).

My objective was to assess patterns of genetic diversity in the state threatened Texas kangaroo rat (*Dipodomys elator*). Specific aims were to: 1) document levels of genetic diversity at multiple localities across the historical distribution of the Texas kangaroo rat, 2) document the degree and patterns of genetic divergence across the historical distribution of the Texas kangaroo rat, 3) compare levels of genetic diversity between two different time periods at the same locality to document potential loss of diversity within the past few decades.

Materials and Methods

Sample collection, DNA extraction, and data collection

Samples of *D. elator* were obtained from four locations representing a large portion of the geographic distribution of the species and from different time periods (Fig. 1, Table 1, Appendix). Tissue samples included ear clips or toe clips from specimens captured and released by other researchers (obtained during previous investigations) or toe clips of museum voucher specimens. The Quanah and Iowa Park localities are the only ones with sufficient numbers of specimens to conduct rigorous population genetic analyses and are represented by two temporal periods. Vernon and Harrold localities are represented by fewer individuals and from only one temporal period and therefore were included in only some analyses. DNA was extracted using the Qiagen DNeasy Blood and Tissue Kit and following the modified protocol of Iudica et al. (2001).

Mitochondrial DNA sequencing and analysis

In order to determine the extent of mitochondrial DNA (mtDNA) sequence variation, two regions of the mtDNA genome were amplified using the polymerase chain reaction (PCR)—the control region and the cytochrome c oxidase subunit I (COI) gene. Because DNA in museum specimens is degraded, short segments (between 200 and 300 base pairs) are recommended (Wandeler et al. 2007). PCR primer pairs were designed to amplify the entire control region (from specimens having high-quality DNA) and two separate, shorter sections corresponding to the two hypervariable regions (necessary to amplify DNA from museum specimens with degraded DNA). To design primers that would be most likely to amplify the entire control region of *D. elator*, complete mitochondrial genomes from seven species of rodents were obtained from Genbank and aligned to identify conserved sequences flanking the control region. For primers used to amplify the short sections, sequences of the entire control region were first obtained from multiple specimens of *D. elator* having high-quality DNA. These sequences were then used to design internal primers specific to *D. elator*. PCR primers were tested on a subset of *D. elator* specimens, and successful primer pairs were used for the remaining specimens. PCR products

were either sequenced in my lab using a Beckman-Coulter CEQ8000 Genetic Analysis System or sent to the Genomics Core Lab at the Texas A&M University–Corpus Christi for sequencing using an ABI 3730XL DNA Analyzer. After aligning mtDNA sequences using ClustalW, as implemented in the software MEGA 6 (Tamura et al. 2013), the number of differences among sequences were quantified.

Initial data showed a striking lack of diversity at the control region of *D. elator*; therefore, in order to reduce the likelihood that the observed lack of mtDNA control region variation was due to amplification of a nuclear mitochondrial translocation (numt; Lopez et al. 1994), a portion of the mitochondrial COI gene was amplified and sequenced. Because the COI gene is opposite the control region on the mtDNA molecule, a numt containing both regions would have to include over one-half of the mtDNA molecule. The majority of numts are much smaller (<1 kb; Richly and Leister 2004).

Given the lack of mtDNA variation, with no pattern associated with geography or time of sampling, I did not collect mtDNA data from all specimens, but rather focused my resources on generating microsatellite data. Therefore, mtDNA control region sequences were obtained from only 57 individuals from Quanah and Iowa Park.

Microsatellite marker generation

Microsatellite loci were isolated specifically from *D. elator*. Briefly, DNA from a single specimen was sent to Research and Testing Laboratory, Lubbock, TX (www.researchandtesting.com). The laboratory generated DNA libraries enriched for a variety of microsatellite motifs, including di-, tri-, and tetra-nucleotides using a protocol modified from Malusa et al. (2011). Amplified libraries were sequenced using an Illumina MiSeq, providing DNA sequences from 6588, 1354, and 1585 di-, tri-, and tetra-nucleotide loci, respectively. Upon receipt of the resulting sequences, I identified microsatellites using the software SSR_pipeline (Miller et al. 2013). Resulting sequences containing single, simple repeat motifs flanked by sequences of sufficient length and quality for the design of primers were selected. Multiple loci were screened by PCR amplification of a small subset of specimens. 12 loci produced good results (exhibiting single amplicons with scorable fragment profiles) and were PCR amplified from the remaining specimens, but ultimately, data from only 11 loci were used for the final dataset.

Genotypes were obtained by PCR using a three-primer system which included a fluorescently labelled universal primer, a modified locus-specific forward primer with a 5' universal primer sequence tail, and a reverse primer in a 0.4:0.3:1 ratio. The universal primer sequences were derived from Blacket et al. (2012); specifically, their primer/tails A, B, and C. PCRs were performed using either the Qiagen© Multiplex PCR Kit (for those in which multiple loci were amplified in a single reaction) or Promega GoTaq© Flexi DNA polymerase (for those

in which a single locus was amplified). PCR products were separated and visualized using a Beckman-Coulter CEQ8000 Genetic Analysis System with an internal size standard.

Microsatellite analyses

The software MICRO-CHECKER V.2.2.3 (van Oosterhout et al. 2004) was used to test for PCR and genotyping errors due to stuttering, large allele drop-out, and null alleles. Deviations from Hardy-Weinberg equilibrium were tested using GENEPOP 4.4 (Rousset 2008).

Estimates of genetic diversity, including number of alleles/locus (*Na*) and observed (H_O) and expected (H_E) heterozygosity were calculated using the software GenAlEx v.6.5 (Peakall and Smouse 2012). Allelic richness (*Ar*) was calculated using the software HP-RARE (Kalinowski, 2005), which uses a rarefaction method that compensates for differences in sample size among populations. This parameter was calculated based on the smallest sample size. Differences between historical and more recent samples for Quanah and Iowa Park were tested with a one-tailed Wilcoxon signed-rank test.

Effective population size (*Ne*) was estimated using several methods implemented in the software NeEstimator v.2.01 (Do et al. 2014). Single-sample estimates were made from moment-based *F*-statistics using three different estimators of *F*: *Fc* (Nei & Tajima 1981), *Fk* (Pollak 1983), and *Fs* (Jorde & Ryman 2007). The two-sample (temporal) method of Waples (1989) was conducted using the default Plan II sampling strategy for the two temporal population pairs (Quanah 1969-1970/1985-1986 and Iowa Park 1966-1969/2005-2008). Temporal analyses were conducted using two estimates of mean generation time (one year and three years) based on empirical estimates from *D. spectabilis* (1.7 years; Swanson 2001) and *D. merriami* (up to 3.7 years; Zeng and Brown, 1987). *Ne* was also estimated from sibship assignments using the software COLONY v.2.0.6.1 (Jones and Wang 2010).

Divergence among populations and sampling times was examined with the fixation index (pairwise F_{ST}) using GENEPOP, Principal Coordinates Analysis (PCoA) using GenAlEx v.6.5 (Peakall and Smouse 2012); and a Bayesian clustering approach within STRUCTURE v.2.3.4 (Pritchard et al. 2000). STRUCTURE implements a model-based clustering method for inferring population structure and infers the posterior probability of the number of clusters (*K*) and membership coefficients (Q) for each individual. The number of MCMC iterations was set to 500,000 and burn-in was set to 100,000 with 5 replicates per K (the assumed number of populations). Analyses were conducted using the admixture model with correlated allele frequencies and the LOCPRIOR model (taking into account sampling locations) as it is more efficient at detecting subtle population structure (Hubisz et al. 2009).

Results

Mitochondrial DNA sequences

57 mtDNA control region sequences from the two hypervariable regions, 419 bp in combined length, were obtained (35 from Iowa Park 2005-2008 and 22 from Quanah 1985-1986). Only two haplotypes were observed, differing by a single nucleotide substitution. Both haplotypes occurred in both populations, with haplotype A (control region) occurring at a frequency of 22% in Iowa Park and 54% in Quanah. 21 sequences of the mtDNA COI gene, 647 bp in length, were obtained from the same two populations. Again, only two haplotypes were observed, differing by a single substitution. Haplotype A (COI) occurred at a frequency of 100% and 85.7% in Iowa Park and Quanah, respectively.

Microsatellites

In contrast to mtDNA sequences, microsatellites provided sufficient diversity for the objectives of this study to be met. One of the 12 loci exhibited homozygosity excess and was omitted from the dataset. There was no evidence of scoring error due to stuttering, large allele drop-out, or null alleles in the remaining 11 loci. After controlling for false discovery rate following the correction of Benjamini and Hochberg (1995), none of the 66 Hardy-Weinberg tests (11 loci from six populations) were significant at $P \leq 0.05$, and two of the 55 tests for genotypic linkage was significant at $P \leq 0.05$. Estimates of genetic diversity were very similar for each geographic and temporal sample (Tables 2 and 3). No comparisons of *Ar* and *He* between temporal samples were significantly different at $P \leq 0.05$. Estimates of *Ne* ranged considerably among populations and among methods (Tables 4, 5, and 6) but were generally low, with the highest estimate being 856. For most estimates, *Ne* was lower for Iowa Park than Quanah.

Pairwise *Fst* values among geographic and temporal populations ranged from 0.009-0.115 (Table 7). *Fst* values between the temporal samples of Quanah and Iowa Park was 0.009 and 0.047, respectively. PCoA analyses showed subtle population structure among some sampled populations (Fig. 2). The PCoA plot of the 1966-1986 samples showed clusters that overlapped to varying degrees, with Iowa Park showing the most divergence. The two PCoA plots comparing Quanah with Iowa Park (two time periods) both showed population structure, with clusters only slightly overlapping. Vernon and Harrold were more closely associated with Quanah than Iowa Park. The two PCoA plots comparing temporal samples of the same location showed no evidence of divergence for Quanah (1969-1970 vs. 1985-1986), and only slight separation for Iowa Park (1966-1969 vs. 2005-2008).

Several combinations of populations were analyzed using STRUCTURE. Samples of all four populations from 1966-1986 were run for values of K = 2, 3, and 4 (Fig. 3). Only values of 2 and 3 produced population assignments that made sense relative to sampling localities. For K = 2, individuals from Quanah, Vernon, and Harrold grouped together separately from Iowa Park, but individuals from Vernon and Harrold shared membership between the Quanah and Iowa Park clusters (with q-values between 10-20% for the Iowa Park cluster and 80-90% for the Quanah cluster). For K = 3, individuals from Quanah, Vernon, and Iowa Park formed separate groupings. Individuals from Harrold showed shared membership between Quanah and Vernon (with Q-values between 25-40% for the Quanah cluster and 35-50% for the Vernon cluster). For K = 4, individuals from Iowa Park exhibited mixed membership between two groups that were uncorrelated with geography, indicating that 4 groups was not biologically meaningful. Four additional STRUCTURE analyses were conducted with pairs of populations for K = 2 , and showed some degree of population structure (Fig. 4), though the temporal comparison for Quanah had lower Q-value support for the two clusters.

Discussion

D. elator is characterized by strikingly low mtDNA diversity, with only two control region haplotypes identified out of 57 individuals sequenced and two COI haplotypes out of 21 individuals (with haplotypes for both differing by only one substitution). Despite a thorough literature review, I am aware of only a few studies that have documented such a striking lack of mtDNA variation within an entire species. These include harrier (Fuchs et al. 2014), alligator (Glenn et al. 2002), salamander (Riberon et al. 2002), viper (Ujvari et al. 2005), and European bison (Wójcik et al. 2009). There are a few additional reports of insular species or isolated populations/subspecies of more widely distributed species that have very low mtDNA variation. An insular species includes the kakerori [an endemic bird]--Chan et al., 2011. Populations or subspecies include the giraffe (Fennessy et al. 2013), Eld's deer (Pang et al. 2003), and a kangaroo rat (*D. heermanni morroensis*; Matocq and Villablanca, 2001).

The lack of mtDNA variation in *D. elator* is suggestive of a population bottleneck within the past few thousand years or an extreme selective sweep (Meiklejohn et al. 2007). Very slow control region evolutionary rate has been implicated in some studies (e.g. Ujvari et al. 2005), but this seems unlikely since there is extensive control region variation in other species of *Dipodomys* having both wide and restricted geographic distributions (Good et al. 1997, Metcalf et al. 2001, Jezkova et al. 2011, Jezkova et al. 2015, Matocq and Villablanca 2001). An additional, potential explanation may be that that a nuclear insert (numt) of the control region has been sequenced rather than the mtDNA itself (Hazkani-Covo et al. 2010). Copies of the mtDNA genome that are translocated into the nucleus have a lower mutation rate and exhibit less

variation than their mtDNA counterparts. However, a numt is unlikely given the corresponding lack of COI sequence diversity, as it would be unlikely that a numt would be long enough to contain both control region and COI genes (~11,000 bp) as most numpts are <1000 bp. Although a numt of this size has been reported in *Panthera* (Kim et al. 2006) and this possibility cannot be excluded with certainty. However, an evolutionary recent, species-wide bottleneck is supported by the relatively low microsatellite diversity (and correspondingly low *Ne* values).

The presence of mtDNA haplotypes differing by only a single substitution could be explained by a species-wide bottleneck having occurred a few thousand years ago, reducing mtDNA diversity to a single haplotype, followed by an expansion of the species and a mutation creating the second haplotype. A less significant bottleneck (in which two or more haplotypes remained) is predicted to result in the survival of haplotypes differing by more than a single nucleotide—given that loss of haplotypes by genetic drift is random and that genetically diverse (pre-bottleneck) populations exhibit haplotypes with greater degrees of divergence. Following a bottleneck, mtDNA diversity is expected to be lower than microsatellite diversity because the loss of mtDNA diversity occurs more quickly than that of nuclear loci due to its lower effective population size (Birky et al. 1989). Given that the two closely related control region haplotypes occurred across the geographic distribution of *D. elator*, the bottleneck must have happened prior to the establishment of the current distribution and involved the entire species. In contrast, independent bottlenecks among isolated populations would have resulted in fixation of different haplotypes in each population.

Several studies have documented genetic diversity in other species of *Dipodomys*. Jezkova et al. (2011) examined 902 bp of the mtDNA control region in *D. microps*, and found 243 haplotypes (differing by 205 variable positions) in 364 individuals. Jezkova et al. (2015) found 149 unique control region haplotypes from 328 individuals of *D. deserti* and 161 haplotypes from 210 individuals of *D. merriami*. All three of these species exhibit geographic distributions much larger than that of *D. elator*. Matocq and Villablanca (2001) obtained control region sequences from pre-bottleneck and post-bottleneck samples of the critically endangered subspecies *D. herrmanii morroensis* and from the more common *D. h. arenae* (n = 8 for each of the three samples). They found the pre- and post-bottleneck samples of *D. h. morroensis* to have only two haplotypes (differing by a single substitution within each sample). In comparison, *D. h. arenae* had 6 haplotypes differing by 15 substitutions. The endangered subspecies had declined from an estimated 8000 individuals in 1957 to 50 individuals in 1986, with its habitat being reduced from 567 ha to 12.6 ha over that time period. The authors attributed low mtDNA diversity in pre-bottleneck samples to a historical bottleneck preceding the contemporary population decline. Nuclear genetic diversity has yet to be examined in any of the above species of *Dipodomys*. Good et al. (1997) examined 295 bp of the control region in *D. ingens*, an endangered species occupying an estimated 3% of its historical distribution, and found 50 haplotypes (differing by 54 variable positions) in 95 individuals. Lowe et al. (2005) examined variation at six microsatellite loci of *D. ingens*, and their estimates of microsatellite diversity

(*He*) were slightly higher than what I found for *D. elator* (although comparisons with other studies must be made cautiously as they can be confounded by choice of microsatellite loci). All of these species of *Dipodomys* exhibited much higher levels of mtDNA diversity than *D. elator*, despite two of them having similar historical range sizes as *D. elator*.

Microsatellite data of *D. elator* showed low to moderate allelic diversity and heterozygosity in all populations, with remarkable consistency across temporal and geographic samples. Iowa Park had the lowest *Ar* and *He* (the two most reliable indicators of genetic diversity), though these values were only slightly lower compared to other populations. These indications of low to moderate nuclear diversity, combined with the low mtDNA diversity, are consistent with an historical bottleneck combined possibly with low *Ne*.

The various analyses of population structure of *D. elator* based on microsatellite data largely support one another, despite their very different underlying methodologies. Pairwise *Fst*, PCoA, and STRUCTURE all show evidence of population structure among the four geographic samples, with Iowa Park being the most divergent population. However, the degree of divergence is low, with the highest *Fst* being 0.115 and incomplete separation of clusters in the PCoA plot. The only other population genetic study of *D. elator* used allozymes (Hamilton et al. 1987) and reported *Fst* values ranging from 0.085 to 0.193 for 21 individuals representing the same (or nearby) populations as those included in my study.

All analyses showed much less divergence between temporal samples compared to geographic samples, indicating that genetic drift has had only minimal impacts in shifting allelic frequencies over the time periods examined here. Given that the time periods differ for the Quanah and Iowa Park temporal samples, it is difficult to interpret their apparent differences in divergence (Iowa Park samples showed slightly greater divergence compared to Quanah samples). The Quanah temporal samples are separated by only ~17 years compared to the ~36 years separating the Iowa Park samples, thus less time for genetic drift to act could explain this difference. Alternatively, the Iowa Park population could have a lower *Ne*, causing genetic drift to be more severe regardless of time. All but 1 of the 7 estimations of *Ne* showed Iowa Park to have a lower *Ne* relative to Quanah (for both temporal samples). This supports the hypothesis that lower *Ne* is responsible for the greater divergence of Iowa Park temporal samples, though it seems likely that both factors have played a role.

Limited gene flow, combined with lower *Ne*, could explain the slightly greater divergence of Iowa Park relative to the other three geographic samples (Quanah, Vernon, and Harrold). STRUCTURE analysis for all four populations when K=2 showed individuals from Vernon and Harrold sharing membership between the Quanah and Iowa Park clusters as would be expected given their intermediate geographic position. However, *Fst*, PCoA, and Q-values from STRUCTURE show them to have a closer affiliation with Quanah than to Iowa Park despite their closer proximity to Iowa Park. This pattern does not conform to isolation by

distance, thus genetic drift appears to have played a greater role than gene flow in establishing genetic structure.

Given the difficulty of sampling *D. elator* over much of its range, it is not known if the genetic patterns identified here are due to actual geographic separation of populations or just limited sampling. Ecological niche modeling (Newbold 2010), combined with the results presented here, may shed light on the likelihood of these two alternatives. If ecological niche modeling predicts less gene flow between Iowa Park and the other populations, this would support an actual separation of populations.

Assuming that the remarkably low mtDNA diversity and low *Ne* is due to a historical bottleneck involving the entire species, at some time in the past *D. elator* was able to disperse across the landscape from a single location to its present distribution as the population expanded. It remains to be seen if the low genetic divergence across the geographic distribution is due to recent expansion (and minimal subsequent genetic drift among now-isolated populations) or to fairly high levels of contemporary gene flow. The presence of both control region haplotypes differing by only one substitution occurring at opposite ends of the distribution suggests to me that that latter may be more likely. Otherwise, a post-bottleneck mutation having occurred in one population would not have dispersed into a second, isolated population.

Given the cryptic nature of *D. elator*, its apparently patchy distribution, and the vast expanses of privately owned land on which it may (or may not) occur, it seems unlikely that reasonably confident estimates of census population sizes (nor demographic estimates of *Ne*) will ever be made. This leaves genetic estimates of *Ne* to inform conservation management. There is evidence that low *Ne* increases the likelihood of extinction because diversity is required for maintaining fitness and future evolutionary adaptation to changing environments (Frankham 2005), with current recommendations of $Ne > 100$ to avoid inbreeding depression and $Ne > 1000$ to maintain evolutionary potential (Frankham et al. 2014). All estimates of *Ne* for *D. elator* are < 1000, with several being < 100, suggesting that conservation efforts that maximize census population size to minimize the loss of genetic diversity should be a priority for this species.

Assuming that the mtDNA data is accurately documenting an historical bottleneck (prior to the arrival of Europeans), rather than a mitochondrial selective sweep, *Ne* would have been impacted by this event and would not have recovered substantially following population expansion (only mutations can increase *Ne* in a closed population). This implies that the ratio of effective to actual population size (*Ne/N*) may be lower in *D. elator* than for most species (Frankham 2007). Because of this, correlation of potential recent or future population declines with loss of genetic diversity is expected to be difficult (Matocq and Villablanca 2001).

Interpreting genetic indicators of population diversity and change (such as those used here) can be challenging because they are influenced by multiple interacting ecological and evolutionary processes, leading to a reluctance by managers to use these indicators to inform

management. The application of genetic indicators in wild populations, including their potential, pitfalls, and strategies for use, were reviewed by Pierson et al. (2015). Briefly, the use of a variety of methods for inferring demography may help avoid misinterpretations of any one indicator. Additionally, the choice of indicator and sampling/experimental design of a genetic monitoring program should be guided by the specific conservation program, requiring close collaboration between conservation managers, ecologists, and geneticists. The results of my study should serve as a starting point for further investigation of the demographics of *D. elator* which together will provide evidence for making well-informed conservation management decisions.

Acknowledgments

Funding was provided by a Texas Parks & Wildlife Department Section 6 grant (TX E-163-R); the Department of Biological Sciences, Tarleton State University; and the Office of Student Research & Creative Activities, Tarleton State University. Special thanks to Jim Goetze, Robert Martin, Kenneth Matocha, Allan Nelson and others for their prior work with this species which produced the museum specimens and tissues used in the current study and to Elizabeth Gilliland for assistance in the laboratory. Toe clips were obtained by Robert Martin and Kenneth Matocha as part of a population study of the species from 1985 to 2000. Ear clips were obtained by Jim Goetze and Allan Nelson as part of a population study from 2005-2008. Toe clips from museum voucher specimens were obtained from the Collection of Recent Mammals, Midwestern State University and the Natural Sciences Research Laboratory, Texas Tech University.

Literature Cited

Benjamini, Y. and Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the royal statistical society. Series B (Methodological): 289-300.

Birky, C.W., Fuerst, P. and Maruyama, T., 1989. Organelle gene diversity under migration, mutation, and drift: equilibrium expectations, approach to equilibrium, effects of heteroplasmic cells, and comparison to nuclear genes.Genetics, 121:613-627.

Blacket, M.J., Robin, C., Good, R.T., Lee, S.F. and Miller, A.D., 2012. Universal primers for fluorescent labelling of PCR fragments—an efficient and cost-effective approach to genotyping by fluorescence. Molecular Ecology Resources, 12:456-463.

Chan, C.H., Robertson, H.A., Saul, E.K., Nia, L.V., Luong, V.P., Kong, X., Zhao, Y. and Chambers, G.K., 2011. Genetic variation in the kakerori (*Pomarea dimidiata*), an endangered endemic bird successfully recovering in the Cook Islands. Conservation genetics, 12:441-447.

Dalquest, W.W., and G. Collier. 1964. Notes on *Dipodomys elator*, a rare kangaroo rat. Southwestern Naturalist, 9:146-150.

Dalquest, W.W., and N.V. Horner. 1984. Mammals of north-central Texas. Midwestern State University Press, Wichita Falls, Texas.

Diamond, D.D., and D.M. Shaw. 1990. GIS and remote sensing for Texas kangaroo rat habitat characterization. Project E-1-2. Unpublished report prepared for the Texas Parks and Wildlife, Austin, Texas.

Do, C., Waples, R.S., Peel, D., Macbeth, G.M., Tillett, B.J. and Ovenden, J.R., 2014. NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. Molecular Ecology Resources, 14:209-214.

Fennessy, J., Bock, F., Tutchings, A., Brenneman, R. and Janke, A., 2013. Mitochondrial DNA analyses show that Zambia's South Luangwa Valley giraffe (Giraffa camelopardalis thornicrofti) are genetically isolated. African Journal of Ecology, 51:635-640.

Frankham, R., 2005. Genetics and extinction. Biological conservation, 126:131-140.

Frankham, R., 2007. Effective population size/adult population size ratios in wildlife: a review. Genetical research, 89:491-503.

Frankham, R., Ballou J. D., Briscoe D. A. 2002. Introduction to Conservation Genetics, Cambridge University Press.

Frankham, R., Bradshaw, C.J. and Brook, B.W., 2014. Genetics in conservation management: revised recommendations for the 50/500 rules, Red List criteria and population viability analyses. Biological Conservation, 170:56-63.

Fuchs, J., Simmons, R.E., Mindell, D.P., Bowie, R.C. and Oatley, G., 2014. Lack of mtDNA genetic diversity in the Black Harrier Circus maurus, a Southern African endemic. Ibis, 156:227-230.

Garza, J.C. and Williamson, E.G., 2001. Detection of reduction in population size using data from microsatellite loci. Molecular ecology, 10:305-318.

Glenn, T.C., Staton, J.L., Vu, A.T., Davis, L.M., Bremer, J.R.A., Rhodes, W.E., Brisbin, I.L. and Sawyer, R.H., 2002. Low mitochondrial DNA variation among American alligators and a novel non-coding region in crocodilians. Journal of Experimental Zoology, 294:312-324.

Goetze, J.R., Stasey, W.C., Nelson, A.D., and Sudman, P.D., 2007. Mapping burrows of Texas kangaroo rats to examine soil types, vegetation, and estimate population size on an intensely grazed pasture in Wichita County, Texas. Texas Journal of Science, 59:11-22.

Hamilton, M.J., Chesser, R.K. and Best, T.L., 1987. Genetic variation in the Texas kangaroo rat, *Dipodomys elator* Merriam. Journal of Mammalogy, 68:775-781.

Good, S.V., Williams, D.F., Ralls, K. and Fleischer, R.C., 1997. Population structure of *Dipodomys ingens* (Heteromyidae): the role of spatial heterogeneity in maintaining genetic diversity. Evolution,51:1296-1310.

Hazkani-Covo, E., Zeller, R.M. and Martin, W., 2010. Molecular poltergeists: mitochondrial DNA copies (numts) in sequenced nuclear genomes. PLoS Genet, 6:1-11.

Hubisz, M.J., Falush, D., Stephens, M. and Pritchard, J.K., 2009. Inferring weak population structure with the assistance of sample group information. Molecular ecology resources, 9:1322-1332.

Iudica, C.A., Whitten, M. Williams, N. H., 2001. Small bones from dried mammal museum specimens as a reliable source of DNA. BioTechniques, 30:733-736.

Jezkova, T., Olah-Hemmings, V. and Riddle, B.R., 2011. Niche shifting in response to warming climate after the last glacial maximum: inference from genetic data and niche assessments in the chisel-toothed kangaroo rat (*Dipodomys microps*). Global Change Biology, 17:3486-3502.

Jezkova, T., Riddle, B.R., Card, D.C., Schield, D.R., Eckstut, M.E. and Castoe, T.A., 2015. Genetic consequences of postglacial range expansion in two codistributed rodents (genus *Dipodomys*) depend on ecology and genetic locus. Molecular ecology, 24:83-97.

Jones, C., Bogan, M.A. and Mount, L.M., 1988. Status of the Texas kangaroo rat (*Dipodomys elator*). The Texas Journal of Science, 40:249-258.

Jones, O.R. and Wang, J., 2010. COLONY: a program for parentage and sibship inference from multilocus genotype data. Molecular ecology resources, 10:551-555.

Jorde P.E., Ryman N., 2007. Unbiased estimator for genetic drift and effective population size. Genetics, 177:927–935.

Kalinowski, S.T., 2005. hp-rare 1.0: a computer program for performing rarefaction on measures of allelic richness. Molecular Ecology Notes, 5:187-189.

Kim, J.H., Antunes, A., Luo, S.J., Menninger, J., Nash, W.G., O'Brien, S.J. and Johnson, W.E., 2006. Evolutionary analysis of a large mtDNA translocation (numt) into the nuclear genome of the Panthera genus species. Gene, 366:292-302.

Lopez, J.V., Yuki, N., Masuda, R., MOdi, W., and O'Briens, J., 1994. Numt, a recent transfer and tandem amplification of mitochondrial DNA to the nuclear genome of the domestic cat. Journal of Molecular Evolution, 39:174–190.

Malausa, T., et al., 2011. High-throughput microsatellite isolation through 454 GS-FLX Titanium pyrosequencing of enriched DNA libraries. Molecular Ecology Resources, 11:638-644.

Martin, R.E., 2002. Status and long term survival of the Texas kangaroo rat, *Dipodomys elator*. Sect. 6, Project 70. Unpublished report prepared for Texas Parks and Wildlife Department, Austin, Texas.

Matocq, M.D. & Villablanca, F.X., 2001. Low genetic diversity in an endangered species: recent or historic pattern? Biological Conservation 98:61-68.

Meiklejohn, C.D., Montooth, K.L. and Rand, D.M., 2007. Positive and negative selection on the mitochondrial genome. Trends in Genetics, 23:259-263.

Metcalf, A.E., Nunney, L. and Hyman, B.C., 2001. Geographic patterns of genetic differentiation within the restricted range of the endangered Stephens' kangaroo rat *Dipodomys stephensi*. Evolution, 55:1233-1244.

Miller, M.P., Knaus, B.J., Mullins, T.D. and Haig, S.M., 2013. SSR_pipeline: A bioinformatic infrastructure for identifying microsatellites from paired-end Illumina high-throughput DNA sequencing data. Journal of Heredity, 104:881-885.

Moss, S.P., and Mehlhop-Cifelli., P., 1990. Status of the Texas kangaroo rat, *Dipodomys elator* (Heteromyidae), in Oklahoma. Southwest Naturalist 35:356-358.

Nei M. and Tajima F., 1981. Genetic drift and estimation of effective population size. Genetics, 1981: 625-640.

Nelson, A.D., Goetze, J. R. Watson, E. and Nelson, M., 2009. Changes in vegetation patterns and its effect on Texas Kangaroo Rats (*Dipodomys elator*). Texas Journal of Science 61:119-130.

Newbold, T., 2010. Applications and limitations of museum data for conservation and ecology, with particular attention to species distribution models. Progress in Physical Geography, 34:3-22.

Pang, J., Hoelzel, A.R., Song, Y., Zeng, Z. and Zhang, Y., 2003. Lack of mtDNA control region variation in Hainan Eld's deer: Consequence of a recent population bottleneck? Conservation Genetics, 4:109-112.

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

Pierson, J., Luikart, G. and Schwartz, M., 2015. The application of genetic indicators in wild populations: potential and pitfalls for genetic monitoring. Indicators and Surrogates of Biodiversity and Environmental Change, p.149.

Pollak E., 1983. A new method for estimating the effective population size from allele frequency changes. Genetics, 104:531–548.

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

Richly, E. and Leister, D., 2004. Numts in sequenced eukaryotic genomes. Molecular Biology and Evolution 21:1081–1084.

Roberts, J.D., and Packard R.L., 1973. Comments on movements, home range and ecology of the Texas kangaroo rat, *Dipodomys elator* Merriam. Journal of Mammalogy, 54:957-962.

Rodríguez-Ramilo, S.T. and Wang, J., 2012. The effect of close relatives on unsupervised Bayesian clustering algorithms in population genetic structure analysis. Molecular Ecology Resources, 12:873-884.

Rodríguez-Ramilo, S.T., Toro, M.A., Wang, J. and Fernandez, J., 2014. Improving the inference of population genetic structure in the presence of related individuals. Genetics Research, 96.

Rousset, F., 2008. Genepop'007: a complete re-implementation of the genepop software for Windows and Linux. Molecular Ecology Resources, 8:103-106.

Roy, M.S., Girman, D.J., Taylor, A.C., and Wayne, R.K., 1994. The use of museum specimens to reconstruct the genetic variability and relationships of extinct populations. Experientia 50:551-7.

Smith, T.B. and Wayne, R.K., eds. 1996. Molecular Genetic Approaches in Conservation, Oxford University Press.

Stangl Jr., F.B. and Schafer, T.S., 1990. Report on the current status of the Texas kangaroo rat, *Dipodomys elator*, in north-central Texas. Unpublished report prepared for Texas Parks and Wildlife Department, Austin, Texas.

Stangl Jr., F.B., Schafer T.S., Goetze J.R., and Pinchak W., 1992. Opportunistic use of modified and disturbed habitat by the Texas kangaroo rat (*Dipodomys elator*). The Texas Journal of Science 44:25-35.

Stasey, W.C., Goetze J.R., Sudman P.D., and Nelson, A.D., 2010. Differential use of grazed and ungrazed plots by *Dipodomys elator* (Mammalia: Heteromyidae) in North Central Texas. Texas Journal of Science, 62:3-14.

Swanson B.J., 2001. An empirical evaluation of effective population size estimators. PhD Thesis, Purdue University, West Lafayette, Indiana.

Tamura K., Stecher G., Peterson D., Filipski A., and Kumar S., 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Molecular Biology and Evolution, 30:2725-2729.

Thomas, W.K., Paabo, S., Villablanca, F.X. & Wilson, A.C., 1990. Spatial and temporal continuity of kangaroo rat populations shown by sequencing mitochondrial DNA from museum specimens. Journal of Molecular Evolution, 31:101-112.

Riberon, A., Sotiriou, E., Miaud, C., Andreone, F. and Taberlet, P., 2002. Lack of genetic diversity in *Salamandra lanzai* revealed by cytochrome b gene sequences. Copeia, 2002: 229-232.

Ujvari, B., Madsen, T. and Olsson, M., 2005. Discrepancy in mitochondrial and nuclear polymorphism in meadow vipers (*Vipera ursinii*) questions the unambiguous use of mtDNA in conservation studies. Amphibia-Reptilia, 26:287-292.

U.S. Fish and Wildlife Service. 2011. Endangered and threatened wildlife and plants; 90-day finding on a petition to list the Texas kangaroo rat as endangered and threatened. Federal Register 76(45):12683-12690.

Van Oosterhout, C., Hutchinson, W.F., Wills, D.P. and Shipley, P., 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes, 4:535-538.

Wandeler, P., Hoeck, P.E. & Keller, L.F., 2007. Back to the future: museum specimens in population genetics. Trends in ecology & evolution 22:634-642.

Waples, R.S., 1989. A generalized approach for estimating effective population size from temporal changes in allele frequency. Genetics, 121:379-391.

WildEarth Guardians. 2010. Petition to list the Texas kangaroo rat (*Dipodomys elator*) under the U.S. Endangered Species Act. WildEarth Guardians. Denver Colorado. 32 pp.

Wójcik, J.M., Kawałko, A., Tokarska, M., Jaarola, M., Vallenback, P. and Pertoldi, C., 2009. Post-bottleneck mtDNA diversity in a free-living population of European bison: implications for conservation. Journal of Zoology, 277:81-87.

Zeng, Z. and Brown, J.H., 1987. Population ecology of a desert rodent: *Dipodomys merriami* in the Chihuahuan Desert. Ecology, 68:1328-1340.

Table 1. Locations of samples of *D. elator* used in this study, including date collected, number of specimens and source of the samples. Location names refer to the town nearest to the sites of collection, not exact collecting localities.

Location	Date collected	No. of specimens	Source‡
Quanah	1969-1970	41	NSRL
Quanah	1985-1986	49	Martin & Matocha
Iowa Park	1966-1969	30	MWSU
Iowa Park	2005-2008	32	Goetze & Nelson
Vernon	1967-1968	13	MWSU
Harrold	1985	7	NSRL

‡ NSRL—Natural Science Research Laboratory, Texas Tech University (toe clips from museum voucher specimens); Martin & Matocha— obtained as part of a population study of the species (toe clips from catch and release specimens); MWSU—Collection of Recent Mammals, Midwestern State University (toe snips from museum voucher specimens); Goetze & Nelson—obtained as part of a population study of the species (ear clips from catch and release specimens).

Table 2. Estimates of allelic diversity for geographical and temporal samples of *D. elator*. Diversity measures include number of alleles (N_a) and allelic richness (A_r) calculated for each microsatellite locus and the mean (bold) ± SE.

	Quanah, TX				Iowa Park, TX			
	1969-1970 (n = 41)		1985-1986 (n = 49)		1966-1969 (n = 30)		2005-2008 (n = 32)	
Locus	*Na*	*Ar*	*Na*	*Ar*	*Na*	*Ar*	*Na*	*Ar*
3790-di	6	5.9	6	5.9	8	8.0	6	6.0
4614-di	7	6.7	7	6.7	5	5.0	5	5.7
5214-di	4	3.9	5	4.5	7	6.9	5	5.0
8509-di	7	6.6	6	5.4	6	6.0	5	5.0
9013-di	4	4.0	4	4.0	4	4.0	5	5.0
17823-di	5	4.9	4	3.8	5	5.0	4	4.8
21888-di	5	4.7	5	4.6	4	4.0	4	4.0
22928-di	6	5.7	7	6.6	5	5.0	5	5.0
8261-tri	4	4.0	5	4.1	4	4.0	4	4.0
8538-tri	6	5.4	6	5.6	5	5.0	6	5.8
9058-tri	6	5.6	6	5.8	4	4.0	6	5.8
Mean	**5.5**	**5.2**	**5.5**	**5.2**	**5.2**	**5.2**	**5.2**	**4.9**
SE	0.3	0.3	0.3	0.3	0.4	0.4	0.2	0.2

Table 3. Estimates of observed (H_O) and expected (H_E) heterozygosity of *D. elator* calculated for each microsatellite locus and the mean (bold) ± SE.

	Quanah, TX		Iowa Park, TX	
	1969-1970 (n = 41)	1985-1986 (n = 49)	1966-1969 (n = 30)	2005-2008 (n = 32)

Locus	H_O	H_E	H_O	H_E	H_O	H_E	H_O	H_E
3790-di	0.62	0.72	0.65	0.68	0.76	0.83	0.75	0.80
4614-di	0.78	0.76	0.74	0.75	0.80	0.71	0.72	0.72
5214-di	0.59	0.60	0.69	0.59	0.57	0.50	0.63	0.57
8509-di	0.83	0.80	0.78	0.74	0.77	0.79	0.84	0.74
9013-di	0.66	0.63	0.59	0.68	0.63	0.67	0.63	0.68
17823-di	0.68	0.59	0.37	0.49	0.48	0.55	0.38	0.37
21888-di	0.78	0.70	0.74	0.70	0.70	0.74	0.59	0.62
22928-di	0.85	0.75	0.84	0.77	0.80	0.76	0.59	0.65
8261-tri	0.48	0.57	0.57	0.52	0.63	0.67	0.59	0.68
8538-tri	0.59	0.60	0.53	0.48	0.62	0.68	0.56	0.53
9058-tri	0.45	0.45	0.55	0.59	0.47	0.48	0.59	0.55
Mean	**0.63**	**0.65**	**0.64**	**0.64**	**0.66**	**0.67**	**0.63**	**0.63**
SE	0.04	0.03	0.04	0.03	0.04	0.04	0.04	0.04

Table 4. Estimates of effective population size (*Ne*) of *D. elator* based on microsatellites using single-samples inferred via linkage disequilibrium (LD), heterozygosity excess (HE), molecular coancestry (Coancestry), and full-likelihood sibship assignment (Sibship). Relevant confidence intervals (95%) are provided for each method (P=parametric, J=jackknife, CI=bootstrapping).

	LD			HE		Coancestry		Sibship	
Population	*Ne*	P	J	*Ne*	P	*Ne*	J	*Ne*	CI
Quanah (1969-1970)	856	115-∞	97-∞	69	12-∞	13	5-25	70	46-109
Quanah (1985-1986)	52	36-81	34-89	∞	15-∞	∞	∞-∞	86	56-139
Iowa Park (1966-1969)	24	17-38	17-38	∞	23-∞	6	3-11	48	29-81
Iowa Park (2005-2008)	12	9-16	9-16	∞	13-∞	5	3-7	32	19-59

Table 5. Estimates of effective population size (*Ne*) of *D. elator* using temporal samples assuming an average generation time of 1 year (17 generations elapsed for Quanah, 36 generations for Iowa Park). Inferences were made using three different estimators of *F* (F_k, F_c, and F_s). Confidence intervals (95%) are provided for each method (P=parametric, J=jackknife).

	F_k			F_c			F_s		
Population	*Ne*	P	J	*Ne*	P	J	*Ne*	P	J
Quanah	358	177-835	176-843	405	194-1031	187-1096	490	322-694	208-∞
Iowa Park	237	137-405	126-436	257	147-446	134-486	195	128-275	105-1300

Table 6. Estimates of effective population size (*Ne*) of *D. elator* using temporal samples assuming an average generation time of 3 years (6 generations elapsed for Quanah, 12 generations for Iowa Park). Inferences were made using three different estimators of *F* (F_k, F_c, and F_s). Confidence intervals (95%) are provided for each method (P=parametric, J=jackknife).

	F_k			F_c			F_s		
Population	*Ne*	P	J	*Ne*	P	J	*Ne*	P	J
Quanah	126	62-294	62-296	143	69-364	66-387	173	114-245	73-∞
Iowa Park	79	46-135	42-145	86	49-149	45-162	65	43-92	35-432

Table 7. Pairwise *Fst* among geographic and temporal populations of *D. elator* based on microsatellites.

	Quanah (1969-1970)	Vernon (1967-1968)	Iowa Park (1966-1969)	Quanah (1985-1986)	Harrold (1985)
Vernon (1966-1969)	0.097				
Iowa Park (1966-1970)	0.076	0.115			
Quanah (1985-1986)	0.009	0.109	0.090		
Harrold (1985)	0.048	0.039	0.073	0.053	
Iowa Park (2005-2008)	0.087	0.100	0.047	0.098	0.069

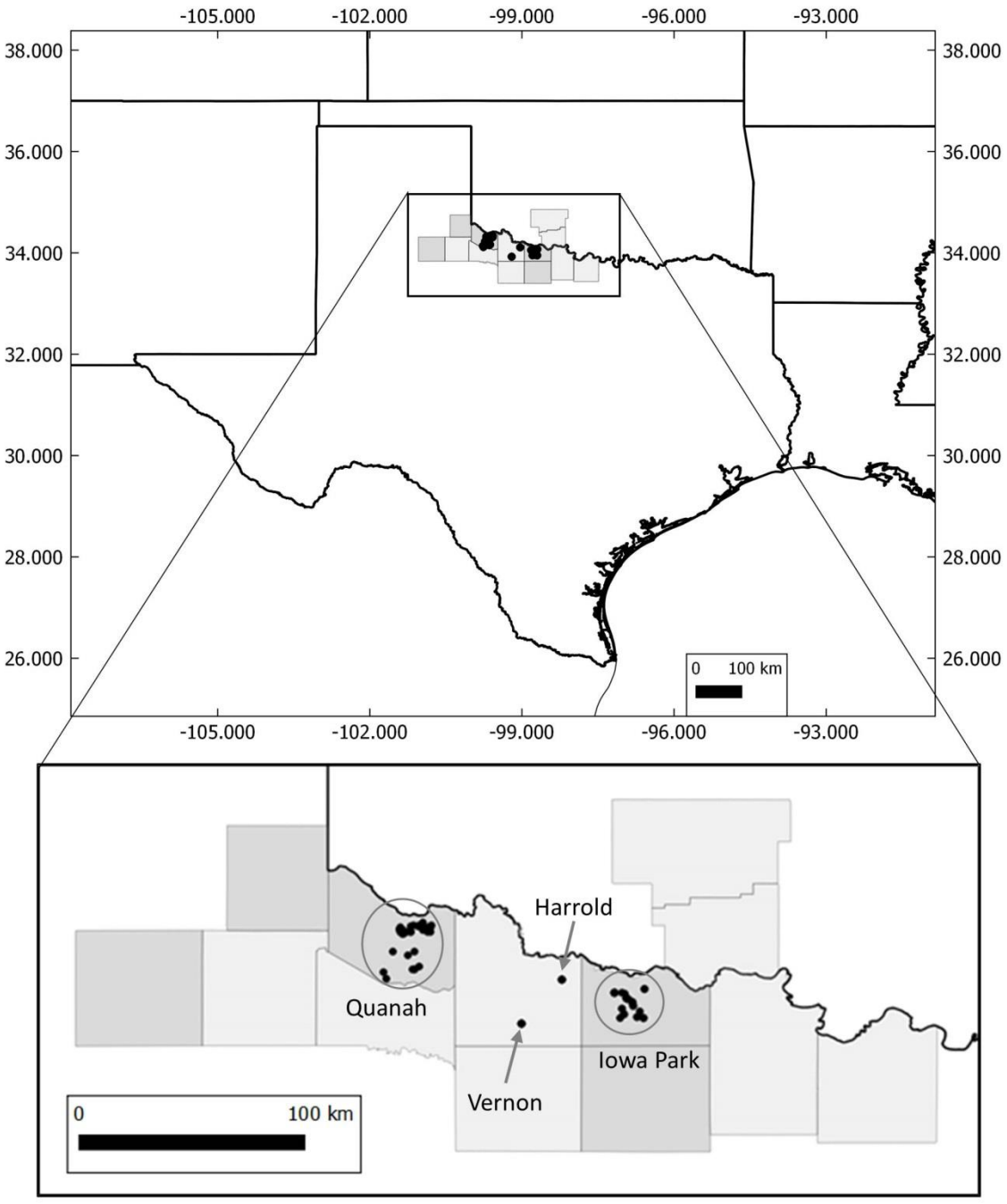

Figure 1. Approximate location of each specimen included in this study (dots) and counties from which *Dipodomys elator* has been recorded historically (shaded). Counties documented by Martin (2002) as having populations of *D. elator* are indicated by dark gray.

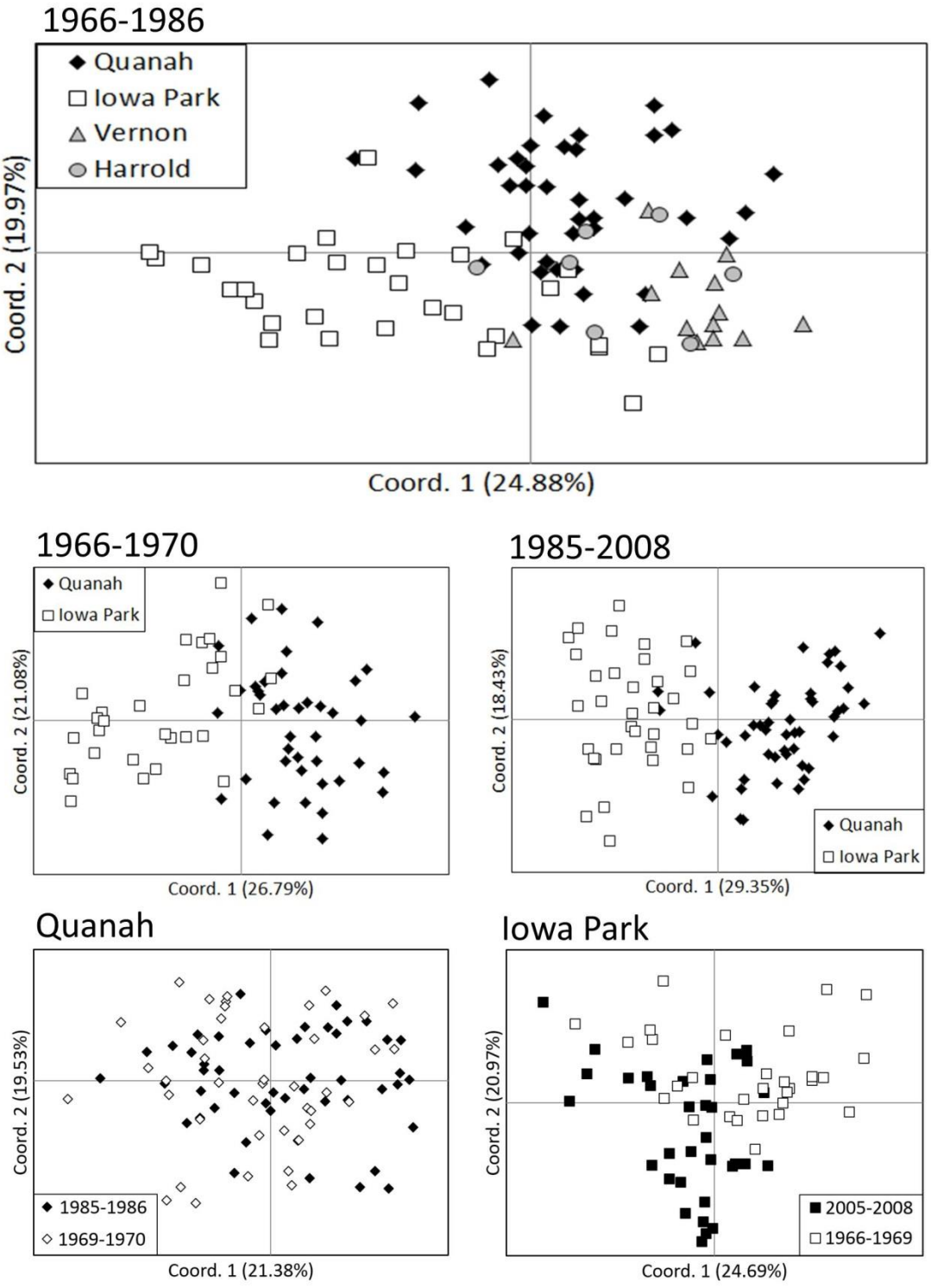

Figure 2. Principal coordinates analysis of microsatellites for various combinations of samples of *D. elator* .

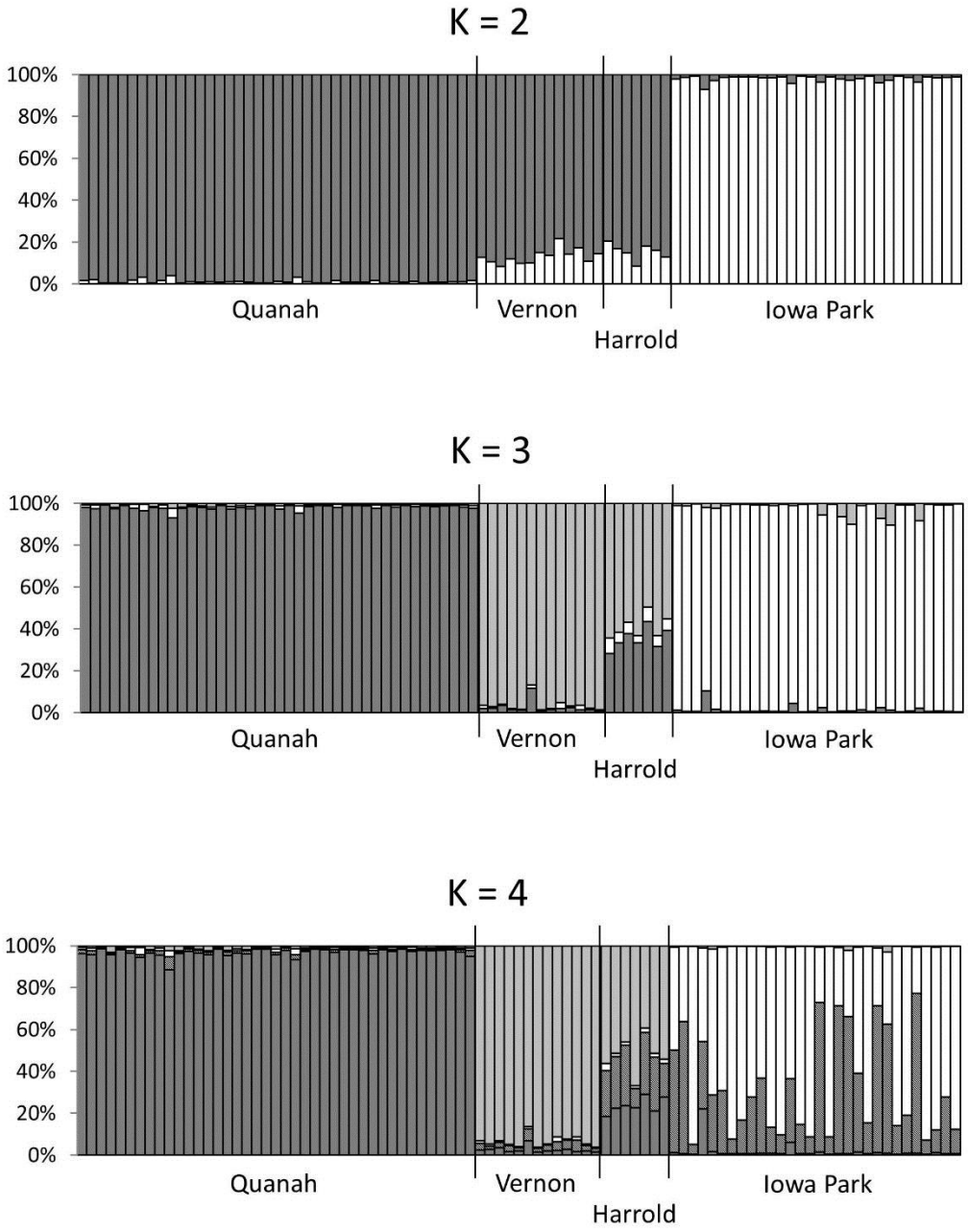

Figure 3. Results of STRUCTURE analyses of microsatellite data from *D. elator* specimens collected from 1966-1985 only. Each vertical bar represents the membership coefficient (Q) of an individual in one of K clusters for K = 2, 3, and 4.

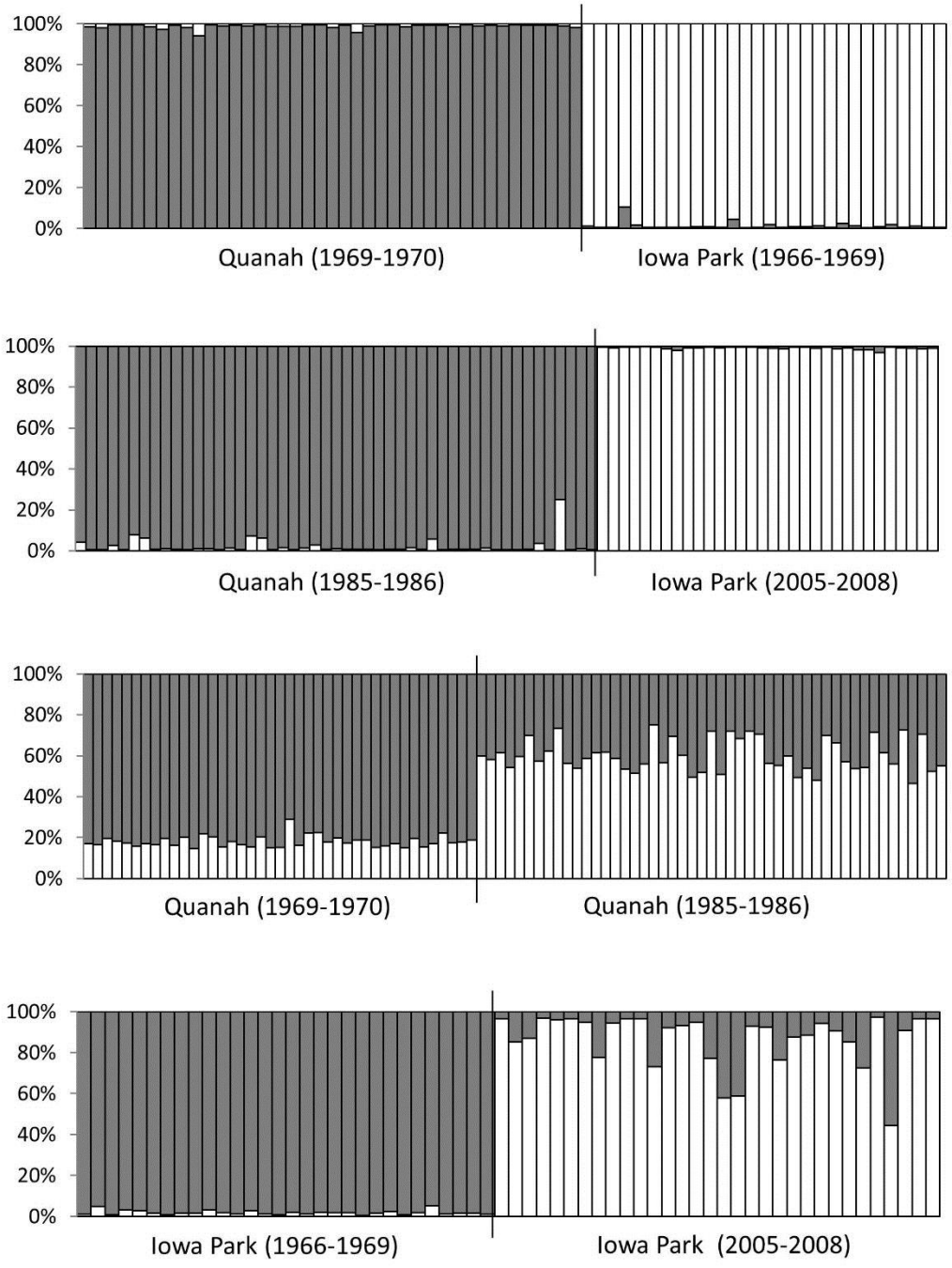

Figure 4. Results of STRUCTURE analyses of microsatellite data from *D. elator* specimens from several combinations of geographical and temporal samples. Each vertical bar represents the membership coefficient (Q) of an individual in one of K= 2 clusters.

Appendix. Locality information for *D. elator* specimens included in this study, including year of collection. TTU and TK numbers (specimen IDs) are catalog numbers from the NSRL, Texas Tech University. MWSU numbers are from Midwestern State University. Population ID refers to population names used within the text. Coordinates are approximate for many specimens, having been estimated from locality data.

Specimen ID	Population ID	Year	County	Locality	Latitude	Longitude
M&M1	Quanah 1985-1986	1985	Hardeman		34.30623	-99.65863
M&M105	Quanah 1985-1987	1986	Hardeman		34.30167	-99.68970
M&M106	Quanah 1985-1988	1986	Hardeman		34.31314	-99.70106
M&M109	Quanah 1985-1989	1986	Hardeman		34.30732	-99.69148
M&M110	Quanah 1985-1990	1986	Hardeman		34.31268	-99.70079
M&M111	Quanah 1985-1991	1986	Hardeman		34.30792	-99.69364
M&M112	Quanah 1985-1992	1986	Hardeman		34.30806	-99.69249
M&M113	Quanah 1985-1993	1986	Hardeman		34.30676	-99.69117
M&M114	Quanah 1985-1994	1986	Hardeman		34.30811	-99.69170
M&M115	Quanah 1985-1995	1986	Hardeman		34.29909	-99.68953
M&M116	Quanah 1985-1996	1986	Hardeman		34.30764	-99.69157
M&M117	Quanah 1985-1997	1986	Hardeman		34.31237	-99.70070
M&M118	Quanah 1985-1998	1986	Hardeman		34.31175	-99.70051
M&M119	Quanah 1985-1999	1986	Hardeman		34.30779	-99.69996
M&M120	Quanah 1985-2000	1986	Hardeman		34.30749	-99.69152
M&M122	Quanah 1985-2001	1986	Hardeman		34.31298	-99.70098
M&M17	Quanah 1985-2002	1986	Hardeman		34.30227	-99.68937
M&M18	Quanah 1985-2003	1986	Hardeman		34.30350	-99.68511
M&M19	Quanah 1985-2004	1986	Hardeman		34.30393	-99.68578
M&M2	Quanah 1985-2005	1985	Hardeman		34.30612	-99.65864
M&M20	Quanah 1985-2006	1986	Hardeman		34.30426	-99.68651
M&M21	Quanah 1985-2007	1986	Hardeman		34.30670	-99.69113
M&M22	Quanah 1985-2008	1986	Hardeman		34.30458	-99.68804

M&M23	Quanah 1985-2009	1986	Hardeman		34.30670	-99.69113
M&M24	Quanah 1985-2010	1986	Hardeman		34.30749	-99.69645
M&M25	Quanah 1985-2011	1986	Hardeman		34.30053	-99.68943
M&M27	Quanah 1985-2012	1986	Hardeman		34.30765	-99.69760
M&M28	Quanah 1985-2013	1986	Hardeman		34.30749	-99.69645
M&M29	Quanah 1985-2014	1986	Hardeman		34.30753	-99.69591
M&M30	Quanah 1985-2015	1986	Hardeman		34.30766	-99.69502
M&M31	Quanah 1985-2016	1986	Hardeman		34.30808	-99.69243
M&M32	Quanah 1985-2017	1986	Hardeman		34.30775	-99.69157
M&M33	Quanah 1985-2018	1986	Hardeman		34.30735	-99.69140
M&M34	Quanah 1985-2019	1986	Hardeman		34.30670	-99.69113
M&M35	Quanah 1985-2020	1986	Hardeman		34.30616	-99.69052
M&M36	Quanah 1985-2021	1986	Hardeman		34.30520	-99.68917
M&M39	Quanah 1985-2022	1986	Hardeman		34.30870	-99.69979
M&M52	Quanah 1985-2023	1986	Hardeman		34.30800	-99.69181
M&M54	Quanah 1985-2024	1986	Hardeman		34.30330	-99.68919
M&M55	Quanah 1985-2025	1986	Hardeman		34.30343	-99.68910
M&M6	Quanah 1985-2026	1985	Hardeman		34.30509	-99.68904
M&M64	Quanah 1985-2027	1986	Hardeman		34.30835	-99.68245
M&M65	Quanah 1985-2028	1986	Hardeman		34.30609	-99.67871
M&M68	Quanah 1985-2029	1986	Hardeman		34.30498	-99.68882
M&M69	Quanah 1985-2030	1986	Hardeman		34.30545	-99.68257
M&M7	Quanah 1985-2031	1985	Hardeman		34.30603	-99.65865
M&M70	Quanah 1985-2032	1986	Hardeman		34.30613	-99.68252
M&M73	Quanah 1985-2033	1986	Hardeman		34.11250	-99.75905
M&M98	Quanah 1985-2034	1986	Hardeman		34.30071	-99.69259
TTU12080	Quanah 1969-1970	1970	Hardeman	3 mi NE Quanah	34.31874	-99.70376
TTU13524	Quanah 1969-1971	1969	Hardeman	2.5 mi N, 4.75 mi E Quanah	34.33203	-99.65685
TTU13525	Quanah 1969-1972	1969	Hardeman	2.5 mi N, 6.5 mi E Quanah	34.33117	-99.62539
TTU13530	Quanah 1969-1973	1969	Hardeman	3.5 mi N, 7.5 mi E Quanah	34.34679	-99.60840

TTU13531	Quanah 1969-1974	1969	Hardeman	2.75 mi N, 9.5 mi E Quanah	34.33599	-99.57277
TTU13532	Quanah 1969-1975	1969	Hardeman	5 mi S, 0.5 mi E Quanah	34.22421	-99.73162
TTU13533	Quanah 1969-1976	1969	Hardeman	11 mi S, 2 mi E Quanah	34.13861	-99.77197
TTU13539	Quanah 1969-1977	1969	Hardeman	2.25 mi N, 7.5 mi E Quanah	34.32809	-99.60858
TTU13547	Quanah 1969-1978	1970	Hardeman	3 mi NE Quanah	34.32715	-99.70085
TTU13548	Quanah 1969-1979	1969	Hardeman	6 mi S, 4 mi E Quanah	34.21052	-99.67048
TTU13549	Quanah 1969-1980	1969	Hardeman	9.25 mi S, 6.5 mi E Quanah	34.16260	-99.62468
TTU13550	Quanah 1969-1981	1969	Hardeman	10.5 mi S, 5 mi E Quanah	34.15226	-99.65138
TTU13551	Quanah 1969-1982	1969	Hardeman	5 mi S, 5.5 mi E Quanah	34.22539	-99.64285
TTU24726	Quanah 1969-1983	1970	Hardeman	2.6 mi N, 6.7 mi E Quanah	34.33375	-99.62219
TTU24727	Quanah 1969-1984	1970	Hardeman	2.5 mi N, 5.25 mi W Quanah	34.33268	-99.64809
TTU24728	Quanah 1969-1985	1969	Hardeman	2.5 mi N, 7.25 mi E Quanah	34.32809	-99.60858
TTU24729	Quanah 1969-1986	1970	Hardeman	2.25 mi N, 8.75 mi E Quanah	34.32775	-99.58619
TTU24730	Quanah 1969-1987	1969	Hardeman	2.25 mi N, 9.25 mi E Quanah	34.32786	-99.57811
TTU24732	Quanah 1969-1988	1969	Hardeman	2 mi N, 9.5 mi E Quanah	34.32786	-99.57811
TTU24733	Quanah 1969-1989	1969	Hardeman	2 mi N, 9.5 mi E Quanah	34.32786	-99.57811
TTU24735	Quanah 1969-1990	1969	Hardeman	1.6 mi N, 8.8 mi E Quanah	34.32012	-99.58625
TTU24736	Quanah 1969-1991	1969	Hardeman	1.6 mi N, 8.8 mi E Quanah	34.32012	-99.58625
TTU24737	Quanah 1969-1992	1970	Hardeman	1.5 mi N, 2.5 mi E Quanah	34.31882	-99.69511
TTU24738	Quanah 1969-1993	1970	Hardeman	1.5 mi N, 2.5 mi E Quanah	34.31882	-99.69511
TTU24740	Quanah 1969-1994	1970	Hardeman	1.5 mi N, 2.5 mi E Quanah	34.31882	-99.69511
TTU24748	Quanah 1969-1995	1969	Hardeman	1.5 mi N, 2.5 mi E Quanah	34.31882	-99.69511
TTU24749	Quanah 1969-1996	1970	Hardeman	1.5 mi N, 2.5 mi E Quanah	34.31882	-99.69511
TTU24750	Quanah 1969-1997	1970	Hardeman	1.5 mi N, 2.5 mi E Quanah	34.31882	-99.69511
TTU24752	Quanah 1969-1998	1970	Hardeman	1.5 mi N, 2.5 mi E Quanah	34.31882	-99.69511
TTU24753	Quanah 1969-1999	1970	Hardeman	3 mi NE Quanah	34.31882	-99.69511
TTU24772	Quanah 1969-2000	1970	Hardeman	1 mi N, 7.5 mi E Quanah	34.31617	-99.60834
TTU24773	Quanah 1969-2001	1969	Hardeman	1 mi N, 8.25 mi E Quanah	34.31351	-99.59633
TTU24774	Quanah 1969-2002	1969	Hardeman	1 mi N, 8.3 mi E Quanah	34.31329	-99.59427
TTU24775	Quanah 1969-2003	1969	Hardeman	1 mi N, 8.3 mi E Quanah	34.31329	-99.59427

TTU24777	Quanah 1969-2004	1969	Hardeman	1 mi N, 8.5 mi E Quanah	34.31224	-99.58930
TTU24779	Quanah 1969-2005	1969	Hardeman	1 mi N, 9 mi E Quanah	34.31273	-99.58384
TTU24780	Quanah 1969-2006	1969	Hardeman	1 mi N, 9 mi E Quanah	34.31273	-99.58384
TTU24781	Quanah 1969-2007	1969	Hardeman	1 mi N, 9 mi E Quanah	34.31273	-99.58384
TTU24782	Quanah 1969-2008	1969	Hardeman	1 mi N, 9.25 mi E Quanah	34.31273	-99.58384
TTU24783	Quanah 1969-2009	1970	Hardeman	1 mi N, 9.5 mi E Quanah	34.31273	-99.58384
TTU24784	Quanah 1969-2010	1969	Hardeman	0.9 mi N, 8.6 mi E Quanah	34.30843	-99.58835
TTU24785	Quanah 1969-2011	1969	Hardeman	0.75 mi N, 9.5 mi E Quanah	34.30695	-99.57529
krat10	Quanah 1969-2012	2005	Wichita		34.03023	-98.76671
krat11	Iowa Park 2005-2008	2005	Wichita		34.03023	-98.76671
krat12	Iowa Park 2005-2009	2005	Wichita		34.03023	-98.76671
krat13	Iowa Park 2005-2010	2005	Wichita		34.03023	-98.76671
krat14	Iowa Park 2005-2011	2005	Wichita		34.03023	-98.76671
krat15	Iowa Park 2005-2012	2005	Wichita		34.03023	-98.76671
krat16	Iowa Park 2005-2013	2005	Wichita		34.03023	-98.76671
krat17	Iowa Park 2005-2014	2005	Wichita		34.03023	-98.76671
krat18	Iowa Park 2005-2015	2005	Wichita		34.03023	-98.76671
krat19	Iowa Park 2005-2016	2005	Wichita		34.03023	-98.76671
krat20	Iowa Park 2005-2017	2005	Wichita		34.03023	-98.76671
krat21	Iowa Park 2005-2018	2007	Wichita		34.05423	-98.81721
krat22	Iowa Park 2005-2019	2007	Wichita		34.05423	-98.81721
krat23	Iowa Park 2005-2020	2007	Wichita		34.05423	-98.81721
krat24	Iowa Park 2005-2021	2007	Wichita		34.05423	-98.81721
krat25	Iowa Park 2005-2022	2007	Wichita		34.05423	-98.81721
krat26	Iowa Park 2005-2023	2007	Wichita		34.05423	-98.81721
krat27	Iowa Park 2005-2024	2007	Wichita		34.05423	-98.81721
krat28	Iowa Park 2005-2025	2007	Wichita		34.05423	-98.81721
krat29	Iowa Park 2005-2026	2007	Wichita		34.05423	-98.81721
krat3	Iowa Park 2005-2027	2007	Wichita		34.02883	-98.75862
krat30	Iowa Park 2005-2028	2007	Wichita		34.05423	-98.81721

krat33	Iowa Park 2005-2029	2008	Wichita		34.07219	-98.69315
krat34	Iowa Park 2005-2030	2008	Wichita		34.07219	-98.69315
krat35	Iowa Park 2005-2031	2008	Wichita		34.07219	-98.69315
krat4	Iowa Park 2005-2032	2008	Wichita		34.03023	-98.76671
krat41	Iowa Park 2005-2033	2008	Wichita		34.05446	-98.78699
krat5	Iowa Park 2005-2034	2008	Wichita		34.03023	-98.76671
krat6	Iowa Park 2005-2035	2008	Wichita		34.03023	-98.76671
krat7	Iowa Park 2005-2036	2008	Wichita		34.03023	-98.76671
krat8	Iowa Park 2005-2037	2008	Wichita		34.03023	-98.76671
krat9	Iowa Park 2005-2038	2008	Wichita		34.03023	-98.76671
MWSU2671	Iowa Park 1966-1969	1966	Wichita	6.5 mi NW of Iowa Park	34.01493	-98.75540
MWSU5101	Iowa Park 1966-1970	1967	Wichita	8 mi N of Iowa Park	34.06899	-98.66952
MWSU5132	Iowa Park 1966-1971	1967	Wichita	6 mi W. Iowa Park then 1 mi N.	33.96665	-98.77647
MWSU5135	Iowa Park 1966-1972	1967	Wichita	1 mi N, 6 mi W of Iowa Park	33.96794	-98.74025
MWSU5136	Iowa Park 1966-1973	1967	Wichita	5 mi NW of Iowa Park	34.01405	-98.73999
MWSU5333	Iowa Park 1966-1974	1967	Wichita	5 miles NW of Iowa Park	34.00203	-98.74063
MWSU5334	Iowa Park 1966-1975	1967	Wichita	Buffalo Creek Reservoir	34.00203	-98.74063
MWSU5337	Iowa Park 1966-1976	1967	Wichita	5 mi. N.W. Iowa Park	34.00203	-98.74063
MWSU5338	Iowa Park 1966-1977	1967	Wichita	3 mi. W. Iowa Park	33.95443	-98.72505
MWSU5341	Iowa Park 1966-1978	1967	Wichita	7 mi WNW of Iowa Park	33.99080	-98.78762
MWSU5344	Iowa Park 1966-1979	1967	Wichita	7 mi. W. Iowa Park	33.95015	-98.79266
MWSU5345	Iowa Park 1966-1980	1967	Wichita	6 mi. N.W. Iowa Park	34.01473	-98.74423
MWSU5842	Iowa Park 1966-1981	1967	Wichita	8 mi N of Iowa Park	34.06882	-98.66835
MWSU5843	Iowa Park 1966-1982	1967	Wichita	3 mi. NW of Iowa Park	33.97892	-98.71037
MWSU5844	Iowa Park 1966-1983	1967	Wichita	6 mi W. Iowa Park, then 1 mi N.	33.96665	-98.77647
MWSU5847	Iowa Park 1966-1984	1967	Wichita	3 mi. W. Iowa Park	33.97892	-98.71037
MWSU5849	Iowa Park 1966-1985	1967	Wichita	6 mi. N.W. Iowa Park	34.01473	-98.74423
MWSU5851	Iowa Park 1966-1986	1967	Wichita	3 mi. W. Iowa Park	33.97892	-98.71037
MWSU5852	Iowa Park 1966-1987	1967	Wichita	3 mi. W. Iowa Park	33.97892	-98.71037

MWSU5853	Iowa Park 1966-1988	1967	Wichita	6 mi NW of Iowa Park	34.01473	-98.74423
MWSU5854	Iowa Park 1966-1989	1967	Wichita	6 mi. W. Iowa Park then 1 mi N.	33.96665	-98.77647
MWSU5857	Iowa Park 1966-1990	1967	Wichita	7 mi west north west Iowa Park, Wichita Falls	33.99080	-98.78762
MWSU7009	Iowa Park 1966-1991	1966	Wichita	9 mi. NW Iowa Park	34.04973	-98.77406
MWSU7288	Iowa Park 1966-1992	1969	Wichita	Lake Buffalo	34.00203	-98.74063
MWSU7292	Iowa Park 1966-1993	1969	Wichita	0.5 mi E of Lake Buffalo	34.00203	-98.74063
MWSU7297	Iowa Park 1966-1994	1969	Wichita	5 mi NW of Iowa Park	34.00203	-98.74063
MWSU7319	Iowa Park 1966-1995	1969	Wichita	1 mi. W. Iowa Park	33.94977	-98.69751
MWSU7676	Iowa Park 1966-1996	1967	Wichita	Not recorded		
MWSU8420	Iowa Park 1966-1997	1967	Wichita	7 mi. WNW Iowa Park	33.99080	-98.78762
MWSU8423	Iowa Park 1966-1998	1967	Wichita	1 mi. W. Iowa Park	33.94977	-98.69751
MWSU5331	Vernon	1967	Wilbarger	16 mi SSE Vernon	33.92553	-99.20124
MWSU5332	Vernon	1967	Wilbarger	16 mi SSE Vernon	33.92553	-99.20124
MWSU5845	Vernon	1967	Wilbarger	16 mi SSE Vernon	33.92553	-99.20124
MWSU6797	Vernon	1968	Wilbarger	16 mi SSE Vernon	33.92553	-99.20124
MWSU6798	Vernon	1968	Wilbarger	16 mi SSE Vernon	33.92553	-99.20124
MWSU6799	Vernon	1968	Wilbarger	16 mi SSE Vernon	33.92553	-99.20124
MWSU7007	Vernon	1968	Wilbarger	16 mi SSE Vernon	33.92553	-99.20124
MWSU7008	Vernon	1968	Wilbarger	16 mi SSE Vernon	33.92553	-99.20124
MWSU7060	Vernon	1968	Wilbarger	16 mi SSE Vernon	33.92553	-99.20124
MWSU7061	Vernon	1968	Wilbarger	16 mi SSE Vernon	33.92553	-99.20124
MWSU7062	Vernon	1968	Wilbarger	16 mi SSE Vernon	33.92553	-99.20124
MWSU7063	Vernon	1968	Wilbarger	16 mi SSE Vernon	33.92553	-99.20124
MWSU7064	Vernon	1968	Wilbarger	16 mi SSE Vernon	33.92553	-99.20124
TK174871	Harrold	1985	Wilbarger	2 mi N Harrold	34.10920	-99.03426
TK174872	Harrold	1985	Wilbarger	2 mi N Harrold	34.10920	-99.03426
TK174873	Harrold	1985	Wilbarger	2 mi N Harrold	34.10920	-99.03426
TK174874	Harrold	1985	Wilbarger	2 mi N Harrold	34.10920	-99.03426
TK174875	Harrold	1985	Wilbarger	2 mi N Harrold	34.10920	-99.03426

TK174876	Harrold	1985	Wilbarger	2 mi N Harrold	34.10920	-99.03426
TK174877	Harrold	1985	Wilbarger	2 mi N Harrold	34.10920	-99.03426