GENETIC ANALYSIS OF BEAVER REINTRODUCTIONS IN TEXAS

_____

A Thesis

Presented to

The Faculty of the Department of Biological Sciences

Sam Houston State University

_____

In Partial Fulfillment

of the Requirements for the Degree of

Master of Science

_____

by

Drew R. Neyland

December, 2022

GENETIC ANALYSIS OF BEAVER REINTRODUCTIONS IN TEXAS

by

Drew R. Neyland

_____

APPROVED:

Monte L. Thies, PhD
Committee Director

Juan D. Daza, PhD
Committee Member

Christopher P. Randle, PhD
Committee Member

Alexandra Herrera-Martinez, PhD
Committee Member

William Godwin, PhD
Committee Member

John B. Pascarella, PhD
Dean, College of Science & Engineering Technology

ABSTRACT

Neyland, Drew N., *Genetic analysis of beaver reintroductions in Texas.* Master of Science (Biology), December, 2022, Sam Houston State University, Huntsville, Texas.

The restoration of *Castor canadensis* in Texas is one of the state's greatest conservation success stories. By 1900, overexploitation by fur trappers decimated beaver numbers in the state and the species was thought to be extirpated from east Texas. Between 1939 and 1942, 129 beavers were translocated from source populations along the South Llano River of Edwards and Kimble Counties in southwest Texas were relocated into 27 eastern counties in an effort to restore the species. How this extirpation and subsequent reintroductions has impacted the genetic composition of present-day beaver populations is currently not known. Given the local extirpation in east Texas prior to 1900, our working hypothesis for this study was that current east Texas populations are wholly connected genetically to populations from southwest Texas. To address this question, we used mitochondrial DNA and microsatellite markers to determine the genetic effect of this bottleneck and connect present day populations to relict populations. To make this determination, we obtained samples from wildlife services, live trapping, incidental finds, and museum specimens from various regions across the state. Using mitochondrial markers, haplotype network analyses were used to reconstruct gene-flow patterns and historical events of current Texas populations. This reconstruction supports the hypothesis that significant gene flow has occurred across Texas beaver populations as a result of past reintroductions and reduced any observable bottleneck effect in current populations. This indicates that while past conservation actions may have had some effect on repopulation, subsequent recolonization was the main factor in population recovery of *Castor Canadensis.*

KEY WORDS: Beaver, *Castor canadensis*, Conservation, Reintroduction, Mitochondrial DNA, Microsatellites, Haplotypes, Texas

ACKNOWLEDGEMENTS

I would first like to thank my advisor and committee chair, Dr. Monte Thies, for his guidance throughout this whole project. Dr. Thies never discouraged me from taking challenges head on and exploring new methods and opportunities during my time as his student. I would also like to express my gratitude to my committee members, who each provided valuable contributions to this study. I would specifically like to acknowledge Dr. Alexandra Herrera-Martinez for sharing her knowledge and laboratory resources as well as her exceptional patience and guidance in the laboratory. This project would not have been possible without the valuable information from historical documents and records that Dr. William Godwin provided through resources at the Sam Houston State University Natural History Collections. I also want to thank Brian Mesenbrink with APHIS Wildlife Services, in addition to Dan Hepker, for the tissue samples that they provided for this project. For the live trapping effort, I would like to acknowledge to Alan Byboth and David Farris who were always willing to help get a beaver out of a trap whenever I called. I'm deeply grateful to all my family for providing support throughout this whole process and occasionally acting as unpaid wildlife technicians. Finally, I want to thank my amazing wife, Kris, who was always there to keep me going. From encouraging me when things got challenging to waking up early on cold mornings to help check traps, I truly could not have done this without her love and support. Funding for this project was provided by Texas Academy Science and Sam Houston State Department of Biological Sciences.

TABLE OF CONTENTS

Page

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

CHAPTER I

Introduction

The North American beaver (*Castor canadensis*) is a wide-spread species across Texas. It is found in the waterways of the Trans Pecos region of West Texas, the Edwards Plateau of Central Texas, and is even expanding into the Llano Estacado in the Panhandle region (Langlois et al., 2022; Schmidly & Bradley, 2016). However, populations are most abundant in East Texas where, due to its semi-aquatic lifestyle, beaver are well suited to the dense forests and wet conditions that result from higher annual rainfall. As ecosystem engineers, beavers increase biodiversity in the wetland systems of this region by creating geomorphological complexity, widening river corridors, and increasing habitat diversity (Naiman et al., 1988; Rossell et al., 2005)

Although present-day populations are increasing, *C. canadensis* historically suffered from near state-wide extirpation following European colonization (Lay, 1944). During the 1700s and 1800s, beaver populations declined rapidly due to overexploitation across the continent (Badger, 2018). In Texas, beavers had been eradicated from much of the state by 1850 (Schmidly & Bradley, 2016). This was especially true in East Texas, where the fur trade was extremely active. Like much of the rest of North America, beaver had been completely extirpated from East Texas prior to the 20th century. By the 1930s, regulations and game laws were introduced limiting when beavers could be hunted (Lay, 1944).

In 1937, the Pittman-Robertson Act granted Texas, along with the other states, funds to hire wildlife professionals and begin restoration projects (Swanson, 1987). One of the first projects included restoring the North American beaver in Texas. At the time,

the South Llano River, near Junction in Kimble County, was still densely populated with beavers, and was selected as the source population for reintroductions. In 1939, 45 individuals were live trapped and translocated to rivers and streams in mostly East Texas counties (Texas Game, Fish, and Oyster Commission, 1939). This program continued for several years and was extremely successful. Between 1939 and 1942, 129 beavers were released in 27 Texas counties (Lay, 1944). Further research has shown that after the Llano River System/East Texas project was ended in 1942, subsequent translocations took place across the state. Between 1942 and 1961, Texas Parks and Wildlife Department animal release records indicate that over 140 beavers were translocated across the state. Many of these were likely nuisance beavers being released elsewhere within the same county. However, a number of animals were moved across counties and at least 11 came from Alabama and eight from Louisiana were introduced into East Texas (Texas Parks and Wildlife Department, 2009). Figure 1, taken from an article published in the September 1944 Texas Game and Fish Magazine, shows a Texas Fish, Game, and Oyster Commission biologist releasing a translocated beaver in Anderson County while a group of school children observed.

Figure 1

Texas Game and Fish Article Photo

A beaver being released in Anderson County. The school at Montalba adjourned to the creek-bank for the occasion and supported the project with an abundance of community good will.

SEPTEMBER, 1944

Note: From "Dr. Beaver, Specialist" by D.W. Lay, 1944, *Texas Game and Fish.* Copyright 1944 by the Texas Parks and Wildlife Department (Originally: Texas Fish Game and Oyster Commission).

Today, beaver populations are once again numerous throughout East Texas (Schmidly & Bradley, 2016). They thrive in the thick forests of this region, building dams and diversifying plant communities in various rivers and streams. While it is unlikely that populations have returned to the levels prior to fur trapping, many regional populations are stable or are still growing (Baker & Hill, 2008). Populations have recovered so well that due to their relatively high localized abundance, many landowners now consider the species a nuisance because of their landscape altering habits. This population recovery shows that past conservation efforts significantly benefited beaver populations. The severe bottlenecking event associated with extirpating beaver in much of Texas undoubtedly resulted in an initial loss of allelic diversity and heterozygosity in the introduced populations; however, rapid population growth and gene flow from outside sources after 1942 may have helped to alleviate these genetic pressures (Nei et al., 1975).

In addition to beavers, the reintroduction of extirpated species into their former distributional ranges has aided in the recovery of other species including wolves, turkeys, Texas horned lizards, and many others (Latch & Rhodes, 2006; Vonholdt et al., 2007; Williams et al., 2019). However, the genetic effects of reintroducing organisms into new populations or new environments have not always been considered in these programs, as was the case with *C. canadensis* in Texas. Presently, genetic considerations have become a part of reintroduction programs (Seddon et al., 2007), and several studies have examined genetic effects on populations that have been restored by reintroductions (Ferrando et al. 2008; Frosch et al. 2014; Latch & Rhodes 2006; Rhodes et al. 2001).

Haplotype networks are a widely used method for analyzing and visualizing the

relationships among DNA sequences within a population or species and can reveal patterns of ancestry (Paradis, 2018). Rhodes et al. (2001) analyzed the haplotypes of pronghorn antelope populations containing reintroduced individuals from different origins. By identifying separate haplotypes, they were able to identify which portion of each population came from certain sources. Latch and Rhodes (2005) investigated how gene flow among reintroduced populations of wild turkey in Indiana could have obscured genetic signatures left by source populations. They found that these signatures were detectable decades after reintroduction. Their data showed that there was little gene flow even in regions where populations were in close proximity with each other: reintroduced populations, for the most part, retained the haplotypes found in their source populations. Studies like these were also done with otters (Ferrando et al., 2008), roe deer (Vernesi et al., 2002), and bighorn sheep (Olson et al., 2013).

Much like *C. canadensis* in Texas, the Eurasian beaver (*C. fiber*) suffered from regional extirpations in western and central Europe until the early 1900's (Frosh et al., 2014). Reintroductions occurred using several source populations, resulting in viable populations characterized by eight haplotypes that were identified through a genetic analysis of present-day Eurasian beaver populations (Frosch et al., 2014). Their haplotype data indicated that there were four distinct sources from which translocated European beavers originated. Results also showed that the populations were admixed throughout the study region.

Beaver reintroductions in Texas were done before genetic considerations could be taken into account. Therefore, there is no way to accurately know the impact that these reintroductions had on beaver recovery in East Texas. In this study, we utilize

mitochondrial DNA (mtDNA) and microsatellite markers to identify the genetic structuring of populations of *C. canadensis* in Texas and to investigate the genetic signature(s) left by past reintroductions of *C. canadensis*. Our results may provide a better understanding into how the beaver populations were able to rebound from its near catastrophic declines over the last 150-200 years. This study will also provide insight into the factors that may drive further population growth and range expansion in the state.

The objectives of this study were to 1) assess the genetic composition of present populations of *C. canadensis* in Texas, and 2) investigate the genetic signature(s) left by past reintroductions of *C. canadensis*. We hypothesized that there would be genetic similarity present among beaver populations in East Texas, and southwest Texas near the Llano River, the source of the translocated populations, despite significant gene flow among beaver populations in Texas. We also hypothesized that there would be little differences among populations detected through a comparison of the haplotype and microsatellite data, but that the data would provide evidence of population groupings based on geography.

CHAPTER II

Methods

Sample Collection

To examine the genetic structure of *C. canadensis,* tissue samples were obtained from beaver across the state of Texas. Overall, samples were collected from 68 separate individuals. 45 samples were provided by Wildlife Services, a state agency that manages nuisance wildlife and 10 samples were obtained via tissue loans from the Angelo State Natural History Collections (Angelo State University, San Angelo, TX) and the Natural Science Research Laboratory (Texas Tech University, Lubbock, TX). The remaining samples came from private landowners, professional trappers, incidental finds, and live trapping.

Live Trapping Protocol

Live trapping was conducted at Sam Houston State University's Pineywoods Environmental Research Laboratory (PERL) in Walker County, TX. In addition to PERL, live trapping was also conducted at Big Bend National Park under the National Park Service Scientific Research and Collecting Permit Number BIBE-2021-SCI-0010. Field work was carried out from January to May 2021. All trapping and handling methods were approved by the Sam Houston State University Institutional Animal Care and Use Committee (IACUC Protocol No. 921991-3) and Texas Parks & Wildlife Scientific Permit Number SPR-1020-178.

Double door Comstock traps were placed in areas of high activity such as channels and trails within a pond ecosystem (Figure 2, left). The traps were baited with a mixture of fruit and vegetables including cabbage, apples, carrots, and twigs of preferred

forage species (i.e., *Salix nigra*), and castor scent was added to the mixture. Traps were opened each day between 17:30 and 19:00, depending on the time of sunset, and were closed after being checked between 06:00 and 07:00 to ensure that no animals were captured during the day. Once captured, beavers were placed in a Beaver Transfer Bag (Tom Bihn, Seattle, WA) and restrained for collection of a tissue sample (Figure 2, right). A topical antibiotic solution was generously applied to the peripheral area of the beaver's tail and a tissue sample was collected using a sterilized 2mm dermal biopsy punch. The tissue sample was then stored in 80% ethanol. Once processed, each individual was immediately released back into the environment in the same location where it was caught.

Figure 2

Live Trapping Photos

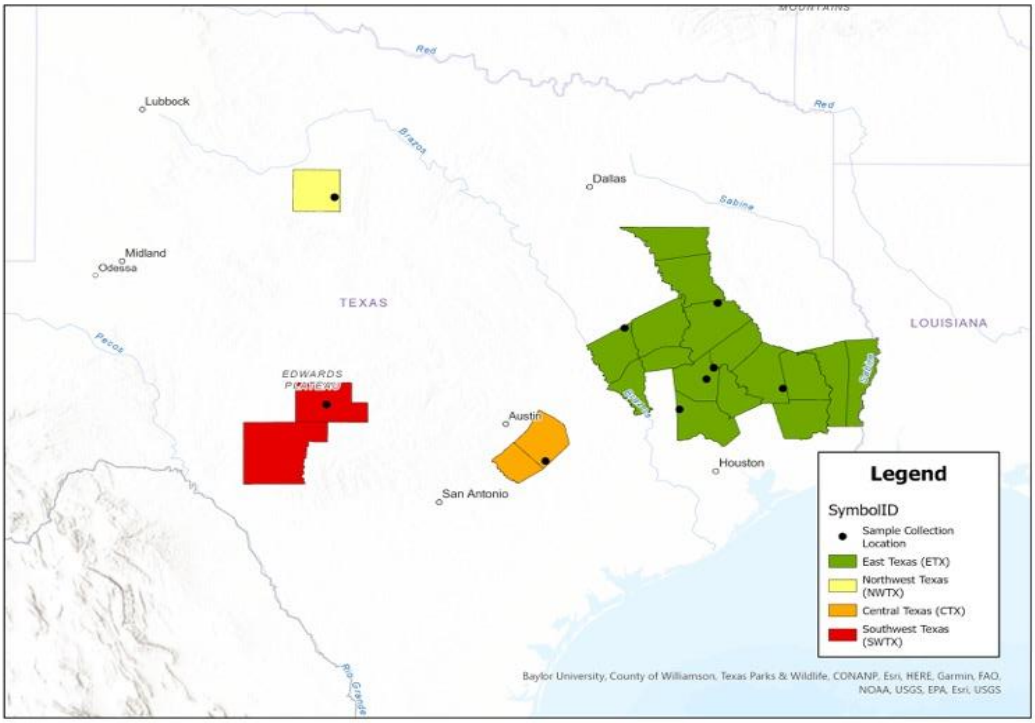
Note: Comstock traps were placed in channels used by beavers and covered in vegetation to make them less visible (Left). A specially designed beaver transfer bag was used to transport and restrain the beaver for sample collection (Right).

Population Grouping

Populations were organized by both geographic region and reintroduction history. The Southwest Texas population (SWTX, Figure 3) represents the area that reintroduced populations historically originated from. The East Texas population (ETX, Figure 3) is represented by counties where beavers were reintroduced between 1939 and 1942 (Texas Parks and Wildlife Department, 2009). This grouping area is geographically larger because it can be reasonably inferred that there would have been higher levels of gene flow in East Texas based on environmental conditions. With higher rainfall and an abundance of wetland habitat, beavers can disperse across multiple watersheds more efficiently in this region as compared to the drier regions of central and West Texas. The other two populations, Northwest and Central Texas (NWTX and CTX, respectively; Figure 3), were not a part of the reintroduction project according to available records but were included to help us draw inferences about how beavers may have dispersed across the state.

Figure 3

Population Sampling Map



Note. Populations are designated by color and include one to 16 counties based on number of samples collected, ecological conditions of the region, and reintroduction history. Approximate sites of sample collection are indicated by black dots.

Mitochondrial DNA Amplification and Analysis

Following sample collection, mitochondrial DNA was extracted using the Qiagen Blood and Tissue Kit following the manufacturer's instructions. The control region of the mitochondrial DNA was amplified using a GoTaq Flexi kit. The control region is the longest non-coding regions of the DNA and exhibits high variability (Bronstein et al., 2018). Non-coding sequences can provide the most information about changes in the population's genetic structure over time, (Frosch et al., 2014). Primers, 1F (5'-AATTACTTTGGTCTTGGTAAACC-3') and 6R (5'-

GCCCTGAAGTAAGAACCACATG-3') were used (Frosch et al., 2014). Polymerase chain reaction (PCR) took place in 50µl reactions containing 31.5 µl of H_2O, 0.5 µl of *Taq* polymerase, 10 µl green buffer, 1.0 µl $MgCl_2$, 1.0 µl of dNTP mix, and 2.0 µl each primer. We then applied the following thermal cycle: 5 min at 94°C, 40 PCR cycles (55 s at 94°C, 45 s at 54°C, 45 s at 72°C) plus 10 min at 72°C (Frosch et al., 2014). An agarose gel with ethidium bromide was run to check for successful and failed samples. Successful PCR products were cleaned following a PEG purification protocol. The amplified samples were sent to Eton Bioscience lab for sequencing (Eton Bioscience, Inc.). Sequences were edited and aligned using a MUSCLE alignment in Sequencher (Sequencher® version 5.4.6 DNA sequence analysis software, Gene Codes Corporation, Ann Arbor, MI, USA). Sequences were inputted into BLAST (Basic Local Alignment Search Tool) to verify species identity. DnaSP was used to reconstruct haplotypes and assign haplotypes to populations (Rozas et al., 2017). Finally, a parsimony haplotype network was created in PopART to visualize haplotype sharing between populations using the TCS method (Clement et al., 2002; Leigh and Bryant, 2015).

Microsatellite DNA Amplification and Analysis

For microsatellite analysis, we originally chose nine microsatellites that have been identified for *Castor canadensis* (Crawford et al., 2008). PCR was performed with each individual marker using Qiagen master mix in 10 µl reactions including 1.0 µl DNA template, 5.3 µl H_2O, 2.0 green buffer, 0.5 µl $MgCl_2$, 0.5 µl dNTP, 0.3 µl of 10 µM forward and reverse primer, and 0.1 µl *Taq*-Polymerase (Qiagen). Fragments were amplified under the following cycle conditions: 15 min at 95°C, 45 cycles of 30 s at 94°C, 90 s annealing at the specific temperature used for each marker, 60 s at 72°C, and

final elongation of 30 min at 72°C. For all PCR reactions positive and negative controls were included. Before beginning PCR with all 68 samples, each marker was tested with a small subset of DNA samples. Seven of the nine markers failed to amplify a product. The two markers chosen for analysis of the full sample set are shown in Table 1.

Table 1

Characterization of the Two Markers Chosen for Analysis

Locus	Primer Sequence (5'-3')	Repeat motif	T_a	N	ASR
Cca9	TCTTTCTTGTTGGTCCTGGAA TGGGAGAGTGGTTGCCTATC	$TG_{(19)}$	60	10	136-156
Cca13	CCCTAGACTTTGATTATACGG AGGTTGCCTAGAGAGAGGTGTG	$GT_{(11)}GT_{(7)}$	60	6	277-297

Note: Shown is the reaction-specific annealing temperature (T_a), the number of alleles (N), and approximate size range (ASR) along with locus, primer sequence, and repeat motif of the two microsatellite marker used for microsatellite analysis (Crawford et al., 2008)

A multiplex PCR reaction was performed with the Cca9 and Cca13 markers using 10 µl Multiplex Master Mix (ThermoFisher Scientific) in 20 µl reactions including 10 µl H_2O, 0.5 µl of 10 µM HEX and FAM fluorescent primer, 0.5 µl of 10 µM Cca9 and Cca13 reverse primers, and 2 µl of DNA extract. Following gel electrophoresis, 52 samples were chosen for fragment analysis (Eton Bioscience, Inc.). Resulting chromatograms were inputted into Microsatellite Analysis Software to score peaks, determine allelic diversity, identify heterozygotes within the population (ThermoFisher Scientific).

After analyzing peaks and identifying heterozygotes in the sample set, our goal was to perform two statistical analyses (STRUCTURE and Principal Coordinates Analysis) to examine population structure across the whole sample set. STRUCTURE was to be used to assign individuals to a population based on multilocus genotype data. The software would illustrate any admixture within individual beavers and identify the number of groups present within the samples data along with the probability that each individual can be assigned to a distinct genetic group. A Principal Coordinates Analysis (PCoA) would also illustrate any natural groupings within the data. It would allow for analysis of genetic distance between samples. The results of this analysis would then be compared to the geographic groupings of the samples to determine if there is a geographic correlation with genetic distance between samples, as well as any indication that the past reintroductions have affected these distances. These two analyses would be combined to fully analyze the population structure of beavers across the state.

.

CHAPTER III

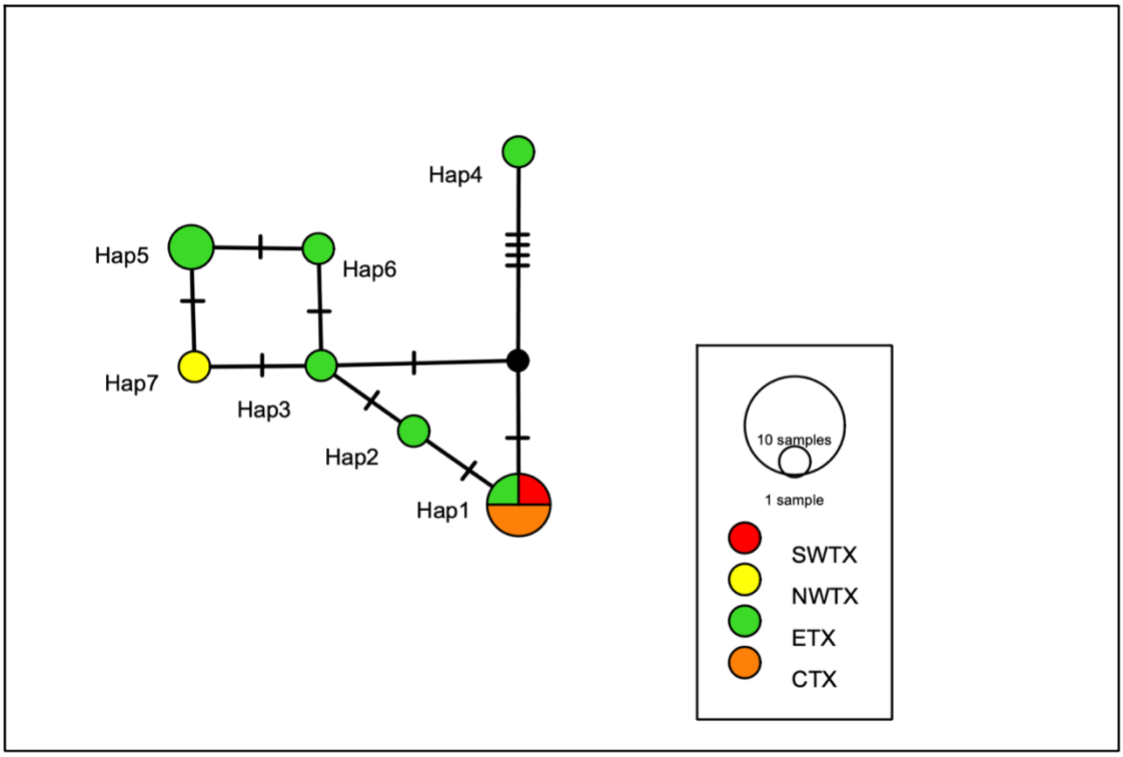
Results

Mitochondrial DNA Analysis

After amplification and sequencing, mitochondrial sequence data were obtained from only 11 of the 68 individuals sampled. Those data represented four separate populations and were included in further analysis based on the quality of the sequences obtained (Table 2). All sequences will be submitted to GeneBank for public use. We analyzed a sequence length of 557 bp of the control region of mitochondrial DNA and identified seven haplotypes (Figure 4, Table 2). One haplotype, (Hap1) was shared among four out of 11 individuals examined and was the only haplotype common to three population groups (SWTX, CTX, and ETX). The ETX population had six haplotypes present in seven individuals. One haplotype (Hap4) was differentiated from the rest of the ETX populations by four mutations (Figure 4). The NWTX populations presented a haplotype (Hap7) that was different from any haplotypes found in the other populations.

Figure 4

Haplotype Network



Note. Populations are designated by color. Mutations separating the haplotypes are indicated by tick marks. Larger circles represent more samples that contain that haplotype.

Table 2

Summary of Mitochondrial Data Set

Population	N	C_N	H_N	Reintroduction History
East Texas (ETX)	7	16	6	R
Southwest Texas (SWTX)	1	2	1	S
Northwest Texas (NWTX)	1	1	1	NI
Central Texas (CTX)	2	2	1	NI
Total	11	21	7*	

Note: Shown above is the number of samples in each population (N), number of counties in the sampling area (C_N), and number of haplotypes identified in each population (H_N). The reintroduction history is also shown (R = reintroduction area; S = source population; NI = not involved in the reintroduction project 1939-1942). *One haplotype was shared among ETX, SWTX, and CTX.

Microsatellite DNA Analysis

Due to an unknown error, no peaks appeared in the chromatogram following the fragment analysis. Therefore, the samples could not be analyzed in STRUCTURE and a Principal Coordinates Analysis could not be performed. Results from the fragment analysis were uploaded to Microsatellite Analysis Software (Thermoscientific). Panels were created to outline where peaks should be detected for both Cca9 and Cca13. However, the only peaks that appeared in the chromatogram were present outside of the defined panels and were found at the same location on both markers in most of the samples. We concluded that these peaks were merely white noise from the fragment analysis not associated with the markers that were being analyzed. This made genotyping

these samples impossible. Without genotyping, analysis could not be performed with either STRUCTURE or Principal Coordinates Analysis.

CHAPTER IV

Discussion

In this study, we were able to identify shared genetic history among four geographically distinct beaver populations. While the larger goal of this study was to also examine state-wide population structure using microsatellite data, mitochondrial DNA results allowed us to still make valuable inferences about the genetic composition of beaver populations in Texas. A TCS haplotype network (Figure 4) allowed us to visualize the shared DNA sequences among the sampled populations and examine how past reintroductions and translocations may have impacted the genetic makeup of the state-wide population.

Our mitochondrial data showed that at least six haplotypes exist in the East Texas population (Table 2), which had the greatest number of haplotypes of any of our sampling groups. This result could suggest that more ancestral lineages are present within this population, and could be attributed to both a larger sample size from this region as well as environmental effects on past gene flow. Of the 11 samples we were able to obtain viable sequences from, seven were from the East Texas population. This higher sample size allowed for more opportunity to detect unique haplotypes. Had the data from the full sample set been successfully processed, more conclusive evidence could support the presence of more unique haplotypes in East Texas, or possibly show the opposite with more samples in other poulations. However, previous studies have shown that watershed structure can also greatly affect the genetic composition of beaver populations (Epps et al., 2021). Many watersheds come together in East Texas, especially in Southeast Texas. The East Texas sampling region was comprised of 12 river subbasin (fourth-level, HUC

8) watersheds while the other sampling areas only contained from two to six watersheds. Epps et al. (2021) found that beaver dispersal most commonly occurred within major tributary (fifth-level, HUC 10) watersheds and adjacent watersheds. There are over 30 fifth-level watersheds within the East Texas sampling area, not including counties that we did not samples. The higher number of tributaries may be a reason the higher number of haplotypes in this region.

Precipitation levels and habitat characteristics are also likely contributors to the increased number of unique haplotypes for East Texas. McNew and Wolf (2005) found that beavers dispersed farther from natal colonies in areas with free-flowing water access. East Texas averages about 43-46 inches of rain each year (Bomar, 1996), while the Southeastern United States averages around 50 inches of precipitation per year (National Centers for Environmental Information). This high amount of rainfall, along with lower elevation, creates an abundance of rivers, lakes, and wetlands across the region. These conditions provide prime habitat for beavers as populations recovered across the Southeastern U.S. Young beavers were and still are able to disperse farther, allowing more migration into East Texas from any remaining populations from outside the region or from populations that had been reintroduced in adjacent states.

When compared to East Texas, our other sampling areas are drier and have less connectivity between water sources. This makes migration and gene flow less likely in these areas. Lower migration and gene flow potential results in less introduction of unique haplotypes. Migrants from outside sources could likely explain the higher number of haplotypes found in the East Texas group.

Lastly, translocations done after 1942 into and within East Texas could provide a

further explanation for more haplotypes being identified in the East Texas region. After 1942, TPWD records show that at least 10 relocation or translocation events took place that moved beavers into East Texas counties (Texas Parks and Wildlife Department, 2009). Most of these efforts were relocation efforts within East Texas; however, two of those events brought beavers into East Texas from Louisiana and Alabama. These relocations from sources other than Edwards and Kimble counties, combined with natural migration from beaver populations recovering in other southeastern states, likely have resulted in more haplotypes. It is also relevant to point out that sample 21015 exhibits a haplotype that is more genetically distant from the other samples in the network; however, we do not have comparable haplotype data from other studies to aid in determining where this haplotype may have originated. This haplotype is four mutations away from its next closest lineage (Figure 4), and it is likely that this lineage could be the result of a reintroduction from a source population not included in our analysis, such as an out of state source.

In addition to the higher number of haplotypes in the East Texas population, our results indicate that there is, in fact, a common lineage between populations in the translocated population (ETX) and the source population (SWTX). This haplotype was also found within the Central Texas population. Overall, Hap1 contained one individual from Montgomery County (ETX), two from Bastrop County (CTX), and one from Kimble County (SWTX) (Table 3). This supports our hypothesis that there is genetic similarity between source populations and reintroduced populations. However, known reintroduction history does not explain why this haplotype is also found in the Central Texas population. One possible explanation is that Hap1 could be a common haplotype

within the Colorado River watershed. Both populations, CTX and SWTX, are within this major river basin (third-level, HUC 6). If this is the case, the Montgomery County sample exhibiting Hap1 (Table 3) would be consistent with our prediction that genetic evidence can connect present day populations with past reintroduction events. It is difficult to make any further or more conclusive inferences about the structure of these populations or the cause of this genetic similarity without a larger sample size or data from microsatellite DNA population structure analyses.

Table 3

Samples and Haplotype Summary

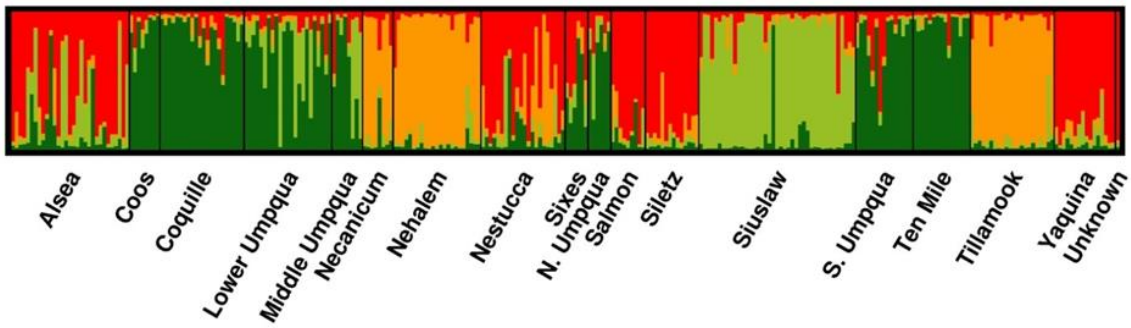
Sample Number	County	Haplotype	Population
20125	Kimble Co.	Hap1	SWTX
21008	Montgomery Co.	Hap1	ETX
21052	Bastrop Co.	Hap1	CTX
21053	Bastrop Co.	Hap1	CTX
21041	Trinity Co.	Hap2	ETX
21027	Houston Co.	Hap3	ETX
21015	Polk Co.	Hap4	ETX
21040	Walker Co.	Hap5	ETX
20126	Robertson Co.	Hap5	ETX
21039	Walker Co.	Hap6	ETX
21043	Jones Co.	Hap7	NTX

The reason that peaks were not detected in the microsatellite data set following fragment analysis is unclear. There are many steps at which the samples could have been compromised. While the samples showed clear product following gel electrophoresis, the product could have deteriorated due to a pipetting error transferring samples to the final plate, inadequate storage temperature, a degraded quality of the fluorescent primers, or poor handling while being shipped to the lab. It is also possible that any combination of these factors could have resulted in the lack of product. Unfortunately, we were unable to attempt to repeat the microsatellite amplification and fragment analysis with the time and resources available.

Epps et al. (2021) conducted a study similar to our planned analysis using microsatellite data from 291 beavers in Coastal Oregon. The goal of this study was to use microsatellite markers to evaluate genetic structure and understand how landscape features have affected gene flow in beaver populations in this region. Like Texas, many legal and unsanctioned translocations of beavers occurred during the past century. As predicted, these translocations obscured the relationship that landscape characteristics had with gene flow. However, they did find that once certain alleles were introduced into a population, landscape features, such as major watersheds, had a significant effect on the spread of those alleles. Their analysis showed that clustering occurs within each major river drainage (Figure 5). Though, there is mixing among river drainages exhibited by multiple colors within certain regions. More colors within a region indicate that more individuals have mixed ancestry. It was found that the regions with higher numbers of recorded translocations displayed the highest levels of mixed ancestry.

Figure 5

STRUCTURE Analysis of Beaver Populations in Coastal Oregon



Note: Individual cluster assignment probabilities from STRUCTURE analysis of American beaver (*Castor canadensis*) sampled in 2014 in western Oregon, USA by Epps et al. (2021). Number of assumed clusters (K) = 4. Each vertical bar represents an individual, and colors show proportional assignment to each cluster. Individuals are grouped by major river drainage. From "Landscape Genetics of American Beaver in Coastal Oregon" by C.W. Epps, V.M. Petro, T.G. Creech, R.S. Crowhurst, M.J. Weldy, and J.D. Taylor, 2021, *The Journal of Wildlife Management, 85*(7), supporting information (https://doi.org/10.1002/jwmg.22102). Copyright 2021 by the Wildlife Society.

The reintroduction of beavers into East Texas was a significant effort to restore a species that had been decimated by human exploitation. Our results indicate that this event may have been so significant in the restoration of the North American beaver that there is still a detectable effect on the genetic composition of present-day populations. It is likely that Hap1 can be attributed to this reintroduction event. There have been no other studies done on the genetic composition of beaver in Texas with which to compare our results. Future studies would benefit in using methods other than mitochondrial DNA, such as microsatellites or SNP analysis, to gain more informative results pertaining to population structure in Texas beavers. Our results support the hypothesis that beavers populations are grouped geographically and that this grouping is affected by past events.

However, heterozygosity, admixture, nucleotide diversity, and other measures of genetic structure could not be analyzed with our methods. Due to the size of Texas, it would also be interesting to analyze population structure at a finer scale, such as watersheds. As this species expands into unoccupied habitat across the state, they will likely be dispersing across watersheds and understanding the genetic composition of populations will help wildlife managers to interpret the dispersal that is driving this species' range expansion.

CHAPTER V

Summary

Overall, this study deepens our understanding of how efforts in the 1930's impact our wildlife today. This past era brought about significant changes in how wildlife was managed in the United States and laid the foundation for the robust populations we see in many species in Texas today. Our results show that there is clearly a genetic signature of past reintroductions still present in East Texas beaver populations. Further studies of this nature focusing on beavers and other species can illustrate how management decisions made over 80 years ago are still affecting wildlife populations. These results can also help to inform present-day biologists and managers as they make genetic considerations when doing beaver translocations and reintroductions. Our study indicates that because of past actions of wildlife professionals, beavers today are expanding their range, reoccupying former habitat, and impacting wetland systems across the state where they were once regionally extirpated. Future studies should strive to include more genetic structure analyses using various marker and methods, further understand landscape and watershed effects on genetic composition, and attempt to gather a more robust sample size from areas of the state that were not sampled in this study. These recommendations combined with our haplotype data would provide a deeper understanding of population genetics of the *Castor canadensis* in Texas today and how the populations is growing and dispersing in the state.

REFERENCES

Badger, J. R. (2018). *Texas in the Southwestern fur trade, 1718-1840.* [Master's thesis, Utah State University]. DigitalCommons. https://doi.org/10.26076/a335-b0d7.

Baker, B.W. & Hill E.P. (2003) Beaver (*Castor canadensis*). In G.A., Feldhamer, B.C., Thompson, & J.A., Chapman (Eds), *Wild mammals of North America: Biology, Management, and Conservation* (288-310). The Johns Hopkins University Press.

Bomar, G.W. (1996) *Weather*. Texas State Historical Association. Retrieved July 29, 2022, from https://www.tshaonline.org/handbook/entries/weather

Bronstein, O., Kroh, A., & Haring, E. (2018). Mind the gap! The mitochondrial control region and its power as a phylogenetic marker in echinoids. *BMC Evolutionary Biology*, *18*(1), 80. https://doi.org/10.1186/s12862-018-1198-x

Clement, M., Posada, D. C. K. A., & Crandall, K. A. (2002). TCS: A computer program to estimate gene genealogies. *Molecular Ecology*, *9*(10), 1657–1659. https://doi.org/10.1046/j.1365-294x.2000.01020.x

Crawford, J. C., Liu, Z., Nelson, T. A., Nielsen, C. K., & Bloomquist, C. K. (2009). Genetic population structure within and between beaver (*Castor canadensis*) Populations in Illinois. *Journal of Mammalogy*, *90*(2), 373–379. https://doi.org/10.1644/08-MAMM-A-146.1

Crawford, J., Liu, Z., Nelson, T., Nielsen, C., & Bloomquist, C. (2008). Isolation and characterization of microsatellite loci in the beaver (*Castor canadensis*). *Molecular Ecology Resources*, *8*(3), 616–618. https://doi.org/10.1111/j.1471-8286.2007.02016.x

Davis, W. B. (1940). Critical notes on the Texas beaver. *Journal of Mammalogy*, *21*(1), 84–86. JSTOR. https://doi.org/10.2307/1374664

Epps, C. W., Petro, V. M., Creech, T. G., Crowhurst, R. S., Weldy, M. J., & Taylor, J. D. (2021). Landscape genetics of American beaver in Coastal Oregon. *The Journal of Wildlife Management*, *85*(7), 1462–1475. https://doi.org/10.1002/jwmg.22102

Frankham, R., Ballou, J. D., and Briscoe, D. A. (2002). *Introduction to conservation genetics.* Cambridge: Cambridge University Press. https://doi.org/10.1017/CBO9780511808999

Ferrando, A., Lecis, R., Domingo-Roura, X., & Ponsà, M. (2008). Genetic diversity and individual identification of reintroduced otters (*Lutra lutra*) in North-Eastern Spain by DNA genotyping of spraints. *Conservation Genetics*, *9*(1), 129–139. https://doi.org/10.1007/s10592-007-9315-1

Frosch, C., Kraus, R. H. S., Angst, C., Allgöwer, R., Michaux, J., Teubner, J., & Nowak, C. (2014). The genetic legacy of multiple beaver reintroductions in Central Europe. *PLOS ONE*, *9*(5), e97619. https://doi.org/10.1371/journal.pone.0097619

Gene Codes Corporation. (2018) *Sequencher®* (Version 5.4.6) [Computer software]. Gene Codes Corporation. http://genecodes.com

Horn, S., Durka, W., Wolf, R., Ermala, A., Stubbe, A., Stubbe, M., & Hofreiter, M. (2011). Mitochondrial genomes reveal slow rates of molecular evolution and the timing of speciation in beavers (*Castor*), One of the largest rodent species. *PLoS ONE*, *6*(1), e14622. https://doi.org/10.1371/journal.pone.0014622

Lay, D.W. (n.d.). *Beaver restocking, 1938-1944.* East Texas Research Center: Stephen F. Austin State University http://archives.sfasu.edu/repositories/2/archival_objects/44947

Lay, D. W (1944, September) Dr. Beaver, Specialist. *Texas Game and Fish,* 4-13.

Langlois, G. D., Cox, R. D., Gipson, P. S., & Stevens, R. D. (2022). The North American beaver (Castor canadensis) is recolonizing the Llano Estacado. *Western North American Naturalist*, *82*(1), 190–195. https://doi.org/10.3398/064.082.0120

Latch, E. K., & Rhodes, O. E. (2006). The effects of gene flow and population isolation on the genetic structure of reintroduced wild turkey populations: Are genetic signatures of source populations retained? *Conservation Genetics*, *6*(6), 981–997. https://doi.org/10.1007/s10592-005-9089-2

Leigh, J. W., & Bryant, D. (2015). Popart: Full-feature software for haplotype network construction. *Methods in Ecology and Evolution*, *6*(9), 1110–1116.

McNew, L. B., & Woolf, A. (2005). Dispersal and survival of juvenile beavers (*Castor canadensis*) in Southern Illinois. *The American Midland Naturalist*, *154*(1), 217–228. https://doi.org/10.1674/0003-0031(2005)154[0217:DASOJB]2.0.CO;2

Mock, K. E., Theimer, T. C., Wakeling, B. F., Rhodes, O. E., Greenberg, D. L., & Keim, P. (2001). Verifying the origins of a reintroduced population of Gould's wild turkey. *The Journal of Wildlife Management*, *65*(4), 871–879. JSTOR. https://doi.org/10.2307/3803036

Naiman, R. J., Johnston, C. A., & Kelley, J. C. (1988). Alteration of North American streams by beaver. *BioScience*, *38*(11), 753–762. https://doi.org/10.2307/1310784

Natural Resource Conservation Service. (2007, June) *Watersheds, hydrologic units, hydrologic unit codes, watershed approach, and rapid watershed assessments*. U.S. Department of Agriculture, Natural Resource Conservation Service. https://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/stelprdb1042207.pdf

Nei, M., Maruyama, T., & Chakraborty, R. (1975). The bottleneck effect and genetic variability in populations. *Evolution, 29*(1), 1–10. https://doi.org/10.1111/j.1558-5646.1975.tb00807.x

NOAA National Centers for Environmental Information. (2022, July) *Climate at a glance: Regional time series*. National Oceanic and Atmospheric Administration, National Centers for Environmental Information. Retrieved August 1, 2022, from https://www.ncei.noaa.gov/cag/

Olson, Z. H., Whittaker, D. G., & Rhodes Jr., O. E. (2013). Translocation history and genetic diversity in reintroduced bighorn sheep. *The Journal of Wildlife Management, 77*(8), 1553–1563. https://doi.org/10.1002/jwmg.624

Rhodes, O. E., Reat, E. P., Heffelfinger, J. R., & Devos, J. C. (2001). Analysis of reintroduced pronghorn populations in Arizona using mitochondrial DNA markers. *Proceedings of the Nineteenth Biennial Pronghorn Antelope Workshop*, 45–54.

Rosell, F., Bozsér, O., Collen, P., & Parker, H. (2005). Ecological impact of beavers *Castor fiber* and *Castor canadensis* and their ability to modify ecosystems. *Mammal Review, 35*(3–4), 248–276. https://doi.org/10.1111/j.1365-2907.2005.00067.x

Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J. C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S. E., & Sánchez-Gracia, A. (2017). DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Molecular Biology and Evolution*, *34*(12), 3299–3302. https://doi.org/10.1093/molbev/msx248

Schmidly, D., & Bradley, R.D. (2016) *The mammals of Texas* (7th ed.) The University of Texas Press.

Swanson, G. A. (1987). Creation and early history. *Wildlife Society Bulletin (1973-2006)*, *15*(1), 9–14. JSTOR.

Texas Game, Fish, and Oyster Commission. (1939). *Annual federal aid report of the* Game, Fish, and Oyster Commission.

Texas Parks and Wildlife Department. (2009). *Combined trap/restock historical data (1932-1989).*

ThermoFisher Scientific. (2021). *Microsatellite Analysis (MSA)* (Version 1.0) [Computer Software] Thermofisher Scientific. http://www.thermofisher.com

Vernesi, C., Pecchioli, E., Caramelli, D., Tiedemann, R., Randi, E., & Bertorelle, G. (2002). The genetic structure of natural and reintroduced roe deer (*Capreolus capreolus*) populations in the Alps and central Italy, with reference to the mitochondrial DNA phylogeography of Europe. *Molecular Ecology, 11(8),* 1285–1297. https://doi.org/10.1046/j.1365-294X.2002.01534.x

Vonholdt, B. M., Stahler, D. R., Smith, D. W., Earl, D. A., Pollinger, J. P., & Wayne, R. K. (2008). The genealogy and genetic viability of reintroduced Yellowstone grey wolves. *Molecular Ecology*, *17*(1), 252–274. https://doi.org/10.1111/j.1365-294X.2007.03468.x

Williams, D.A., Rains, N.D., Hale, A.M. (2019). Population genetic structure of Texas horned lizards: Implications for reintroduction and captive breeding. *PeerJ, 7*(e7746) https://doi.org/10.7717/peerj.7746

VITA

Drew Neyland, AWB®

EDUCATION
B.S. in Wildlife Biology (2018)
Texas State University, San Marcos, Texas
GPA: **3.43**

ACADEMIC RESEARCH EXPERIENCE
Graduate Researcher
Sam Houston State University | Huntsville, TX | 2019-2022
- Designed a unique research project and performed data analysis.
- Trapped live beavers and collected tissue samples.
- Analyzed mitochondrial and microsatellite DNA to determine haplotypes of Texas beaver populations.
- Assessed genetic composition of populations across the state.

Undergraduate Researcher
Texas State University | San Marcos, TX | 2018
- Trapped small mammals.
- Measured population abundance and activity in various habitat types.
- Used statistical analyses to measure activity.

EMPLOYMENT EXPERIENCE
Natural Resource Specialist II
Texas Military Department | Austin, TX | January 2022-Present
- Wildlife:
  - Perform in-house planning level surveys focusing on large mammals, reptiles, bats, and other wildlife on military lands.
  - Design and implement surveys for endangered species of bats and amphibians.
  - Accomplish yearly game surveys with a team of biologists
- Habitat:
  - Perform native warm season grass restoration
  - Participate in prescribed burn activities
  - Apply herbicide to combat woody vegetation encroachment
- Administrative:
  - Compiled data to create reports to be used in an Integrated Natural Resource Management Plan on military lands
  - Acted as project manager on contracted wildlife surveys and habitat projects

Wildlife field intern
Texas Parks and Wildlife Department | New Waverly, TX | May 2021-September 2021

- Wildlife:
  - Assisted WMA staff and district 6 biologists with spotlight surveys for white-tail deer in SHNF and Liberty county.
  - Gained extensive knowledge of threatened and endangered species management in the SHNF, such as the Red-cockaded Woodpecker.
  - Assisted in daily maintenance and wildlife management activities on the Sam Houston wildlife management area located in the Sam Houston National Forest.
- Habitat:
  - Performed native warm season grass restoration techniques on remnant blackland prairie ecosystems.
  - Operated a New Holland skid steer with a mulcher attachment to control woody encroachment into prairie and forest ecosystems.
  - Gained knowledge of common silvicultural practices and stand survey techniques used for wildlife management in upland pine forests.

Ecology teaching assistant
Sam Houston State University | Huntsville, TX | Aug. 2020-May 2021

- Education/Outreach:
  - Instructed up to 25 students in an outdoor lab setting as well as virtual meetings.
  - Led group discussions and student assignments focused on ecological principles at the organismal to ecosystem level.
  - Demonstrated ecological field work techniques and allowed students opportunities for hands-on field experience.

Urban wildlife intern
Texas Parks and Wildlife Department | Houston, TX | June 2020-Aug. 2020

- Wildlife:
  - Collected and analyzed coyote scat samples from urban greenspaces.
  - Used bone fragments, hair, and other remains to identify prey items in urban coyote diets.
  - Used a kayak to locate alligator snapping turtles using radio telemetry.
  - Assisted in mitigating human-wildlife conflict in an urban interface.
- Education/Outreach:
  - Presented management and educational programs focused on urban wildlife to children and adult groups.
  - Coordinated 15+ volunteers to collect project data throughout the field season.

Archive technician
SHSU Natural History Collections and Archives | Huntsville, TX | Aug. 2019-May 2020

- Created and utilized a finding aid to organize various scientific reports, TPWD correspondence, and miscellaneous archives related to natural history.
- Prepared the archives and museum for public opening. Uploaded images and documents into an accessible database.
- Conducted archival research using wildlife articles and reports dating as far back as the 1930's.

Foundations of Science teaching assistant
Sam Houston State University | Huntsville, TX | Aug. 2019-May 2020

- Education/Outreach:
    - Instructed up to 25 students in a classroom setting.
    - Led group discussions and student assignments.
    - Taught critical thinking in a scientific context. Subjects ranged from scientific method, geology, evolution, and biology.

Conservation intern
Bamberger Ranch Preserve | Johnson City, TX | Jan. 2019-July 2019

- Wildlife:
    - Collected insects and other invertebrates for an ongoing taxonomic survey.
    - Assisted with feeding and maintenance of a live collection of venomous and non-venomous snakes.
    - Assisted with a bi-annual bird survey.
    - Assisted with all research activities focused on a ~300,000 member bat colony, including acoustic monitoring and WNS detection.
- Habitat:
    - Managed a cedar-dominated forest to promote herbaceous understory.
    - Used mechanical equipment and herbicide to manage woody encroachment on a central Texas grassland ecosystem.
- Education/Outreach:
    - Designed and implemented outreach for adults and children about habitat restoration, ecology, and animal identification in the Texas Hill Country.
    - Coordinated volunteers on habitat management and data collection projects.

Farming assistant
Thomas Seed Farm | Maxwell, TX | Aug. 2016-Dec. 2018

- Operated maintenance and farming equipment used for harvesting wildflower seeds.
- Assisted in managing central Texas grasslands to promote growth of native wildflowers.
- Performed duties in adverse weather conditions.
- Completed regular maintenance on harvesting equipment.

VOLUNTEER WORK

Alligator Surveys | Huntsville, TX | 2021

- Assisted TPWD District 6 biologists with annual alligator surveys at Huntsville State Park.
- Used a spotlight to count alligators and estimate size.

Alligator Snapping Turtle Radio Telemetry | Houston, TX | 2020

- Continued a research project on Buffalo Bayou as a volunteer.
- Used radio telemetry to gather location data on tagged alligator snapping turtles.

Red-Cockaded Woodpecker habitat restoration | Huntsville, TX | 2019

- Used chainsaws and tractors to remove woody vegetation and promote an herbaceous understory.
- Created RCW habitat at Pineywood Environmental Research Laboratory (SHSU).

Bird banding | San Marcos, TX | 2017

- Assisted a PhD student banding chicks of cavity nesting birds at Freeman Ranch.

PEER-REVIEWED PUBLICATIONS

Lutterschmidt, W.I., Z.E. Perelman, **D.R. Neyland**, M.L. Thies, and E.D. Roth. 2021. *AGKISTRODON PISCIVORUS* (Northern Cottonmouth) - DIET. Herpetological Review 52(2).

PROFESSIONAL PRESENTATIONS

Neyland, D.R. and Thies, M.L. (2021, Feburary). *Genetic Analysis of Beaver Reintroductions in Texas.* Poster presentation given at the 124th annual meeting of Texas Academy of Sciences, Virtual.

Neyland, D.R. (2021, May). *Diet Composition of Urban Coyotes in the Greater Houston Area.* Poster presentation given at the 2021 International Urban Wildlife Conference, Virtual.

OUTREACH

Neyland, D.R. (2020, October) *Urban Bats: Benefits, Threats, and How to Help*. Presentation given to Girl Scout Troop 122251, Houston, TX.

Neyland, D.R., Norrid, K. (2020, July) *Urban Coyote Diet Project Training.* Presentation given to Houston-area chapters of Texas Master Naturalists as advanced training credits, Houston, TX.

PROFESSIONAL MEMBERSHIPS

Biological Sciences Graduate Students Organization: Vice President, 2020
Texas Society of Mammalogists: Member, 2020
Texas Academy of Science: Member, 2020
Texas Chapter Wildlife Society: Member, 2015-2021
Bat Association at Texas State: Member, 2017-2018

LICENSES/PERMITS

Type II Wildland Firefighter ("Red Card")
TPWD Scientific Research Permit

AWARDS/GRANTS

Texas Academy of Science Research Award, 2020
Texas Grant, 2015-2018
Commendable Academic Achievement in Biology, 2017