UNIVERSITY OF CENTRAL OKLAHOMA

Edmond, Oklahoma

Department of Biology

Reexamination of a Pocket Gopher (*Geomys*) Contact Zone in Oklahoma and Hybrid Identification Using Microsatellites and Mitochondrial DNA Markers

A THESIS

SUBMITTED TO THE GRADUATE FACULTY

In partial fulfillment of the requirements

for the degree of

MASTER OF SCIENCE IN BIOLOGY

By

Kristy Meyer

Edmond, OK

2019

Reexamination of a Pocket Gopher (*Geomys*) Contact Zone in Oklahoma and Hybrid Identification Using Microsatellites and Mitochondrial DNA Markers

A THESIS

APPROVED FOR THE DEPARTMENT OF BIOLOGY

July 2019

By _____
Dr. Michelle Haynie Committee Chairperson

Allyson M. Fenwick
Dr. Allyson Fenwick Committee Member

Nikki Seagraves
Dr. Nikki Seagraves Committee Member

Acknowledgments:

I would like to thank Dr. Michelle Haynie for her assistance and guidance on my project. She believed in me before I even started my degree and was constant with her support and advice. Cristina Coffman was invaluable in teaching me the basics of gopher trapping to get this project started. Dr. Seagraves and Dr. Fenwick were quick to offer suggestions as needed. Various skills and knowledge from my graduate classes were used during this project, so I want to thank Dr. Caire, Dr. Creecy, Dr. Butler, Dr. Kotturi, Dr. Chooback, and Dr. Watkins. Linda Luna was an amazing TA advisor and shoulder to cry on. I also want to thank the Office of High Impact Practices at the University of Central Oklahoma for two RCSA grants funding this project. Additionally, the College of Mathematics and Science Dean's Office provided additional funding support.

On a personal note, I want to thank Mario Franklin who was my constant support and always believed in me. Quinn Franklin was my biggest motivation to get lab work completed before her delivery. Bruce and Dixie Meyer were the supportive parents I am so grateful to have. Austin, Justin, and Mitchell Meyer were the brothers that kept me grounded and gave me games, concerts, and other things to do besides my graduate work. My graduate cohort was amazing in providing both support and fun throughout the process, so thanks Mariah, Chris, Matthew, Sarah, Kinsey, and Cristina. Jennifer Osborne and Brad Wilson have been my nonscience people for years now, reminding me of the other aspects of life. My trivia team of DJ, Brian, Chris, and Taylor reminding there are other academic things besides science. And without a doubt, I never would have made it through this degree without my daily support and conversations with Jamie Watson.

Table of Contents:

List of Figures or Illustrations:

List of Tables:

ABSTRACT OF THESIS

University of Central Oklahoma

Edmond, Oklahoma

NAME: Kristy Meyer

TITLE OF THESIS: Reexamination of a Pocket Gopher (*Geomys*) Contact Zone in Oklahoma and Hybrid Identification Using Microsatellites and Mitochondrial DNA Markers

DIRECTOR OF THESIS: Dr. Michelle Haynie

PAGES: 73

ABSTRACT: Two species of pocket gophers, *Geomys bursarius* and *G. breviceps*, are found in central Oklahoma. These species were previously recorded as hybridizing in a contact zone located within Cleveland County. This contact zone was first studied in 1951 and most recently in 1985 but has yet to be analyzed using modern molecular techniques. Because pocket gophers are cryptic species, genetic analysis is needed for species identification. I investigated the frequency of hybrid offspring within the contact zone using mitochondrial and nuclear markers, and compared the historical contact zone boundaries to the current boundaries using GPS and GIS. Overall, gophers were found

primarily in the eastern locations of the contact zone and a large distribution was found north of the original trapping locations around Stanley Draper Lake, an area in which they were not previously documented. The species identification conducted using RFLP analysis indicated all specimens were *G. breviceps*. Microsatellite data suggested two distinct genetic clusters. The clusters were divided into a north-south pattern and potentially represent two different species or two populations of *G. breviceps* that are isolated by distance. Three admixture individuals were identified using STRUCTURE. Future research including samples of both *Geomys* species outside of contact zones and further microsatellite analysis is needed to elucidate the nature of the two clusters.

Chapter I - Introduction

Species Concepts

The definition of a species has been difficult to convey and has included such identifiers as the smallest, most exclusive taxonomic group being distinguished by similar morphological traits and the inability to produce healthy offspring with members outside of this group. The use of genetic analysis allowed the scientific community to no longer rely solely upon morphological or reproductive traits for identification, which can be misleading, as primary definitions for species (Heckman et al. 2006). The ability to analyze DNA for species identification exposed some of the inadequacies of previously used methods. For example, genetic studies have shown that organisms that differ in appearance are in fact closely related or, conversely, organisms that are similar in appearance have divergent genomes. However, the dichotomy of splitting versus lumping, which increases or decreases the number of species identified and was common when using morphological methods, was not solved when using genetic methods.

Reclassifying organisms has become commonplace in light of genetic analysis. Markolf et al. (2011) discuss the increase in recognized lemur species; 36 species were recognized in 1982 compared to 101 species as of 2011. They question if the increasing species numbers are a result of better trapping methods or from overemphasis on genetic diversity. The difficulty in classifying species based on genetic analysis is a lack of criteria for the amount of diversity needed to identify the population as a distinct species (Groves 2012). Tattersall (2013) states a loss of recognized subspecies has occurred by

elevating them to the species level, which also partially accounts for the explosion of newly identified species.

The species inflation issue is further compounded when different levels of genetic diversity can be found within the same population by using different molecular markers, such as mitochondrial DNA (mtDNA) versus nuclear markers or two nuclear markers with different evolutionary rates. Small sample size also can impact genetic diversity. There is not a minimum specimen count required before labeling a genetically unique population a new species. The main criticisms of the review of the lemur species explosion were the use of small sample size and the total reliance on mtDNA analysis (Markolf et al. 2011). Best practice will include multiple molecular markers as well as a robust sample size which is what most current genetic research is adopting. These factors have led to debates and ambiguity within the field of taxonomy and the need for a unified species concept.

de Queiroz (1998) attempts to tackle the species definition problem by proposing a unified species concept. He outlines how various definitions of species are influenced by the authors' field of expertise with some being more genetic based, others focused on reproduction and mating behaviors, and still others relying on evolutionary history. While all definitions have valid points of emphasis, de Queiroz simply defines species as separately evolving lineages. He continues by not specifying what kinds of data (e.g., reproductive isolation; or phylogenetic, ecological, morphological, or genetic changes) are required to show populations are evolving. While this concept may seem a step backward in specificity, the brilliance in this definition is the acknowledgment that

species evolve at different rates and likely through different mechanisms or different sequences of mechanisms. It also provides allowances for how quickly in the evolutionary process two populations are relabeled as two species.

Contact Zones

As a result of sometimes ambiguous genetic data, further research is needed to help define what makes a group a distinct species and how new species are formed. One type of study examining the species question involves contact zones. Contact zones are locations in which two species' distributions overlap. These contact zones can be old, resulting from glaciation or climatic fluctuations as seen in Europe and North America after the last ice age, or more recent, resulting from anthropogenic impacts (Barton and Hewitt 1985). Some contact zones have rapidly changing boundaries (Bilton and Foster 2016). In one example, a *Blarina* (shrew) contact zone was documented shifting 2.4 km in 22 months (Benedict 1999). Barton and Hewitt (1985), however, argue most zones have been located in essentially the same area for a millennium.

There are two types of contact zones: primary and secondary (Hewitt 1988). A primary contact zone is created within an established, sympatric species distribution. Genetic differences in the species arise within this distribution, splitting the area into two distinct overlapping zones. These zones might originate from an environmental factor impacting phenotypic fitness. They also might originate from the genesis of an advantageous allele which creates gradients within a species distribution; if this is the case, these clines can become further established by selection acting on the new allele.

A secondary contact zone is created when two previously isolated and allopatric species distributions suddenly overlap, most often due to an environmental change (Hewitt 1988). Most current hybrid zones are hypothesized to have formed through secondary contact. Many of these zones were established after glaciers receded during the last ice age. The warmer climate allowed for distribution expansion resulting in new interactions and overlap of species. Hewitt (1988) lists 20 documented hybrid zones which arose after glacial retreat. While conceptually the two different contact zones are distinct, in practice designating a current contact zone as either primary or secondary can be difficult if not impossible when the historic distributions are unknown (Barton and Hewitt 1985).

Studies of contact zones often explore speciation mechanisms and look for unique genetic traits in the species involved (e.g., Bradley et al. 1993). Hewitt (1988) describes contact zones as "natural laboratories for evolutionary studies." These zones are unique in allowing the interactions of different species to be studied because the species often are closely related and likely a result of divergent evolution; however, convergent evolution also is a possibility in these zones. Studying the genetic similarities and dissimilarities of parapatric species in contact zones can illuminate the various evolutionary processes through which speciation has occurred. To date, speciation has been documented in these zones as a result of intracistronic recombination (Ohno et al. 1969; Watt et al. 1972; Sage and Selander 1979), gene conversion (Hillis et al. 1991), transposable element activity (Bregliano et al. 1983; Kidwell 1990), and posttranslational modification of proteins (Murphy et al. 1984; Woodruff 1989).

A common question addressed when studying contact zones is the rate of divergence observed. Genetic studies of these zones often analyze gene flow, unique alleles and gene combinations, as well as new genes. A molecular clock analyzes mutation rates to estimate the time elapsed since divergence. Generally, these events are assumed to be gradual, taking thousands to millions of years (Futuyma 1998). Another looming question of speciation is whether a specific gene or suite of genes are involved in the divergence of a species across all forms of life. Baker et al. (1989) suggested that genetic studies have indicated speciation is not correlated to changes in specific genetic markers and the genetic variation found likely excludes the future discovery of a universal speciation marker.

Hybridization

In some contact zones, the interaction between species results in hybridization and the formation of hybrid zones. Hybrid zones are similar to contact zones between sympatric species, however, their defining characteristic is mating between the parental species and the presence of hybrid offspring, often both F_1 and F_2 generational specimens. Also, like contact zones, hybrid zones have been intensely studied for decades and recently have been found to be in higher frequencies than previously estimated (Barton and Hewitt 1985, 1989; Hewitt 1988; Harrison 1990, 1993; Ferris et al. 1993; Virdee and Hewitt 1994; Hodges et al. 1996; Foltz 1997; Wang et al. 2017). Hybrid zones typically are narrow stretches within contact zones, from hundreds of meters to kilometers in width, sometimes only becoming obvious after research within a contact zone (Benedict 1999). Several factors can influence the width of hybrid zones including

habitat structure, selection against hybrids, and an organism's mobility. Short (1969) states unlike most hybrid zones, large and broad avian hybrid zones can be found on most of the continents along with the more typical smaller and narrower contact zones. One broad hybrid zone of orioles (*Icterus galbula*) is approximately 200 miles wide.

The use of the term hybrid can be broad. Here, a hybrid refers to an individual with parents of different species. This event also is called secondary introgression (Merrell 1977). Hybridization has been observed in a large variety of organisms including seaweed (Coyer et al. 2002), Louisiana irises (Hodges et al. 1996), green toads (Stöck et al. 2010), short-tailed shrews (Rickert 2009), sea stars (Foltz 1997), and warblers (Short 1969).

The importance of hybridization in speciation often is debated. Harrison (1993) points out this debate is greatly impacted by being either a botanist or zoologist; hybridization is largely seen within the botany community as a constructive and common speciation process, whereas zoologists view hybridization as a rare and less common speciation mechanism. For example, Lotsy (1916), a botanist, wrote a book not only emphasizing hybridization but suggested it as the primary basis for evolutionary changes such as speciation. The genesis of many plant species comes from the hybridization of two parent species. One process, known as allopolyploidy, occurs when hybrids have their chromosome numbers doubled, creating a new species. To illustrate this point, when discussing the prevalence of plant hybrids Wissemann (2007) states, "Hybridization is important because life on earth is predominately a hybrid plant phenomenon."

Before genetic studies, finding naturally occurring animal hybrids was rare compared to plants. The mule is the most well-known example of animal hybrids, being the offspring of a female horse and male donkey and illustrates many issues hybrids face. Mules are sterile due to nonhomologous chromosomes that cannot segregate properly for viable gamete formation (Hartwell et al. 2008). Mules often are cited as examples of heterosis, however, being described as having "greater size, more endurance and stress tolerance, longer work life, and survive on poorer nutrition than their parents" (Troyer 2006). Inheritance patterns of mules are established enough that breeders can accurately predict the offspring's overall mature size and weight. This is a result of the larger female influence on the hybrid offspring's phenotype (Ensminger 1990).

Hybrids are sometimes less fit than their parental species and can be found in areas called tension zones. Examples of this include fire-bellied toads (*Bombina*), grasshoppers (*Caledia*, *Chorthippus,* and *Podisma*), pine trees (*Pinus*), leopard frogs (*Rana*), lizards (*Sceloporus*), mole rats (*Spalax*), and pocket gophers (*Thomomys*; Hewitt 1988). Plant hybrids, however, have been shown to neither suffer decreased fitness, negative selection pressure, nor reduced fertility (Wissemann 2007). Additionally, some hybrids have been found to be equal or greater in fitness compared to the parents (Arnold 1988). One study found first generation hybrids to be phenotypically inferior compared to the parental species, but after 20 generations the hybrids were found to have superior fitness (Hwang et al. 2011). Grant and Grant (1992), when reviewing bird hybrids, found a range of fitness outcomes with disadvantaged hybrids with decreased or no offspring produced at one extreme to hybrids with similar fitness outcomes as the parental species

at the other extreme. Moore (1977) argues some hybrid zones could exist in which selection pressure favors hybrids in an intermediate habitat, however, Barton and Gale (1993) argue this is unlikely.

It often is assumed hybrids will have equally shared parental traits. Hybrids sometimes do have a combination of parental traits; however, they also can strongly resemble one parental species over another or may not look like either parental species. Ellstrand (1987) found a hybrid in the heather plant genus (*Ericaceae*) which had a blended appearance of both parental species, yet was physiologically much more similar to one parent. Several bird hybrids in different genera with intermediate phenotypes of the parental species were found to outcompete the parents in extreme environments and better cope with environmental disturbances (Grant and Grant 1992). In a survey of hybrids known to exhibit phenotypic variation, Rieseberg et al. (1999) found 91% of the hybrid populations had at a minimum one extreme phenotype which allowed them to thrive in a unique niche neither parental species inhabited.

Hybrids are not always easy to identify in the field using obvious phenotypic markers. Many hybrids are identified after analyzing DNA and finding mixed allelic combinations from both parental species. For this reason, hybrid research often involves molecular analysis. Being similar to contact zones, the molecular data from hybrid zone studies can be analyzed to study gene flow, adaptations, isolating mechanisms, epistasis, interspecific competition, adaptation of parasites, and the impact of climate on fitness (Benedict 1999).

Cryptic Species

Another result of analysis of genetic diversity within groups is the identification of cryptic species. Cryptic species are two or more distinct species that lack identifying morphological or other obvious traits to distinguish one from another, that were previously clustered into one taxonomic group (Bickford et al. 2006). The concept of cryptic species was first documented in letters written by William Derham in 1718 (Winker 2005). Despite the concept predating the Linnaean classification system, the requirements for the cryptic species label are still up for debate; there also is currently ambiguity on differentiation between cryptic species and sister species (Bickford et al. 2006). In general, sister species are two taxa which have evolved from the same immediate ancestor with specific species denotations; cryptic species are also generally closely related but differ by commonly being confused for one another due to a previous taxonomic designation.

Struck et al. (2018), in reviewing the current literature on cryptic species, found inconsistent and ambiguous use of the cryptic species label. They proposed criteria for cryptic species which included statistically divergent genotypes and limited morphological differences compared to other species and to ancestors over time. The existence of cryptic species is likely a result of several factors. One explanation for lack of morphological differences is fitness within a sympatric habitat. Because cryptic species are found in similar habitats, large morphological trait changes are unlikely because the overall body form is likely ideally adapted for the current habitat. Additionally, Schönrogge et al. (2002) argue selection acting upon a species could

prevent morphological changes despite speciation, leading to the creation of cryptic species. This could be especially true of species living in extreme habitats.

Another explanation for the formation of cryptic species is nonvisual mating signals. Sister species have been found to have different auditory or pheromone cues despite similar appearance (Narins 1983; Byers and Strubble 1990; Henry 1994; Cicero 1996; Kozlov et al. 1996; Jones and Barlow 2003). Mayr (1963) argues cryptic species are found in higher numbers in organisms where visual ability is surpassed by olfactory ability. Additionally, cryptic species are rarer in animals with strong reliance on visual cues like birds. However, there is a bias of cryptic species research focusing on animals, so it is unknown how ubiquitous this phenomenon is.

Research on cryptic species has greatly increased over the last 20 years, likely a result of DNA analysis and the need for conservation strategies (Bickford et al. 2006). The common method used to discover cryptic species is analyzing molecular genetic data (Harrington and Near 2011; Satler et al. 2013; Hedin 2015; Liu et al. 2018). Sukumaran and Knowles (2017) warn, however, that overreliance on molecular data, specifically the process of genome scanning, can lead to labeling groups as putative species instead of distinct populations when not using other diagnostic criteria like behavior, niche analysis, diet, or other data. Lohman et al. (2010) summarizes the real-life consequences of the species or cryptic species label in terms of conservation policy which is likely dependent upon genetic data (Mace 2004; Bickford et al. 2006; Zou et al. 2007; Meier 2008; Balke et al. 2009; Marks 2010).

Molecular Identification of Species

MtDNA has been the most widely used molecular marker over the past 30 years, in part due to being relatively cheap and easy to analyze (Galtier et al. 2009). MtDNA commonly is used to identify species, especially when studying cryptic species (Herbert et al. 2004; Janzen et al. 2005; Ball and Armstrong 2006; Galtier et al. 2009; Dupuis et al. 2012). MtDNA has many advantages over nuclear DNA including being found in up to thousands of copies per cell, compared to two copies found in the nucleus. Because mtDNA is circular, it also is durable against denaturing when compared to nuclear DNA. MtDNA also does not have genome complications like transposable elements or allele recombination. Additionally, mtDNA overall is highly conserved, with few duplications and no introns (Galtier et al. 2009). Conversely, mtDNA has highly variable regions which can be used to study population dynamics and species interactions. However, one consideration of mtDNA analysis is the bias of maternal inheritance, which eliminates the male parent's DNA contribution.

The mtDNA *Cytochrome b* (*Cytb*) gene commonly is used as a marker for species identification (Lopez-Giraldez et al. 2005), especially in mammals. It can readily be amplified via polymerase chain reaction (PCR) and analyzed using restriction fragment length polymorphism (RFLP) digest or DNA sequencing (Avise et al. 1979; Szalanski and Powers 1996; Armstrong et al. 1997; Miller et al. 1999; Scheffer et al. 2001; Muraji and Nakahara 2002; Toda and Komazaki 2002; Saltonstall 2003; Weathersbee et al. 2003; Szalanski et al. 2004). The RFLP process works by taking advantage of species specific variations within the *Cytb* gene. Restriction endonucleases are enzymes which

cleave DNA at specific sequences called cut sites (Hartwell et al. 2008). These cut sites can be highly variable, with mutations sometimes leading to the restriction endonucleases not recognizing the site, leaving the DNA intact (Tropp 2008). This results in different combinations of fragment sizes when the DNA is digested. The fragments are then analyzed using gel electrophoresis, which separates the fragments within each sample by size, creating a distinctive band pattern. The different fragments can correlate with different species, therefore the banding patterns from gel electrophoresis can be used to identify species (e.g., Harrison et al. 1985; Pfeiffer et al. 2004).

Microsatellite markers are biparentally inherited nuclear markers that are useful for individual identification and have been used recently in the detection of hybrid individuals (Genoways et al. 2008; Välie et al. 2010; Van Haecke et al. 2012; Mauldin et al. 2014). These markers are found throughout the genome and are highly variable. Microsatellites are repeating motifs which are analyzed based on the fragment length. These sites can have different alleles based on the number of repeats and individuals are thought to be heterozygous at most loci. New repeats are created by slippage during replication, both as an artifact of PCR and during natural cell replication. Because this process occurs often, microsatellites show variation at the population level. Because they usually are in noncoding regions, the repeats can accumulate without detriment to the individual. These characteristics allow for the examination of gene flow, paternity, and hybrid breeding events.

Using a combination of nuclear DNA and mtDNA markers to analyze specimens within hybrid zones is especially crucial. Harrison et al. (1987) used a combination of

morphometric, nuclear, and mtDNA data to analyze field crickets within a hybrid zone and found specimens which matched one mtDNA parental species yet were classified as the other parental species based on morphometric and nuclear DNA data. Obtaining various sources of genetic data was significant in this study in light of their finding that viable hybrids occur only with mating of *Gryllus firums* males and *G. pennsylvanicus* females (Harrison et al. 1987). Asymmetric breeding would provide an incomplete picture if only mtDNA markers were used, due to its maternal inheritance. Both asymmetric breeding and mtDNA introgression were investigated by Chan and Levin (2005) in various reproductive models; they found small immigration rates within hybrid zones can lead to rapid mtDNA introgression. They caution against the exclusive use of mtDNA markers in species identification for this reason, despite the practice being commonplace.

Geomys

Geomys (pocket gophers) are small, ubiquitous rodents. They can be found from southern Canada through the central United States to northeast Mexico, extending eastward along the southeastern coast of the United States and westward to the Rocky Mountains (Hall 1981; Fig. 1.1). *Geomys* live a fossorial and mostly solitary lifestyle (Sulentich et al. 1991). This fossorial lifestyle results in soil types being a major influence on their distribution (Davis et al. 1938). In a study of three different pocket gopher genera, Best (1973) found that each genus has a specific soil preference. Additionally, some species within each genus also have different preferences for soil type. For example, *G. bursarius* (plains pocket gopher) typically is found in sandy-loam soil (Best

1973) and *G. breviceps* (Baird's pocket gopher) avoids sandy-loam soil (Davis et al. 1938). Wilkins and Swearingen (1990), using morphological analysis, found that soil type had a stronger correlation to morphological differences compared to geographic isolation.

Recent land usage also can influence the prevalence of gophers. When nitrogen-poor soil is fertilized, there was increased pocket gopher activity as a result of the increasing plant biomass (Inouye et al. 1997). One study found different vegetation types in areas with *G. bursarius*, with larger amounts of annual grasses and annual forbs, compared to controlled areas without gophers (Foster and Stubbendieck 1980). The subspecies *G. personatus maritimus* was found to prefer areas which were mowed or impacted by construction, but not overly manicured (Cortez et al. 2015). This preference could be a result of mowing keeping vegetation from maturation resulting in a constant higher nutritional value.

Over the past 100 years, the number of *Geomys* species and subspecies has been greatly debated, with molecular studies resulting in the recognition of numerous cryptic species (e.g., Genoways et al. 2008; Chambers et al. 2009). Hall (1981) recognized eight species of *Geomys*, which was later expanded to 10 species in response to chromosomal studies (Tucker and Schmidly 1981; Heaney and Timm 1983, 1985; Qumsiyeh et al. 1988; Baker et al. 1989; Dowler 1989; Block and Zimmerman 1991; Bradley et al. 1991b). An eleventh species was included after further revisions in the 1990's and 2000's (Sulentich et al. 1991; Smolen and Bickham 1995; Burt and Dowler 1999; Jolley et al. 2000; Baker et al. 2003). Sudman et al. (2006) completed an extensive study of *Geomys*

using the mtDNA *Cytb* gene and concluded there are 11 species; however, another mtDNA and nuclear study suggested a minimum of 12 species (Chambers et al. 2009).

Geomys contact zones have been studied extensively using different molecular techniques including chromosome comparisons, mtDNA analysis, ribosomal DNA analysis, and allozymic studies (Pembleton and Baker 1978; Tucker and Schmidly 1981; Baker et al. 1989; Bradley et al. 1991a; Genoways et al. 2008). *Geomys* contact zones have included interactions between *G. lutescens* (sand hills pocket gopher) and *G. bursarius majusculus*, *G. knoxjonesi* (Knox Jones's pocket gopher) and *G. bursarius major*, *G. breviceps* and *G. bursarius major*, and *G. breviceps* and *G. attwateri* (Attwater's pocket gopher; Sulentich et al. 1991; Connior 2011). Heaney and Timm (1983) analyzed *Geomys* distribution throughout the central and northern Great Plains, using morphometric measurements, and found three areas of contact between *Geomys* species within Oklahoma. Figure 1.1 shows the parapatric distribution between two *Geomys* species in central Oklahoma.

Oklahoma Geomys

There currently are three identified species of *Geomys* in Oklahoma: *G. breviceps*, *G. bursarius*, and G. *jugossicularis* (Hall's pocket gopher) (Coffman 2018). *Geomys breviceps* is distributed mostly in the southern United States, with a northern boundary in eastern Oklahoma and western Arkansas extending southward to eastern Texas and western Louisiana (Sulentich et al. 1991; Fig. 1.1). It is found in the eastern half of Oklahoma. *Geomys bursarius* has a larger distribution extending northward to Manitoba, Canada, southward to central Texas, westward into New Mexico, and eastward into

Illinois and Indiana (Connior 2011; Fig. 1.1). It is found in western Oklahoma, including portions of the panhandle. *Geomys jugossicularis* is found in southern Nebraska, western Kansas, eastern Colorado, and the panhandles of Oklahoma and Texas (Genoways et al. 2008). In Oklahoma it is restricted to the panhandle. This species is not the focus of this study and will not be discussed further. *Geomys* species found in Oklahoma are considered cryptic because there are no clear and reliable morphological differences that can be used for species identification (Sulentich et al. 1991; Connior 2011).

In Oklahoma, previous work established a contact zone with suspected hybridization between *G. breviceps* and *G. bursarius* in Cleveland County near Norman, Oklahoma (Baker and Glass 1951; Hart 1971; Bohlin and Zimmerman 1982; Heaney and Timm 1983; Cothran and Zimmerman 1985). This zone generally was described as 2 to 3.5 miles east of Norman (Hart 1971). Hart (1971) suggested the presence of two hybrids from this area based on karyological studies. Bohlin and Zimmerman (1982) studied the contact zone in Norman using allozyme electrophoresis to determine the extent of genetic isolation between two chromosomal races, then recognized as *G. bursarius* and which were later designated as *G. bursarius* and *G. breviceps*. They proposed limited interbreeding between these groups and one hybrid was found (Fig. 1.2). Cothran and Zimmerman (1985) claim the only hybrids observed were F_2 or backcrosses, despite two localities having a mixed population of both species. While these studies suggest limited hybridization events, no research has been conducted in this area using modern molecular techniques (i.e., mtDNA or microsatellite analysis). Additionally, the location of the preferred soil type of *G. bursarius* in this contact zone has been impacted by urban

sprawl since the previous studies. Because pocket gophers are considered pests by homeowners, urban sprawl could have resulted in a reduction or elimination of pocket gophers in those areas.

Research into the frequency of hybrid individuals has occurred in other *Geomys* contact zones. Baker et al. (1989) conducted molecular analysis on 75 *Geomys* specimens collected within a New Mexico contact zone and found 41 to have hybrid ancestry. Genoways et al. (2008) conducted research using modern molecular techniques in a Nebraska *Geomys* contact zone described by previous authors as having limited introgression. They found 30 of 39 specimens exhibited a hybrid genotype at one site, suggesting a high level of introgression despite previous research to the contrary.

MtDNA loci are useful species identification markers and have been used widely in pocket gopher studies (Baker et al. 1989; Jones et al. 1995; Hafner et al 2005; Sudman et al. 2006; Genoways et al. 2008; Chambers et al. 2009; Soto-Centeno et al. 2013). *Geomys* hybrids with mtDNA indicating one species and nuclear DNA of another species have been discovered (e.g., Baker et al. 1989; Bradley et al. 1991a; Jones et al. 1995). This makes it best practice to include both mtDNA and microsatellite markers when evaluating specimens of this genus for evidence of hybridization.

Objectives

The purpose of this research was to identify the boundaries of the contact zone between *G. bursarius* and *G. breviceps* in Cleveland County, Oklahoma, using mtDNA and microsatellite markers. The objectives of this research were to: 1) identify the current

boundaries of the contact zone near Norman, Oklahoma, 2) compare the current contact zone boundaries to those established by Cothran and Zimmerman (1985), and 3) identify any hybrid individuals within the contact zone.

Hypotheses

Hypothesis 1a: The contact zone established by Cothran and Zimmerman (1985) will largely remain intact.

Hypothesis 1b: The northern border of the contact zone will have shifted north and east towards Stanley Draper Lake due to the urban sprawl of Norman.

Hypothesis 2: Based on Genoways et al. (2008), hybridization will be common when using both mtDNA and microsatellites as a means of hybrid identification, contrary to previous conclusions by Bohlin and Zimmerman (1982) and Cothran and Zimmerman (1985).

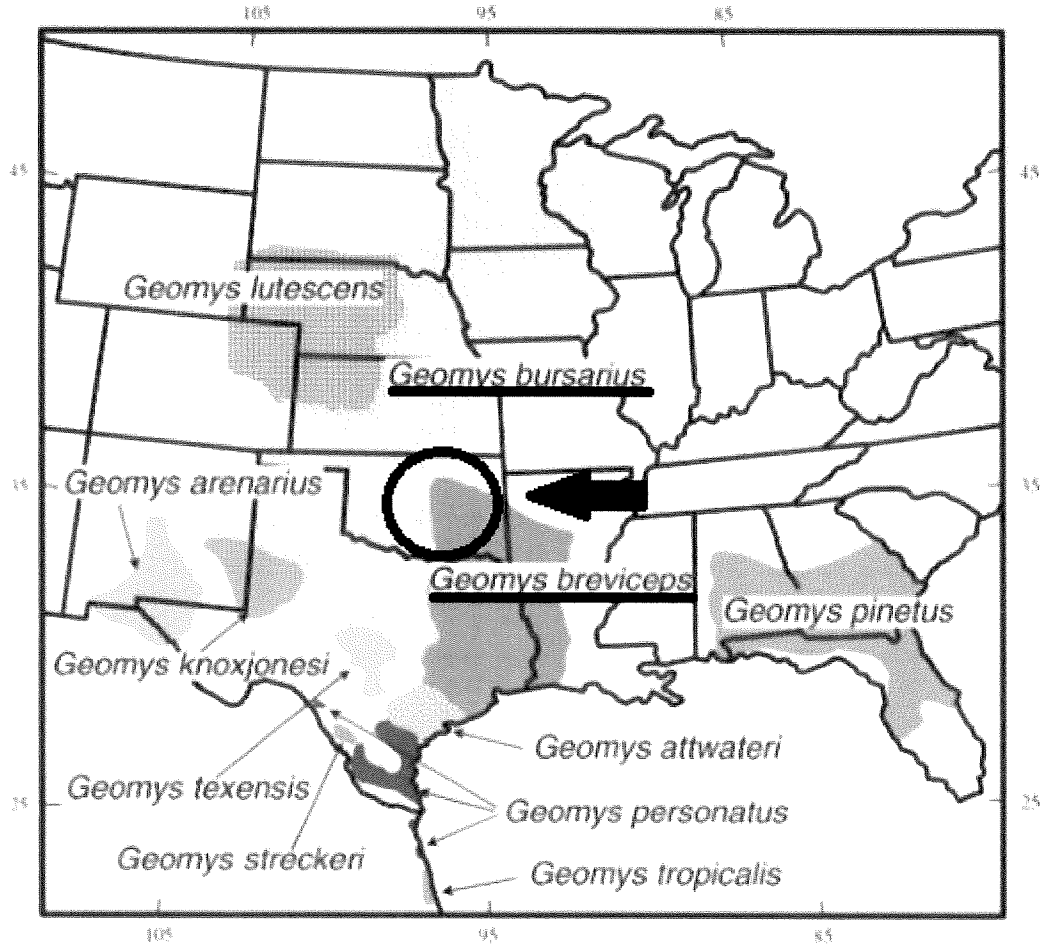

Figure 1.1 - *Geomys* distribution across the United States. The parapatric boundary of *G. bursarius* and *G. breviceps* distribution in central Oklahoma is indicated by the circle. Edited from Sudman et al. (2006).

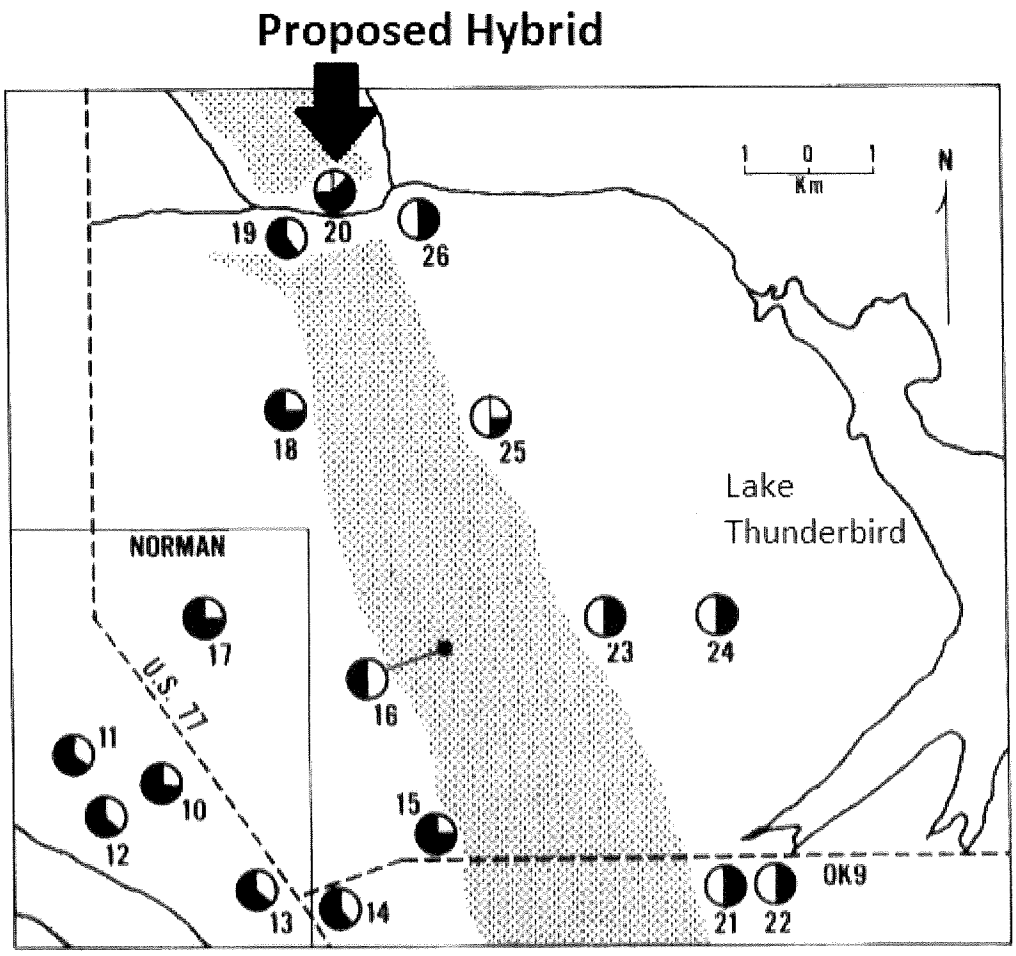

Figure 1.2 - Identification of a potential hybrid between *Geomys breviceps* and *G. bursarius* within a contact zone near Norman, Oklahoma. Figure taken from Bohlin and Zimmerman (1982).

Chapter II - Materials & Methods

Sample Collection

Fieldwork was focused in Cleveland County, Oklahoma, to examine how the boundaries of a previously described contact zone (Bohlin and Zimmerman 1982; Cothran and Zimmerman 1985) had changed over 30 years. Before conducing field work, trapping sites from the previous studies were mapped using Google Maps (Fig. 2.1). In the literature, the previous sites were denoted using a street reference and measuring the distance from the site to the street. The locations of previous trapping sites were approximated using the measuring tool on Google Maps and converted to GPS coordinates. Using ArcGIS (ESRI 2011), the GPS coordinates were used to map the previous trapping sites (Fig. 2.2). A soil type layer was added to the map to show in which soil the mounds were found because soil is a major determining factor for *Geomys* distribution.

A survey of current gopher mound distribution was conducted, with emphasis in the area where two previous hybrids were identified, through personal observations made while driving the area. Once a current gopher mound was identified, GPS coordinates were recorded and later mapped using ArcGIS. The survey was conducted to determine whether a shift in the distribution of *Geomys* had occurred by mapping the current boundaries in the area. For comparison, the previous trapping sites and current mounds were mapped together using ArcGIS (Fig. 2.3). After the survey was complete and the current distribution of gophers was assessed, the study area was divided into transects. Trapping sites were located in three different zones along east-west running transects to

span the changing soil types within the suspected contact zone east of Norman (Fig. 2.4). The area of focus expanded north around Stanley Draper Lake in response to the current distribution of gopher mounds.

Active gopher mounds were identified by sight when driving transects. Once a mound was found, Macabee or Victor kill traps were placed in the tunnel system (Fig. 2.5). These traps are approved by the American Society of Mammalogists (ASM) Institutional Animal Care and Use Committee (IACUC; Sikes et al. 2016). The traps are designed to kill the gopher upon capture; however, if a gopher was found alive in the trap, it was euthanized in the field using a combination of isoflurane or chloroform inhalation, and thoracic compressions if necessary. A combination of chemical inhalation and thoracic compression is approved by the ASM IACUC (Sikes et al. 2016). Traps were set and checked at short time intervals, and they were not left out overnight. The described protocol was approved by the University of Central Oklahoma IACUC (#14011).

GPS coordinates and date were recorded for each trapped gopher. Gophers were processed in the lab, with all collected samples prepared as museum voucher specimens. This included taking standard measurements (total body length, hind foot, tail, and ear) recording sex and reproductive status, taking tissues (heart, kidney, lung, liver, spleen, thigh muscle, and colon), preparing the skin for long-term preservation, and preserving the complete skeleton. The voucher measurements were statistically analyzed using a one-tailed t-test in the R statistical software package (R Core Team 2018). The specimens were grouped in various ways (by sex, species, or genetic clusters) to determine if any

significant morphometric differences were found. Only adult gophers were used for these analyses.

DNA Extraction and PCR

DNA was extracted from liver samples using a DNeasy Blood and Tissue Extraction kit (Qiagen, Valencia, CA). Species identification of samples was obtained using RFLP analysis of the mtDNA *Cytb* gene. The complete mtDNA *Cytb* gene was amplified via PCR for all specimens following the protocol of Sudman et al. (2006), using primers L14735 and H15906 (Elrod et al. 2000). Once amplified, the DNA was incubated with the restriction enzyme EcoRV (New England Biolabs, Ipswich, MA) following the manufacturers protocols. The digested samples were analyzed using gel electrophoresis (2% agarose gel). Species identification was made based on the number of bands present with *G. breviceps* having a 727 base pair (bp) band and a 413 bp band, whereas *G. bursarius* had a single 1140 bp band (Coffman 2018).

A series of nine nuclear microsatellite markers (Table 2.1) were screened for all collected specimens following the protocol of Welborn et al. (2011). Six markers (Gbr09, Gbr10, Gbr15, Gbr25, Gbr27, and Gbr33) were designed specifically for *G. breviceps* (Welborn et al. 2011). The remaining three loci (Tm1, Tm6, and Tm7) were designed for another pocket gopher genus (*Thomomys*; Steinberg 1999), but have been shown to work in *Geomys* (Welborn et al. 2011). All protocols for PCR established by Welborn et al. (2011) were followed except for two altered PCR thermal cycling profiles discussed below. The PCR thermal cycling parameters were as follows with the altered temperatures for Gbr10 and Gbr33 marked in parentheses: initial denaturation at 95°C for

180 seconds; 9 cycles of 95°C denaturation for 30 seconds, 58°C (57°C for Gbr10; 56°C for Gbr33) annealing for 45 seconds, and 72°C extension for 1 minute; 9 cycles of 95°C denaturation for 30 seconds, 56°C (55°C for Gbr10; 54°C for Gbr33) annealing for 45 seconds, and 72°C extension for 60 seconds; 14 cycles of 95°C denaturation for 30 seconds, 52°C (51°C for Gbr10; 50°C for Gbr33) annealing for 45 seconds, 72°C extension for 60 seconds; and a final 72°C extension for 600 seconds. The sample were analyzed using an ABI3500 Genetic Analyzer (ThermoFisher Scientific, Waltham, MA). Genotypes were visualized, edited, and scored using GeneMapper Software 5 (ThermoFisher Scientific).

Data Analyses

Microchecker v2.2.1 (Van Oosterhout et al. 2004) was used to test for the presence of null alleles, large-allele dropout, and stutter-induced typing errors at each microsatellite locus. FSTAT 2.9.3.2 (Goudet 1995, 2001) was used to test for deviations from Hardy-Weinberg Equilibrium (HWE) and linkage disequilibrium, and to estimate F_{ST} values between genetic clusters. Cervus 3.0.7 (Kalinowski et al. 2007) was used for allelic frequency analyses, observed and expected homozygosity, and polymorphic information content for all nine microsatellites.

STRUCTURE 2.3.4 (Pritchard et al. 2000; Falush et al. 2003; Hubisz et al. 2009) was used to estimate the number of clusters represented by the data. Parameters for the STRUCTURE run included the correlated allele frequency model, 50,000 burn-in steps, 100,000 sampling steps, and 5 iterations for each K (number of putative populations tested). K was set at 1 - 5. STRUCTURE result files were uploaded to STRUCTURE

HARVESTER (Earl and vonHoldt 2012) to determine the value of K that best fit the data using the Evanno method (Evanno et al. 2005). STRUCTURE result files for the optimal K were analyzed using CLUMMP 1.1.2 (Jakobsson and Rosenberg 2007) and visualized using *distruct* 1.1 (Rosenberg 2004).

Species and Hybrid Identification

The mtDNA data set was used to identify the species of each specimen within the contact zone. Pure parental samples (*G. bursarius*: G10, G11, G12, G16, and G17 ; *G. breviceps*: G23, G,24 G25, G26, and G28; Coffman 2018) were used with the collected specimens when conducting RFLP. Based on RFLP data, each individual was identified as either *G. bursarius* or *G. breviceps*. This allowed for comparisons of pure parental samples to those collected in the contact zone.

Each individual also was assigned to a genetic cluster based on microsatellite data. Microsatellite alleles can be analyzed using software programs like STRUCTURE to place individuals into clusters based on allelic similarity. Individuals were considered admixed if the estimated membership within the most likely cluster was <80%. Once assigned to clusters, voucher measurements were compared in a t-test to look for statistically significant differences.

Geographic Analysis

After species and hybrid identification of the samples, the geographic area of the contact zone was mapped with clusters denoting locations of each species and putative

hybrid. The sample sites of Bohlin and Zimmerman (1982) and Cothran and Zimmerman (1985) were overlaid with current samples for a temporal comparison.

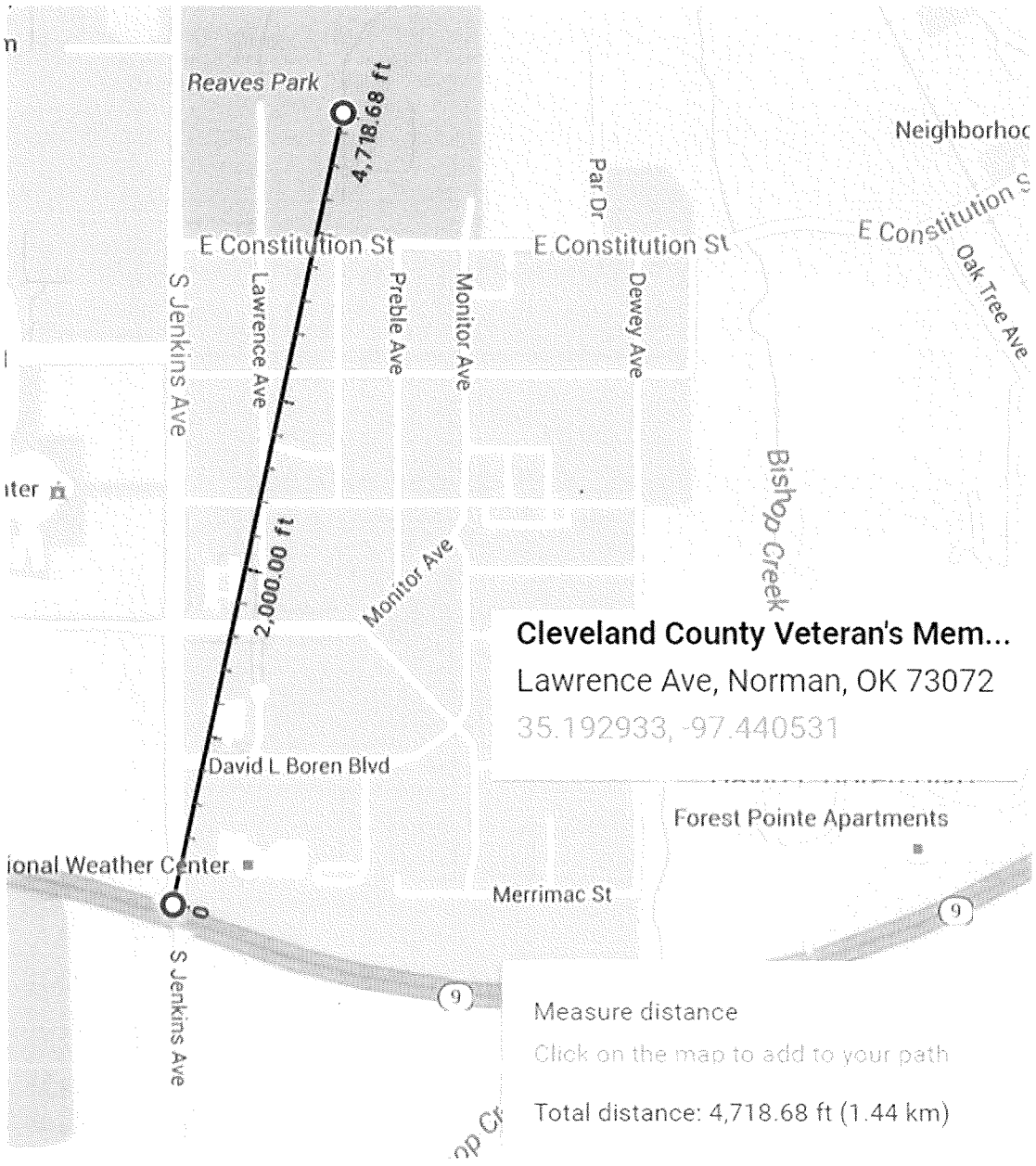

Figure 2.1 - Example of how Google Maps was used to locate previous trapping sites. I used reference trapping locations from Bohlin and Zimmerman (1982) and Cothran and Zimmerman (1985) and converted them into GPS data. For example: 1.4 km northeast of Highway 9 and S Jenkins Ave became 35.192933, -97.440531.

Figure 2.2 - Map of the original trapping sites within the Cleveland County contact zone. Locations from the Bohlin and Zimmerman (1982) study are denoted with black circles. Locations from the Cothran and Zimmerman (1985) study are denoted in white squares. Suspected hybrids are denoted with black stars.

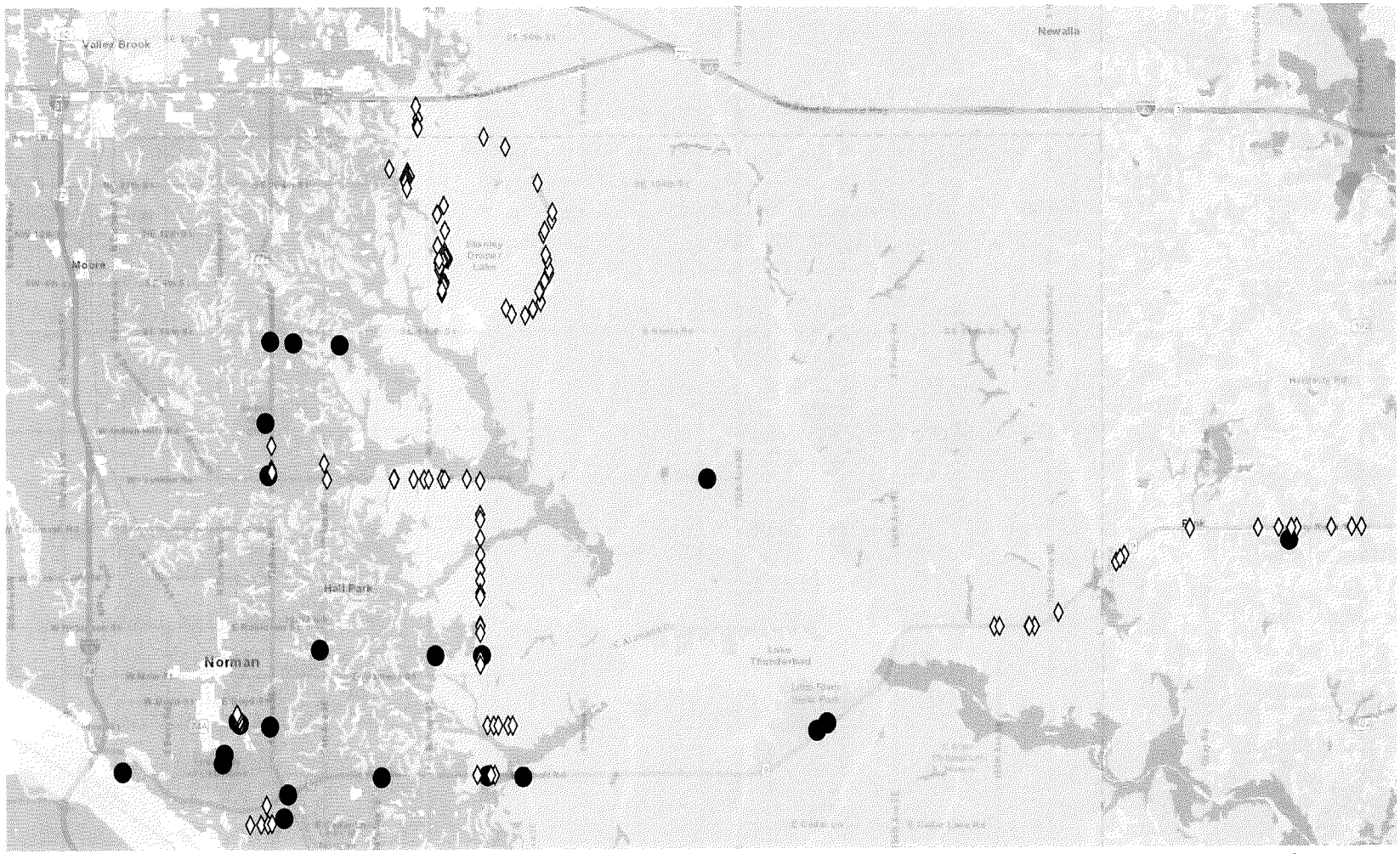

Figure 2.3 - Map of original trapping sites and current mounds in the Cleveland County contact zone. Original mounds are denoted with black circles and current locations of mounds are denoted with white diamonds.

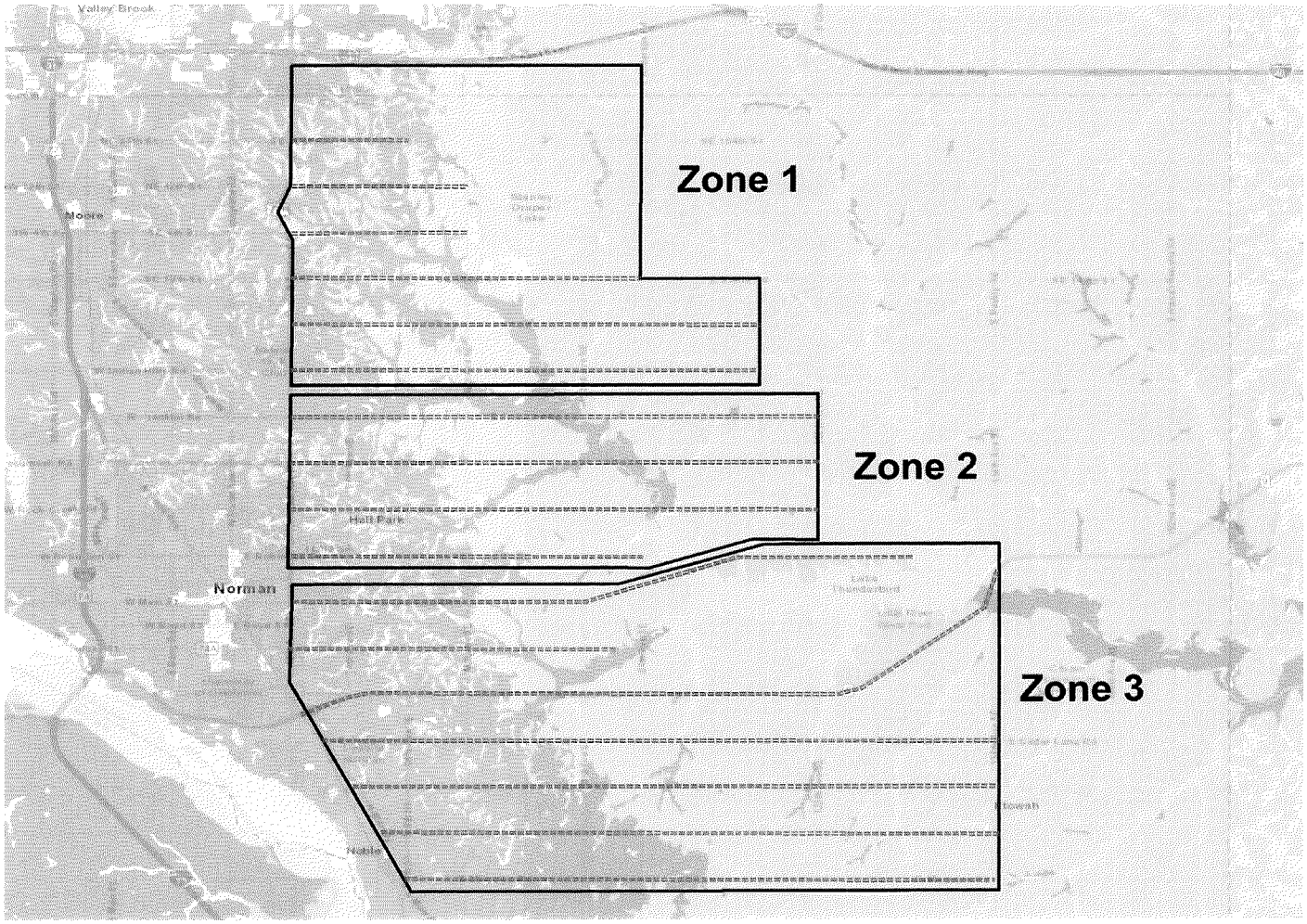

Figure 2.4 – Trapping zones and transects used in this study. Three zones were created based on current gopher distribution and soil type which are denoted in the background in shades of gray. The resulting trapping zones and transects were used to collect specimens. Each transect ran east to west along roadways, however, roads around Lake Thunderbird and Stanley Draper Lake also were used.

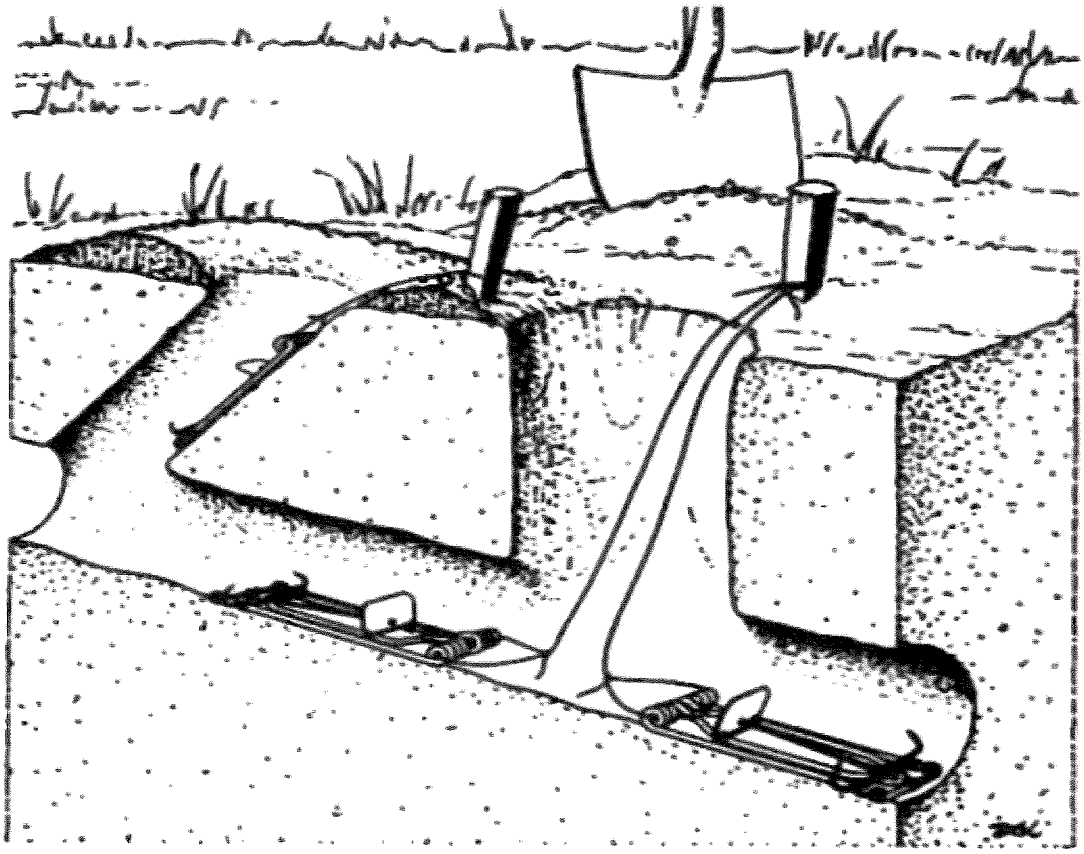

Figure 2.5 - Image of trapping method used with pairs of traps being placed in the lateral tunnels. Adapted from Marsh (1998).

Table 2.1 - Microsatellite primers used in this study. Gbr primers were developed by Welborn et al. (2011), Tm primers were developed by Steinberg (1999). The forward primer of each pair was labeled with a fluorescent dye. These nine markers were used for hybrid identification. Only the forward sequence is provided.

Locus	Repeat Motif	Size (bp)	Primer Sequence
Gbr9	$(CA)_{18}$	214	TGGCTCAAGTGAGAGCATCAGGAGGAGGAACAAGCAATCA
Gbr10	$(CA)_{19}$	235	TAGTGCATGCTCTGGCTTTGAAATGCCCTCCAGAAGGAAC
Gbr15	$(GT)_{14}$	212	CTCTCCCTCAGCTCAGCAGTGTGTCCAGCCCAGTTATGCT
Gbr25	$(GT)_{27}$	227	CCTGGGAGACTAGCATGAGGCACAAGAAAGCCAGAAGTGC
Gbr27	$(GT)_{10}$	229	TGATGACACGCTGACTTTCCTGGAGGTGTAGCTCAAGTGG
Gbr33	$(AAGG)_{14}$	227	GTGGTAGTGGTGGTGTTTGCACACTGGAGTGTCTCATGTGG
Tm1	$(GT)_{28}$	181	TCACATACTAGCCCAAAGTCCTCGTGGTAGAGCAAAAGAAGCTGAA
Tm6	$(GT)_{17}$	173	CCGGATCTTGGATTAGGCATGGCTGTTTTAATTTCCTTCATGT
Tm7	$(CA)_{10...}$ $(GT)_{9}$	288	TCTACTGAACCACCAGAAAATCAAAGCACTGGACTTGAACACAAATAC

Chapter III – Results

Current vs. Historic Gopher Distribution in Cleveland County

When comparing the current distribution of gopher mounds to gopher records used in previous studies, the current distribution was both further east and northeast, located in the more rural areas of Cleveland County. One of the proposed hybrids from the previous studies was collected in the northern most sampling location. Although this survey did not find gopher mounds in that area, a large and concentrated distribution of gopher mounds was observed further northeast around Stanley Draper Lake. Because of this result, sampling was shifted further north compared to previous studies. In total, three trapping zones were created using the survey data: zone 1 included Stanley Draper Lake and the location of a previous hybrid gopher, zone 2 included the outskirts of Norman and the northern half of Lake Thunderbird, and zone 3 included the southern half of Lake Thunderbird and the location of another previous hybrid gopher.

Table 3.1 shows the breakdown of statistics by trapping zone. Overall, there was a bias towards females in the dataset with 21 females, 10 males, and two subadults that were likely male (Fig. 3.1). Trapping zones 2 and 3 each contained pregnant gophers, with one in zone 2 and four in zone 3. The fetuses were collected and included in genetic analyses. Unfortunately, paternity could not be established for any fetus. The specimen mean value for each voucher measurement was similar to those reported in Sulentich et al. (1991) for *G. breviceps*. When specimens were grouped based on the STRUCTURE clusters, however, the southern cluster was found to be significantly larger in total body

size than the northern cluster (one-tailed t-test, $p = 0.04337$). The other three measurements were not found to be significantly different among clusters.

Species Identification and Hybridization

Results of the RFLP analysis indicated all samples were *G. breviceps* (Appendix). Analyses were repeated three times, to confirm results, using pure parental samples of *G. breviceps* and *G. bursarius* for controls (Fig. 3.2).

All samples were analyzed using all nine microsatellite markers and Microchecker was used to look for an excess of homozygotes, presence of null alleles, stuttering, or large allele dropout (Table 3.2). All loci were in HWE and there was no evidence of linkage disequilibrium between any pair of loci. The mean number of alleles per locus was 5.889, mean observed heterozygosity was 0.4272, mean expected heterozygosity was 0.6107, and mean polymorphic information content (PIC) was 0.5688. The number of alleles per locus ranged from 1-9. Cervus results per locus are shown in Table 3.3.

The samples were grouped into two clusters (K = 2), based on STRUCTURE results, with 49% of the samples in cluster 1 and 51% in cluster 2 (Fig. 3.3; Fig. 3.4; Appendix). Three admixture individuals were identified based on STRUCTURE analyses: sample m24 (79.4% cluster 1), sample m25 (77.3% cluster 1), and sample m31 (56.6% cluster 1; Table 3.4; Appendix). None of these admixture individuals were found in previous trapping locations. There was a distinct geographic separation between the two clusters (Fig. 3.4). Cluster 1 represented 100% of specimens from trapping zone 1

and 91.6% of specimens from trapping zone 2 (Table 3.3). Cluster 2 represented 8.4% of specimens from trapping zone 2 and 100% of specimens from trapping zone 3 (Table 3.4). The F_{ST} value between the two clusters was 0.123, indicating moderate genetic differentiation between the groups.

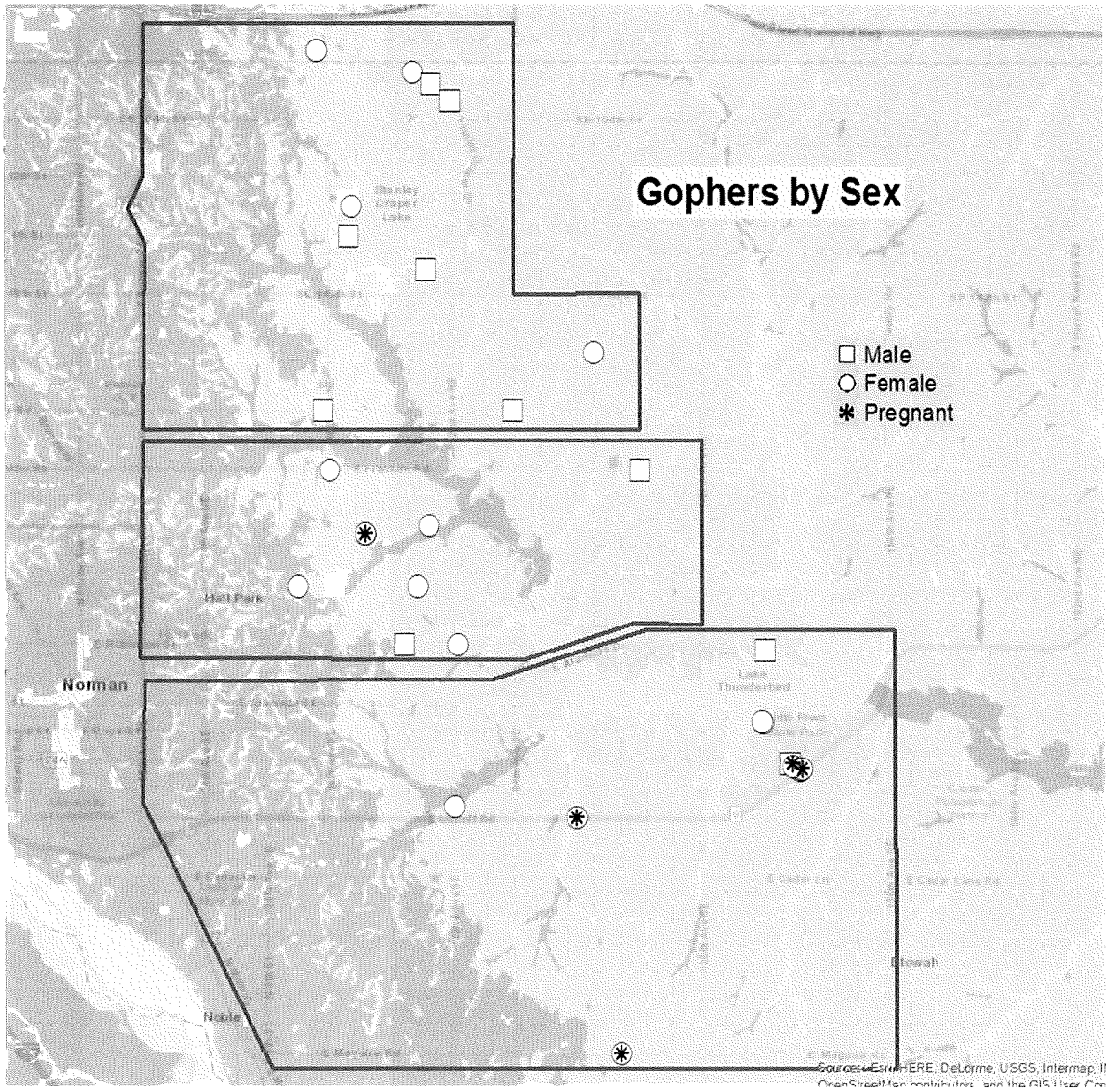

Figure 3.1 - Sampled *Geomys* marked within trapping zones and by sex. White squares represent males, white circles represent females, and white circles with stars represent pregnant females.

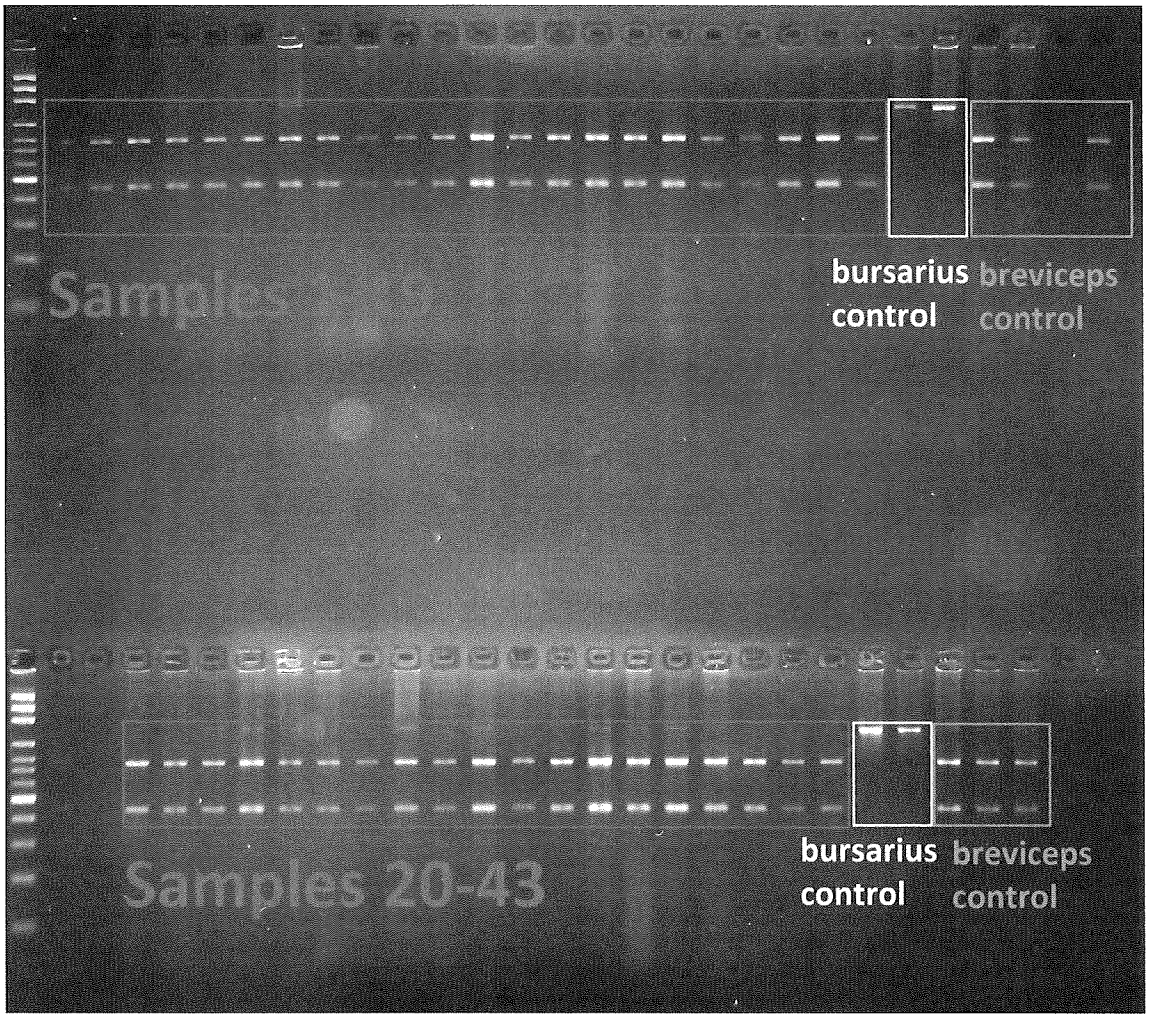

Figure 3.2 - RFLP results with parental controls on the right. All 43 samples matched the banding pattern of *Geomys breviceps*.

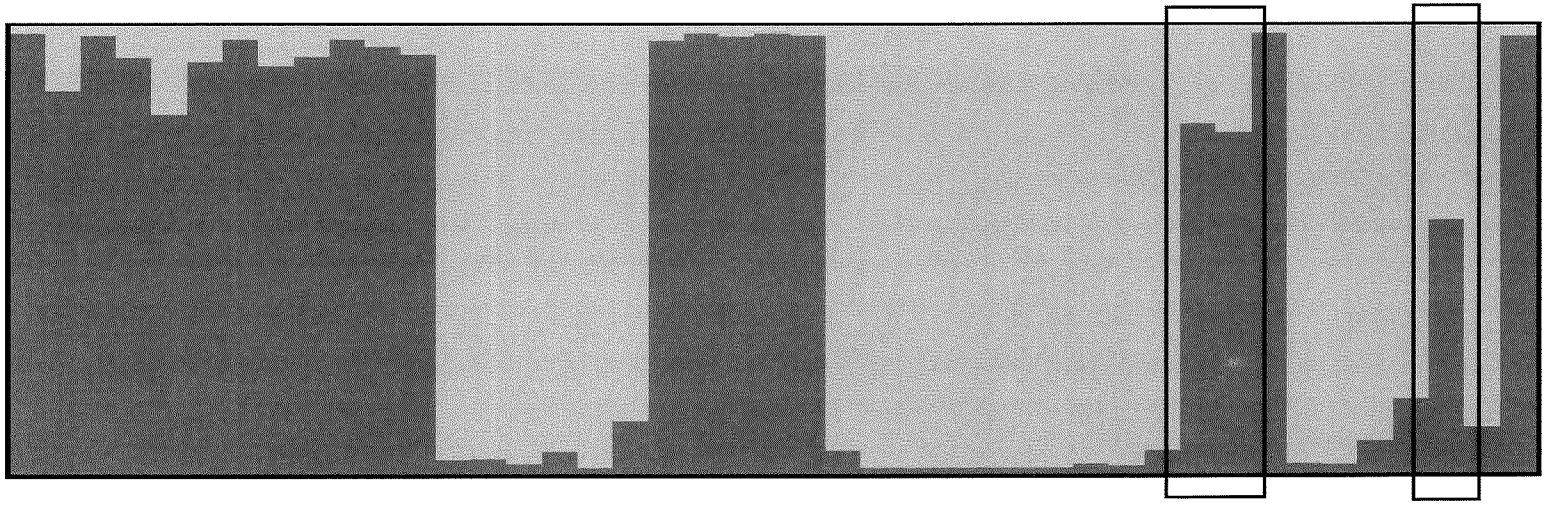

Figure 3.3 - STRUCTURE results with three admixture individuals marked with black boxes. Cluster one is denoted in dark gray and cluster two is denoted in light gray.

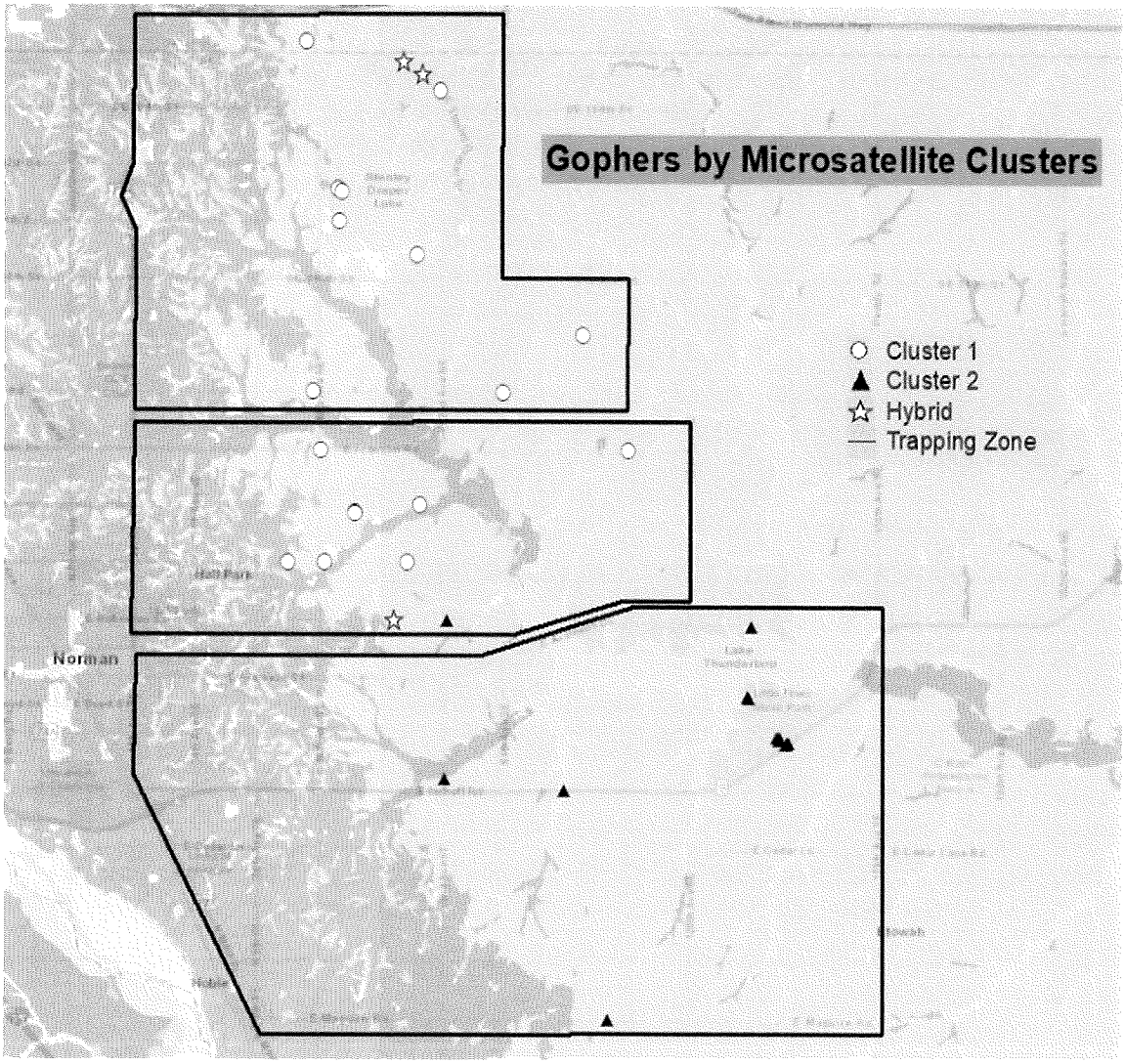

Figure 3.4 - *Geomys* specimens grouped by microsatellite STRUCTURE cluster results. White circles represent cluster 1, black triangles represent cluster 2, and stars represent admixture individuals.

Table 3.1 - Trapping data and voucher specimen measurements. The number of individuals for each age and sex class are provided for each trapping zone and across all samples. Average measurements are provided for all three trapping zones combined and for the two clusters. Cluster measurements were compared to those provided by Sulentich et al. (1991) for *Geomys breviceps*. Tests of significance were performed using R statistical software (R Core Team 2018). *1 of 7 females in this zone were pregnant. **4 of 10 females in this zone were pregnant.

Trapping Zone	**1**	**2**	**3**	**Total**
Age				
Adult	11	10	12	33
Fetus	0	2	8	10
Sex (Adults Only)				
Male	6	2	2	10
Female	4	7*	10**	21
Unknown	1	1	0	2

Voucher Measurements in cm				
	Total length	**Tail**	**Hind Foot**	**Ear**
Average	21.3	6.2	2.5	0.3
Max	24.4	7.4	2.7	0.5
Min	17.2	4.9	2.3	0.2
Sulentich et al.	20.8	6.14	2.56	
Cluster 1 average	20.9	6.1	2.5	0.3
Cluster 2 average	21.8	6.3	2.4	0.3
significance (1 tailed t-test)	**0.04337**	0.1031	0.256	

Table 3.2 – Summary of Microchecker results. Each "X" denotes a possible issue at that locus.

Primer Name	Excess of Homozygotes	Null Alleles	Stutter	Allele Dropout
Gbr09	X	X	-	-
Gbr10	-	-	-	-
Gbr15	X	-	-	-
Gbr25	-	-	-	-
Gbr27	-	-	-	-
Gbr33	X	X	-	-
Tm1	X	X	-	-
Tm 6	X	X	X	-
Tm 7	X	X	-	-

Table 3.3 - Cervus analysis results. The number of alleles, observed heterozygosity (H_O), expected heterozygosity (H_E), and polymorphic information content (PIC) are provided for each locus.

Name	# alleles	Ho	H_E	PIC
Gbr09	4	0.5581	0.6947	0.636
Gbr10	9	0.6905	0.7576	0.713
Gbr15	7	0.6047	0.7008	0.701
Gbr25	7	0.619	0.7212	0.671
Gbr27	1	0	0	0
Gbr33	9	0.5	0.7714	0.737
Tm1	8	0.4884	0.8342	0.802
Tm 6	4	0.1163	0.3609	0.334
Tm 7	4	0.268	0.613	0.525
Average	5.889	0.4272	0.6107	0.5688

Table 3.4 - Summary of STRUCTURE analysis results. Section one shows the three admixture individuals (m24, m25, and m31) provided along with the sex, trapping zone, and percentage designation into each genetic cluster. Individuals with a cluster assignment less than 80% were considered admixture. Section two shows the cluster assignments for specimens analyzed by their original trapping zone locations. Section three is a demographic of clusters by sex and admixture individuals. *one specimen (m32) was trapped in zone 2 but assigned to cluster two unlike the rest of the specimens from zone 2.

Admixture Samples

Sample #	Sex	Trapping Zone	Cluster 1%	Cluster 2%
m24	f	1	0.794	0.206
m25	m	1	0.773	0.227
m31	m	2	0.566	0.434

Cluster Assignment by Trapping Zones

Trapping Zone	Cluster	Cluster Membership	% Cluster Membership
1	1	11 of 11	100%
2	1*	11 of 12	91.67%
3	2	20 of 20	100%

Cluster Specimen Demographics

Cluster	1	2
female	10	11
male	8	2
admixture	3	0

Chapter IV - Discussion and Conclusion

Current vs. Historic Gopher Distribution in Cleveland County

The survey of the previous trapping locations showed they were within residential areas of Norman. Likely because of homeowner intervention, few mounds were found in these locations in this study. Norman also has experienced substantial urban sprawl over the last three decades, which might account for the gopher populations shifting eastward. While the high gopher concentration around Stanley Draper Lake was unexpected, Heaney and Timm (1983) did mention a possible contact zone in Oklahoma City near this location. They analyzed specimens caught two miles east of Tinker Field (now Tinker Air Force Base). This area was at the northern edge of trapping zone 1, which also is where two admixture individuals were identified in this study. It is possible the contact zone always extended this far north and the previous Cleveland County contact zone studies simply did not sample these locations. The specimen from Bohlin and Zimmerman's (1983) study that was identified as a putative hybrid was at their northern most trapping site (see Fig. 1.2).

There was a bias towards female specimens in the dataset. This could be a result of the sexual dimorphism within *Geomys*, with larger males avoiding or escaping the traps. If this is the case, it could also explain why the males caught were smaller specimens; the two gopher specimens which could not readily be sexed were likely subadult males. Thirty-one of the 43 specimens were collected from May to July of 2017. It is of note that all pregnant females were caught during June and 50% of males were caught in the first week of July. The reproductive season of both *Geomys* species is

variable, with *G. bursarius* spanning January to November and *G. breviceps* spanning February to August (Sulentich et al. 1991; Connior 2011). However, the mating season of *G. breviceps* peaks in June and July (Sulentich et al. 1991), which explains the high number of pregnancies. Additionally, males might also be more active during this time looking for mates (Sulentich et al. 1991).

Overall, the gopher distribution seems to have shifted eastward, which is likely a result of Norman's growth. This result questions the hypothesis that the contact zone established by Cothran and Zimmerman (1985) has largely remained intact. A dense gopher distribution and the presence of admixture individuals were found further north in Oklahoma City, Oklahoma County, around Stanley Draper Lake. It is important to note that the admixture individuals may all be *G. breviceps* based on the mitochondrial data. These data provide some support for the hypothesis of the northern border of the contact zone shifting north and east towards Stanley Draper Lake due to the urban sprawl of Norman. However, this result cannot be exclusively attributed to urban sprawl. As mentioned earlier, Heaney and Timm (1983) did suggest a contact zone might occur in this area, although there was not data to support this statement. Therefore, it is possible this zone had been established but previously undocumented. It also is possible that there is no contact zone in this area due to the lack of a signal of *G. bursarius*, indicating it may have been extirpated from the area.

Species Identification and Hybridization

All 43 samples, including the 10 fetuses, were identified as *G. breviceps* based on RFLP analysis. This result was unexpected, especially because the transects and trapping

sites were positioned to include both soil types found within the contact zone and soil type has found to be the most influential factor for gopher distribution. However, these results could be illustrating two known issues that occur when working with mtDNA in hybrid zones: introgression and maternal inheritance bias. MtDNA introgression occurs when mtDNA of one species enters the gene pool of another species through hybridization. This can result in hybrids with mtDNA of one species and nuclear DNA from both species, as well as backcross individuals with nuclear DNA of one species and mtDNA of another. Patton and Smith (1993) cited many instances of mtDNA introgression in *Thomomys* and *Geomys* hybrid zones, therefore my results would support findings from previous work. Additionally, both genera have been shown to exhibit unequal interspecies mating behaviors, with viable offspring only resulting from females of one species and males of the other (Baker et al. 1989). This would result in hybrids having only the RFLP results of the female parent, inaccurately labeling populations as one continuous species despite the presence of hybrids. This issue would be most apparent in mtDNA data because only the female species would be represented.

Because the mtDNA RFLP data showed only one species was present, the nuclear microsatellite data analysis was important in fully investigating hybridization; unfortunately, genotypes of known parental species were unavailable, hampering identification of hybrids. Across all nine microsatellite loci, the observed heterozygosity was lower than expected heterozygosity based on analysis from Cervus which suggests limited gene flow. Analysis of Gbr27 yielded only one allele across all specimens, despite Welborn et al. (2011) describing the presence of five alleles at this locus; this

could be a result of founder effect. Two distinct clusters emerged after STRUCTURE analysis and the presence of three admixture individuals was suggested. With the lower heterozygosity levels observed, it seems the two clusters are somewhat reproductively isolated, although limited admixture was detected.

The clustering pattern seen could be the result of isolation by distance (IBD) or the presence of two species in the dataset. IBD means the clusters were detected as a result of limited amounts of gene flow and the establishment of a north-south allelic gradient. Mapping showed this obvious geographic pattern, with one cluster containing the northern specimens and the second cluster containing the southern specimens (see Fig. 3.4). The geographic direction of this clustering pattern was unexpected. Before mapping, it was assumed the two clusters would instead be divided into eastern and western clusters, similar to the east-west distribution pattern of *G. bursarius* and *G. breviceps* and the east-west variation in soil types. However, the amount of genetic differentiation seen between the two clusters (0.123) is similar to that seen between the two species across Oklahoma (Coffman 2018).

The average voucher measurements for the specimens in cluster 1 were nearly identical to those listed in the *G. breviceps* species account, however, the average body length measurements for those in cluster 2 were statistically significantly larger (one-tailed t-test, $p = 0.04337$). The largest specimen for total length was an admixture individual and all three admixture individuals were in the top 50% of total length. This morphometric variance could be a result of cluster 2 having a high incidence of hybrids which were not detected with the specific nuclear markers used in this study. In a review

of pocket gopher hybrid zones, Patton and Smith (1993) discuss the mating asymmetry documented in *Geomys* and *Thomomys* hybrid zones, suggesting female choice as the explanation for mtDNA introgression with females from smaller species mating with larger males of the parapatric species. Baker et al. (1989), in a study of a *G. bursarius* and *G. knoxjonesi* hybrid zone, found mtDNA from the smaller *G. knoxjonesi* in all putative hybrids. *Geomys breviceps* is the smaller pocket gopher within this hybrid zone which would mirror the findings of Baker et al. (1989). If the two genetic clusters represent different species, the data supports one-way gene flow into cluster 2 with female *G. breviceps* mating with male *G. bursarius*.

From this data, hypothesis 2 that was based on Genoways et al. (2008) and states hybridization will be common when using both mtDNA and microsatellites as a means of hybrid identification, contrary to previous deductions by Bohlin and Zimmerman (1982) and Cothran and Zimmerman (1985), should be further investigated. Three of 43 specimens were denoted as admixture individuals in STRUCTURE. These may be hybrids or a mixture of two genetic clusters of *G. breviceps*. Individuals in cluster 2 also possibly represent hybrids based upon a combination of nuclear and mtDNA data, are a distinct genetic cluster of *G. breviceps,* or represent decreased gene flow across increased geographic distance north to south.

Conclusions

My research has suggested that the contact zone between *G. breviceps* and *G. bursarius* first described by Baker and Glass (1951) might be intact, although the boundaries have shifted eastward. It is unclear if the putative northern boundaries of this

contact zone have shifted further northward or if the suspected zone discussed by Heaney and Timm (1983) was included as part of this study. The species identification conducted using RFLP analysis indicated all specimens were *G. breviceps*. Microsatellite data, however, suggests two distinct genetic clusters. The clusters were divided into a north-south pattern and it is unclear whether these two clusters represent two species or substructure within a single species. The presence of admixture individuals in the microsatellite dataset suggests a need to continue to evaluate possible hybridization in this area. My data suggests the contact zone and potential hybridization might occur further north than previously described. Alternatively, the contact zone may no longer exist with urban sprawl effectively eliminating *G. bursarius* from this area. Additional trapping west of the suspected contact zone to look for *G. bursarius* and further north to investigate admixture individuals found in that area is needed.

Further research using microsatellites to compare specimens in each cluster to known *G. breviceps* and *G. bursarius* samples from outside the contact zone is needed to elucidate if the two clusters indicate separate species that are hybridizing or subpopulation structure. Sequencing the *Cytb* gene in these samples would allow for haplotype analysis as a further means to examine gene flow and population structure. Additional sampling within Cleveland County further east of the locations used in this study is needed to see if the southern cluster population extends northward towards Stanley Draper Lake. This could be one explanation for the two admixture individuals at the most northern trapping sites. Sampling further north around Tinker Air Force Base is needed to investigate the claims of Heaney and Timm (1983) of a contact zone in that

area. Finally, more trapping within Norman to obtain more samples within the other soil type is critical to determine if *G. bursarius* still exists in this area or if the contact zone has vanished due to anthropological impacts.

Literature Cited

Armstrong, K.F., C.M. Cameron, and E.R. Frampton. 1997. Fruit fly (Diptera: Tephritidae) species identification: a rapid molecular diagnostic technique for quarantine application. *Bulletin of Entomological Research* 87:111–118.

Arnold, M.L. 1988. *Natural Hybridization and Evolution.* Oxford University Press, New York.

Avise, J.C., R.A. Lansman, and R.O. Shade. 1979. The use of restriction endonucleases to measure mitochondrial DNA sequence relatedness in natural populations. I. Population structure and evolution in the genus Peromyscus. *Genetics* 92:279–295.

Baker, R.H., and B.P. Glass. 1951. The taxonomic status of the pocket gophers, *Geomys bursarius* and *Geomys breviceps. Proceedings of the Biological Society of Washington* 64:55-58.

Baker, R.J., S.K. Davis, R.D. Bradley, M.J. Hamilton, and R.A. Van Den Bussche. 1989. Ribosomal-DNA, mitochondrial-DNA, chromosomal, and allozymic studies on a contact zone in the pocket gopher, *Geomys. Evolution* 43:63-75.

Baker, R.J., R.D. Bradley, and L.R. McAliley. 2003. Pocket gophers (*Geomyidae*). Pp. 276–287 in *Wild mammals of North America: biology, management, and economics* (J.A. Chapman and G.A. Feldhamer, eds.). Johns Hopkins University Press, Baltimore, Maryland.

Ball, S.L., and K.F. Armstrong. 2006. DNA barcodes for insect pest identification: a test case with tussock moths (Lepidoptera: Lymantriidae). *Canadian Journal of Forest Research* 36:337-350.

Balke, M., I. Ribera, L. Hendrich, M.A. Miller, K. Sagata, A. Posman, A.P. Vogler, and R. Meier. 2009. New Guinea highland origin of a widespread arthropod supertramp. *Proceedings of the Royal Society B: Biological Sciences* 276:2359–2367.

Barton, N.H., and G.M. Hewitt. 1985. Analysis of hybrid zones. *Annual Review of Ecology and Systematics* 16:113-148.

Barton, N.H., and G.M. Hewitt. 1989. Adaptation, speciation and hybrid zones. *Nature* 341:497-503.

Barton, N.H., and K.S. Gale. 1993. Genetic analysis of hybrid zones. Pp. 13-45 in *Hybrid zones and the evolutionary process* (R.G. Harrison, ed.). Oxford University Press, New York.

Benedict, R. 1999. Characteristics of a hybrid zone between two species of short-tailed shrews (*Blarina*). *Journal of Mammalogy* 80:135-141.

Best, T.L. 1973. Ecological separation of three genera of pocket gophers (Geomyidae). *Ecology* 54:1311-1319.

Bickford, D., D.J. Lohman, N.S. Sodhi, P.K.L. Ng, R. Meier, K. Winker, K.K. Ingram, and I. Das. 2006. Cryptic species as a window on diversity and conservation. *Trends in Ecology and Evolution* 22:148–155.

Bilton, D.T., and G.N. Foster. 2016. Observed shifts in the contact zone between two forms of the diving beetle *Hydroporus memnonius* are consistent with predictions from sexual conflict. *PeerJ* 4:E2089.

Block, S.B., and E.G. Zimmerman. 1991. Allozymic variation and systematics of plains pocket gophers (*Geomys*) of south-central Texas. *Southwestern Naturalist* 36:29–36.

Bohlin, R.G., and E.G. Zimmerman. 1982. Genic differentiation of two chromosomal races of *Geomys bursarius* complex. *Journal of Mammalogy* 63:218-228.

Bradley, R.D., S.K. Davis, S.F. Lockwood, J.W. Bickham, and R.J. Baker. 1991a. Hybrid breakdown and cellular-DNA content in a contact zone between two species of pocket gophers (*Geomys*). *Journal of Mammalogy* 72:697-705.

Bradley, R.D., S.K. Davis, and R.J. Baker. 1991b. Genetic control of premating-isolating behavior: Kaneshiro's hypothesis and asymmetrical sexual selection in pocket gophers. *Journal of Heredity* 82:192-196.

Bradley, R.D., J.J. Bull, A.D. Johnson, and D.M. Hillis. 1993. Origin of a novel allele in a mammalian hybrid zone. *Proceedings of the National Academy of Sciences* 90:8939-8941.

Bregliano, J.C., M.G. Kidwell, and J.A. Shapiro. 1983. *Mobile Genetic Elements.* Academic Press, Troy, Missouri.

Burt, M.S., and R.C. Dowler. 1999. Biochemical systematics of *Geomys breviceps* and two chromosomal races of *Geomys attwateri* in eastern Texas. *Journal of Mammalogy* 80:799–809.

Byers, J.R., and D.L. Strubble. 1990. Identification of sex pheromones of two sibling species in dingy cutworm complex I (Lepidoptera: Noctuidae). *Journal of Chemical Ecology* 16:2981–2992.

Chambers, R.R., P.D. Sudman, and R.D. Bradley. 2009. A phylogenetic assessment of pocket gophers (*Geomys*): evidence from nuclear and mitochondrial genes. *Journal of Mammalogy* 90:537-547.

Chan, K.M.A., and S. Levin. 2005. Leaky prezygotic isolation and porous genomes: rapid introgression of maternally inherited DNA. *Evolution* 59:720-729.

Cicero, C. 1996. Sibling species of titmice in the *Parus inornatus* complex (Aves: Paridae). University of California Press, Berkeley.

Coffman, C.N. 2018. Using molecular markers to assess species distribution, contact zones, and hybridization in Oklahoma pocket gophers (*Geomys*). Master's Thesis, University of Central Oklahoma, Edmond.

Connior, M.B. 2011. *Geomys bursarius* (Rodentia: Geomyidae). *Mammalian Species* 43:104-117.

Cortez, J.D., S.E. Henke, D.W. Wiemers, T.E. Fulbright, D.B. Wester, and R. Riddle. 2015. Distribution and habitat selection by the maritime pocket gopher. *Southwestern Naturalist* 14:41-56.

Cothran, E.G., and E.G. Zimmerman. 1985. Electrophoretic analysis of the contact zone between *Geomys breviceps* and *Geomys bursarius*. *Journal of Mammalogy* 66:489-497.

Coyer, J., A. Peters, G. Hoarau, W. Stam, and J. Olsen. 2002. Hybridization of the marine seaweeds, *Fucus serratus* and *Fucus evanescens* (Heterokontophyta: Phaeophyceae) in a 100-year-old zone of secondary contact. *Proceedings: Biological Sciences* 269:1829-1834.

Davis, W.B., R.R. Ramsey, and J.M. Arendale, Jr. 1938. Distribution of pocket gophers (*Geomys* breviceps) in relation to soils. *Journal of Mammalogy* 19:412-418.

De Queiroz, K. 1998. The general lineage concept of species, species criteria, and the process of speciation: a conceptual unification and terminological recommendations. Pp. 57-75 in *Endless forms: species and speciation*. Oxford University Press.

Dowler, R.C. 1989. Cytogenetic studies of three chromosomal races of pocket gophers (*Geomys bursarius* complex) at hybrid zones. *Journal of Mammalogy* 70:253–266.

Dupuis, J.R., A.D. Roe, and F.A.H. Sperling. 2012. Multi-locus species delimitation in closely related animals and fungi: one marker is not enough. *Molecular Ecology* 21:4422-4436.

Earl, D.A., and B.M. vonHoldt. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetic Resources* 4:359-361.

Elrod, D.A., E.G. Zimmerman, P.D. Sudman, and G.A. Heidt. 2000. A new subspecies of pocket gopher (Genus *Geomys*) from the Ozark Mountains of Arkansas with comments on its historical biogeography. *Journal of Mammalogy* 81:852-864.

Ellstrand, N.C, J.M. Lee, J.E. Keeley, and S.C. Keeley. 1987. Ecological isolation and introgression: biochemical confirmation of introgression in an *Arctostaphylos* (Ericaceae) population. *Acta Oecologia Plantarum* 8:299-398.

Ensminger, M.E. 1990. *Horses and Horsemanship: Animal Agriculture Series* (Sixth ed.). Interstate Publishers, Prairie Village, Kansas.

ESRI. 2011. ArcGIS Desktop: Release 10. Environmental Systems Research Institute, Redlands, California.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* 14:2611-2620.

Falush, D., M. Stephens, and J.K. Pritchard. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics* 164:1567–1587.

Ferris, C.J., J.M. Rubio, L. Serrano, J. Gosalvez, and G.M. Hewitt. 1993. One way introgression of a subspecific sex chromosome marker in a hybrid zone. *Heredity* 71:119-12.

Foltz, D.W. 1997. Hybridization frequency is negatively correlated with divergence time of mitochondrial DNA haplotypes in a sea star (*Leptasterias* spp.) species complex. *Evolution* 51:283-288.

Foster, M.A., and J. Stubbendieck. 1980. Effects of the plains pocket gopher (*Geomys bursarius*) on rangeland. *Journal of Range Management* 33:74-78.

Futuyma, D.J. 1998. *Evolutionary biology,* 3rd ed. Sinauer, Sunderland, Massachusetts.

Galtier, N., B. Nabholz, S. Glemin, and G.D.D. Hurst. 2009. Mitochondrial DNA as a marker of molecular diversity: a reappraisal. *Molecular Ecology* 18:4541-4550.

Genoways, H.H., M.J. Hamilton, D.M. Bell, R.R. Chambers, and R.D. Bradley. 2008. Hybrid zones, genetic isolation, and systematics of pocket gophers (Genus *Geomys*) in Nebraska. *Journal of Mammalogy* 89:826-836.

Goudet, J. 1995. FSTAT (vers. 1.2): a computer program to calculate F-statistics. *Journal of Heredity* 86:485–186.

Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices Version 2.9.3. Updated from Goudet (1995). http://www.unil.ch/izea/softwares/fstat.html

Grant, P., and B. Grant. 1992. Hybridization of bird species. *Science* 256:193-197.

Groves, C. 2012. Species concept in primates. *American Journal of Primatology* 74:687-691.

Hafner, M.S., J.E. Light, D.J. Harner, S.V. Brant, T.A. Spradling, and J.W. Demastes. 2005. Cryptic species in the Mexican pocket gopher *Cratogeomys merriami*. *Journal of Mammalogy* 86:1095-1108.

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

Harrington, R.C., and T.J. Near. 2011. Phylogenetic and coalescent strategies of species delimitation in snubnose darters (Percidae: Etheostoma). *Systematic Biology* 61:63-79.

Harrison, R.G., D.M. Rand, and W.C. Wheeler. 1985. Mitochondrial DNA size variation within individual crickets. *Science* 228:1446-1448.

Harrison, R.G., D.M. Rand, and W.C. Wheeler. 1987. Mitochondrial DNA variation in field crickets across a narrow hybrid zone. *Molecular Biology and Evolution* 4:144-158.

Harrison, R.G. 1990. Hybrid zones: windows on evolutionary process. *Oxford Survey in Evolutionary Biology* 7:69-128.

Harrison, R.G. 1993. Hybrids and hybrid zones: historical perspective, Pp. 1-12 in *Hybrid Zones and the Evolutionary Process* (R.G. Harrison, ed.). Oxford University Press, New York.

Hart, E.B. 1971. Karyology and evolution of the plains pocket gopher, *Geomys bursarius*. Ph.D. Dissertation, University of Oklahoma, Norman.

Hartwell, L.H., L. Hood, M.L. Goldberg, A.E. Reynolds, L.M. Silver, and R.C. Veres. 2008. *Genetics: From Genes to Genomes.* McGraw-Hill Companies, New York.

Heaney, L.R., and R.M. Timm. 1983. Relationships of pocket gophers of the genus *Geomys* from the central and northern Great Plains. *Occasional Papers, Museum of Natural History, University of Kansas* 74:1–59.

Heaney, L.R., and R.M. Timm. 1985. Morphology, genetics, and ecology of pocket gophers (genus *Geomys*) in a narrow hybrid zone. *Biological Journal of the Linnean Society* 25:301–317.

Heckman, K.L., E. Rasoazanabary, E. Machlin, L.R. Godfrey, and A.D. Yoder. 2006. Incongruence between genetic and morphological diversity in *Microcebus griseorufus* of Beza Mahafaly. *BMC Evolutionary Biology* 6:98.

Hedin, M. 2015. High-stakes species delimitation in eyeless cave spiders (*Cicurina, Dictynidae, Araneae*) from central Texas. *Molecular Ecology* 24:346-361.

Henry, C.S. 1994. Singing and cryptic speciation in insects. *Trends in Ecology and Evolution* 9:388–392.

Herbert, P.D.N, E.H. Penton, J.M. Burns, D.H. Janzen, and W. Hallwachs. 2004. Ten species in one: DNA barcoding reveals cryptic species in the Neotropical skipper butterfly *Astraptes fulgeratoer. Proceedings of the National Academy of Sciences* 101:14812-14817.

Hewitt, G.M. 1988. Hybrid zones - natural laboratories for evolutionary studies. *Trends in Ecology and Evolution* 3:158-167.

Hillis, D.M., C. Moritz, C.A. Porter, and R.J. Baker. 1991. Evidence for biased gene conversion in concerted evolution of ribosomal DNA. *Science* 251:308-310.

Hodges, S.A., J.M. Burke, and M.L. Arnold. 1996. Natural formation of Iris hybrids: experimental evidence on the establishment of hybrid zones. *Evolution* 50:2504-2509.

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

Hwang, A.S., S.L. Northrup, J.K. Alexander, K.T. Vo, and S. Edmands. 2011. Long-term experimental hybrid swarms between moderately incompatible *Tigriopus californicus* populations: hybrid inferiority in early generations yields to hybrid superiority in later generations. *Conservation Genetics* 12:895–909.

Inouye, R.S., N. Huntly, and G.A. Wasley. 1997. Effects of pocket gophers (*Geomys bursarius*) on microtopographic variation. *Journal of Mammalogy* 78:1144-1148.

Jakobsson, M., and N.A. Rosenberg. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* 23:1801-1806.

Janzen, D.H., M. Hajibabaei, J.M. Burns, W. Hallwachs, E. Remigio, and P.D.N. Herbert. 2005. Wedding biodiversity inventory of a large and complex Lepidoptera fauna with DNA barcoding. *Philosophical Transactions of the Royal Society B: Biological Science* 360:1835-1845.

Jolley, T.W., R.L. Honeycutt, and R.D. Bradley. 2000. Phylogenetic relationships of pocket gophers (genus *Geomys*) based on the mitochondrial 12S rRNA gene. *Journal of Mammalogy* 81:1025–1034.

Jones, J. K., Jr., R.D. Bradley, and R.J. Baker. 1995. Hybrid pocket gophers and some thoughts on the relationship of natural hybrids to the rules of nomenclature and the endangered species act. *Journal of Mammalogy* 76:43-49.

Jones, G., and K.E. Barlow. 2003. Cryptic species of echolocating bats. Pp. 345-349 in *Echolocation in Bats and Dolphins*. University of Chicago Press.

Kalinowski, S.T., M.L. Taper, and T.C. Marshall. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology* 16:1099-1106.

Kidwell, M.G. 1990. Evolutionary aspects of hybrid dysgenesis in *Drosophila*. *Canadian Journal of Zoology* 68:1716-1726.

Kozlov, M.V., J. Zhu, P. Philipp, W. Francke, E.L. Zvereva, B.S. Hansson, and C. Löfstedt. 1996. Pheromone specificity in *Eriocrania semipurpurella* (Stephens) and *E. sangii* (Wood) (Lepidoptera: Eriocraniidae) based on chirality of semiochemicals. *Journal of Chemical Ecology* 22:431-454.

Liu, Z., G. Chen, T. Zhu, Z. Zeng, Z. Lyu, J. Wang, K. Messenger, A. J. Greenberg, Z. Guo, Z. Yang, S. Shi, and Y. Wang. 2018. Prevalence of cryptic species in morphologically uniform taxa – fast speciation and evolutionary radiation in Asian frogs. *Molecular Phylogenetics and Evolution* 127:723-731.

Lohman, D.J., K.K. Ingram, D.M. Prawiradilaga, K. Winker, F.H. Sheldon, R.G. Moyle, P.K.L. Ng, P.S. Ong, L.K. Wang, T.M. Braile, D. Astuti, and R. Meier. 2010. Cryptic genetic diversity in ''widespread" Southeast Asian bird species suggests that Philippine avian endemism is gravely underestimated. *Biological Conservation* 143:1885–189.

Lopez-Giraldez, F., B.J. Gomez-Moliner, J. Marmi, and X. Domingo-Roura. 2005. Genetic distinction of American and European mink (*Mustela vison* and *M. lutereola*) and European polecat (*M. putorius*) hair samples by detection of a species-specific SINE and a RFLP assay. *Journal of Zoology* 265:405-410.

Lotsy, J.P. 1916. *Evolution by means of hybridization.* Springer Netherlands, The Hague.

Mace, G.M. 2004. The role of taxonomy in species conservation. *Philosophical Transactions of the Royal Society B: Biological Sciences* 359:711–719.

Markolf, M., M. Brameier, and P.M. Kappeler. 2011. On species delimitation: yet another lemur species or just genetic variation? *BMC Evolutionary Biology* 11:216.

Marks, B.D. 2010. Are lowland rainforests really evolutionary museums? Phylogeography of the green hylia (*Hylia prasina*) in the Afrotropics. *Molecular Phylogenetics and Evolution* 55:178-184.

Marsh, R.E. 1998. One hundred years of pocket gopher traps and trapping. *Proceedings of the Vertebrate Pest Conference* 18:221–226.

Mauldin, M.R., M.L. Haynie, J.D. Hanson, R.J. Baker, and R.D. Bradley. 2014. Multi-locus characterization of a woodrat (genus *Neotoma*) hybrid zone. *Journal of Heredity* 105:466-476.

Mayr, E. 1963. *Animal species and evolution.* Belknap Press of Harvard University Press, Cambridge, Massachusetts.

Meier, R. 2008. DNA sequences in taxonomy: opportunities and challenges. Pp. 96-127 in *The new taxonomy* (Q.D. Wheeler, ed.). The Systematics Association Special Volume Series 76, CRC Press, Boca Raton, Florida.

Merrell, D.J. 1977. *Ecological genetics.* University of Minnesota Press, Minneapolis.

Miller, L.J., P.G. Allsopp, G.C. Graham, and D.K. Yeates. 1999. Identification of morphologically similar canegrubs (Coleoptera: Scarabaeidae: Melolonthini) using a molecular diagnostic technique. *Australian Journal of Entomology* 38:189–196.

Moore, W.S. 1977. An evaluation of narrow hybrid zones in vertebrates. *The Quarterly Review of Biology* 52:263-278.

Muraji, M., and S. Nakahara. 2002. Discrimination among pest species of Bactrocera (Diptera: Tephritidae) based on PCR–RFLP of the mitochondrial DNA. *Applied Entomology and Zoology* 37:437–446.

Murphy, R.W., F.C. McCollum, G.C. Gorman, and R. Thomas. 1984. Genetics of hybridizing populations of Puerto Rican *Sphaerodactylus*. *Journal of Herpetology* 18:93-105.

Narins, P.M. 1983. Divergence of acoustic communication systems of two sibling species of eleutherodactylid frogs. *Copeia* 4:1089–1090.

Ohno, S., C. Stenius, L. Christian, and G. Schipmann. 1969. De novo mutation-like events observed at the 6PGD locus of the Japanese quail, and the principle of polymorphism breeding more polymorphism. *Biochemical genetics* 3:417-428.

Patton, J.L., and M.F. Smith. 1993. Molecular evidence for mating asymmetry and female choice in a pocket gopher (*Thomomys*) hybrid zone. *Molecular Ecology* 2:3-8.

Pembleton, E.F., and R.J. Baker. 1978. Studies of a contact zone between chromosomally characterized populations of *Geomys bursarius*. *Journal of Mammalogy* 59:233-242.

Pfeiffer, I., J. Burger, and B. Brenig. 2004. Diagnostic polymorphisms in the mitochondrial cytochrome b gene allow discrimination between cattle, sheep, goat, roe buck and deer by PCR-RFLP. *BMC Genetics* 5:30.

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

Qumsiyeh, M.B., C. Sanchez-Hernandez, S.K. Davis, J.C. Patton, and R.J. Baker. 1988. Chromosomal evolution in Geomys as revealed by G- and C-band analysis. *Southwestern Naturalist* 33:1-13.

R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Rickert, K. 2009. Hybridization among short-tailed shrews (*Blarina*) in south central Nebraska. Ph.D. Dissertation, University of Nebraska at Kearney.

Rieseberg, L.H., M.A. Archer, and R.K. Wayne. 1999. Transgressive segregation, adaptation, and speciation. *Heredity* 83:363-372.

Rosenberg, N.A. 2004. Distruct: a program for the graphical display of population structure. *Molecular Ecology Notes* 4:137-138.

Sage, R.D., and R.K. Selander. 1979. Hybridization between species of the *Rana pipiens* complex in central Texas. *Evolution* 33:1069-1088.

Satler, J. D., B.C. Carstens, and M. Hedin. 2013. Multilocus species delimitation in a complex of morphologically conserved trapdoor spiders (*Mygalomorphae, Antrodiaetidae, Aliatypus*). *Systematic Biology* 62:805-823.

Saltonstall, K. 2003. A rapid method for identifying the origin of North American *Phragmites* populations using RFLP analysis. *Wetlands* 23:1043–1047.

Scheffer, S.J., A. Wijesekara, D. Visser, and R.H. Hallett. 2001. Polymerase chain reaction–restriction fragment length polymorphism method to distinguish Liriomyza huidobrensis from *L. langei* (Diptera: Agromyzidae) applied to three recent leafminer invasions. *Journal of Economic Entomology* 94:1177–1182.

Schönrogge, K., B. Barr, J.C. Wardlaw, E. Napper, M.G. Gardner, J. Breen, G.W. Elmes, and J.A. Thomas. 2002. When rare species become endangered: cryptic speciation in myrmecophilous hoverflies. *Biological Journal of the Linnaean Society* 75:291-300.

Short, L.L. 1969. Taxonomic aspects of avian hybridization. *The Auk* 86:84-105.

Sikes, R.S., and the Animal Care and Use Committee of the American Society of Mammalogists. 2016. 2016 guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. *Journal of Mammalogy* 97:663-688.

Smolen, M.J., and J.W. Bickham. 1995. Phylogenetic implications of chromosome evolution in Geomys. *Journal of Mammalogy* 76:50–67.

Soto-Centeno, J.A., L.N. Barrow, J.M. Allen, and D.L. Reed. 2013. Reevaluation of a classic phylogeographic barrier: new techniques reveal the influence of microgeographic climate variation on population divergence. *Ecology and Evolution* 3:1603-1613.

Steinberg, E.K. 1999. Characterization of polymorphic microsatellites from current and historic populations of North American pocket gophers (Geomyidae: *Thomomys*). *Molecular Ecology* 8:1075-1092.

Stöck, M., J. Ustinova, D.K. Lamatsch, M. Schartl, N. Perrin, and C. Moritz. 2010. A vertebrate reproductive system involving three ploidy levels: hybrid origin of triploids in a contact zone of diploid and tetraploid Palearctic green toads (*Bufo virdid* subgroup). *Evolution* 64:944-959.

Struck, T.H., J.L. Feder, M. Bendiksby, S. Birkeland, J. Cerca, V.I. Gusarov, S. Kistenich, K. Larsson, L.H. Liow, M.D. Nowak, B. Stedje, L. Bachmann, and D. Dimitrov. 2018. Finding evolutionary processes hidden in cryptic species. *Trends in Ecology & Evolution* 33:153-163.

Sudman, P.D., J.K. Wickliffe, P. Horner, M.J. Smolen, J.W. Bickham, and R.D. Bradley. 2006. Molecular systematics of pocket gophers of the genus *Geomys*. *Journal of Mammalogy* 87:668-676.

Sukumaran, J., and L.L. Knowles. 2017. Multispecies coalescent delimits structure, not species. *Proceedings of the National Academy of Sciences* 114:1607-1612.

Sulentich, J.M., L.R. Williams, and G.N. Cameron. 1991. *Geomys breviceps*. *Mammalian Species* 383:1-4.

Szalanski, A.L., and T.O. Powers. 1996. Molecular diagnostics of three *Diabrotica* (Coleoptera: Chrysomelidae) pest species. *The Journal of the Kansas Entomological Society* 69:260–266.

Szalanski, A.L., J.W. Austin, R.H. Scheffrahn, and M.T. Messenger. 2004. Molecular diagnostics of the formosan subterranean termite (Isoptera: Rhinotermitidae). *Florida Entomologist* 87:145–151.

Tattersall, I. 2013. Understanding species-level primate diversity in Madagascar. *Madagascar Conservation and Development* 8:7-11.

Toda, S., and S. Komazakim. 2002. Identification of thrips species (Thysanoptera: Thripidae) on Japanese fruit trees by polymerase chain reaction and restriction fragment length polymorphism of the ribosomal ITS2 region. *Bulletin of Entomological Research* 92:359–363.

Tropp, B.E. 2008. *Molecular Biology: Genes to Proteins*, 3rd ed. Jones and Bartlett Publishers Inc., Burlington, Massachusetts.

Troyer, A.F. 2006. Adaptedness and heterosis in corn and mule hybrids. *Crop Science* 46:528-543.

Tucker, P.K., and D.J. Schmidly. 1981. Studies of a contact zone among three chromosomal races of *Geomys bursarius* in east Texas. *Journal of Mammalogy* 62:258-272.

Väli, Ü., P. Saag, V. Dombrovski, B. Meyburg, G. Maciorowski, T. Mizera, T. Rimgaudas, and S. Fagerberg. 2010. Microsatellites and single nucleotide polymorphisms in avian hybrid identification: a comparative case study. *Journal of Avian Biology* 41:34-49.

Van Haecke, D., C.D. Leaniz, G. Gajardo, K. Young, J Sanzana, G. Orellana, ... and S. Consuegra. 2012. DNA barcoding and microsatellites help species delimitation and hybrid identification in endangered galaxiid fishes. *PLoS ONE* 7:1-10.

Van Oosterhout, C., W.F. Hutchinson, D.P.M. Willis, and P. Shipley. 2004. Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* 4:535–538.

Virdee, S.R., and G.M. Hewitt. 1994. Clines for hybrid dysfunction in a grasshopper hybrid zone. *Evolution* 48:392-407.

Wang, H., E.D. McArthur, S.C. Sanderson, J.H. Graham, and D.C. Freeman. 2017. Narrow hybrid zone between two subspecies of big sagebrush (*Artemesia tridentata: Asteraceae*). IV. Reciprocal transplant experiments. *Evolution* 51:95-10.

Watt, W.B. 1972. Intragenic recombination as a source of population genetic variability. *The American Naturalist* 106:737-753.

Weathersbee, A.A., R. C. Bullock, T. D. Panchal, and P. M. Dang. 2003. Differentiation of *Diaprepes abbreviatus* and *Pachnaeus litus* (Coleoptera: Curculionidae) egg masses: PCR–Restriction fragment-length polymorphism and species-specific PCR amplification of 18S rDNA products. *Annals of the Entomological Society of America* 96:637–642.

Welborn, S.R., M.A. Renshaw, and J.E. Light. 2011. Characterization of 10 polymorphic loci in the Baird's pocket gopher (*Geomys breviceps*) and cross-amplification in other gopher species. *Conservation Genetics Resources* 4:467-469.

Wilkins, K.T., and C.D. Swearingen. 1990. Factors affecting historical distribution and modern geographic variation in the south Texas pocket gopher *Geomys personatus. American Midland Naturalist* 124:57-72.

Winker, K. 2005. Sibling species were first recognized by William Derham (1718). *The Auk* 122:706-707.

Wissemann, V. 2007. Plant evolution by means of hybridization. *Systematics and Biodiversity* 5:243-253.

Woodruff, D.S. 1989. Genetic anomalies associated with *Cerion* hybrid zones: the origin and maintenance of new electromorphic variants called hybrizymes. *Biological Journal of the Linnean Society* 36:281-294.

Zou, F., H.C. Lim, B. Marks, R.G. Moyle, and F.H. Sheldon. 2007. Molecular phylogenetic analysis of the grey-cheeked fulvetta (*Alcippe morrisonia*) of China and Indochina: a case of remarkable genetic divergence in a ''species". *Molecular Phylogenetics and Evolution* 44:165–174.

Appendix: Table representing all *Geomys* samples used in this study. A, B, and C designations after the sample name indicate fetuses. RFLP ID results and STRUCTURE clustering results (Cluster) are listed for each individual. In the RFLP column, the number of bands and the species ID (in parenthesis) are provided. GPS coordinates for each specimen also are provided.

Sample Name	**GPS (latitude)**	**GPS (longitude)**	**Trapping Zone**	**RFLP ID**	**Cluster**
m1	35.33400	-97.36500	1	2 (*breviceps*)	1
m2	35.37990	-97.37410	1	2 (*breviceps*)	1
m3	35.27606	-97.37041	2	2 (*breviceps*)	1
m4	35.30501	-97.29560	1	2 (*breviceps*)	1
m5	35.27574	-97.28285	2	2 (*breviceps*)	1
m6	35.29072	-97.31858	1	2 (*breviceps*)	1
m7	35.29085	-97.37243	1	2 (*breviceps*)	1
m8	35.24725	-97.36915	2	2 (*breviceps*)	1
m9	35.24719	-97.34594	2	2 (*breviceps*)	1
m10	35.32562	-97.34300	1	2 (*breviceps*)	1
m11	35.34242	-97.36514	1	2 (*breviceps*)	1
m12	35.34143	-97.36419	1	2 (*breviceps*)	1
m13	35.13141	-97.28850	3	2 (*breviceps*)	2
m13 A	35.13141	-97.28850	3	2 (*breviceps*)	2

m14	35.18961	-97.30096	3	2 (*breviceps*)	2
m14 A	35.18961	-97.30096	3	2 (*breviceps*)	2
m14 B	35.18961	-97.30096	3	2 (*breviceps*)	2
m15	35.19241	-97.33543	3	2 (*breviceps*)	2
m16	35.26199	-97.34245	2	2 (*breviceps*)	1
m17	35.26019	-97.36057	2	2 (*breviceps*)	1
m17 A	35.26019	-97.36057	2	2 (*breviceps*)	1
m17 B	35.26019	-97.36057	2	2 (*breviceps*)	1
m18	35.26000	-97.36059	2	2 (*breviceps*)	1
m19	35.20135	-97.23812	3	2 (*breviceps*)	2
m20	35.20326	-97.24016	3	2 (*breviceps*)	2
m20 A	35.20326	-97.24016	3	2 (*breviceps*)	2
m20 B	35.20326	-97.24016	3	2 (*breviceps*)	2
m20 C	35.20326	-97.24016	3	2 (*breviceps*)	2
m21	35.20165	-97.23795	3	2 (*breviceps*)	2
m22	35.20167	-97.23745	3	2 (*breviceps*)	2
m22 A	35.20167	-97.23745	3	2 (*breviceps*)	2
m22 B	35.20167	-97.23745	3	2 (*breviceps*)	2
m23	35.21325	-97.24847	3	2 (*breviceps*)	2
m24	35.37462	-97.34678	1	2 (*breviceps*)	admixture
m25	35.37137	-97.34146	1	2 (*breviceps*)	admixture
m26	35.36740	-97.33621	1	2 (*breviceps*)	1

m27	35.20314	-97.24081	3	2 (*breviceps*)	2
m28	35.20244	-97.23998	3	2 (*breviceps*)	2
m29	35.23127	-97.24767	3	2 (*breviceps*)	2
m30	35.21355	-97.24898	3	2 (*breviceps*)	2
m31	35.23273	-97.34948	2	2 (*breviceps*)	admixture
m32	35.23270	-97.33450	2	2 (*breviceps*)	2
m33	35.24721	-97.37973	2	2 (*breviceps*)	1