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GENETIC STRUCTURE AND THE POTENTIAL FOR HYBRIDIZATION IN POPULATIONS OF *PEROMYSCUS SPP.* OF PLATEAU REGIONS IN WESTERN OKLAHOMA

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Sarah C. Vrla

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By: _____

Michelle L. Haynie – Committee Chairperson

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Allyson M. Fenwick – Committee Member

_____

Greg M. Wilson – Committee Member

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ABSTRACT OF THESIS

University of Central Oklahoma

Edmond, Oklahoma

NAME: Sarah C. Vrla

TITLE OF THESIS: Genetic structure and the potential for hybridization in populations of *Peromyscus spp*. of plateau regions in western Oklahoma

DIRECTOR OF THESIS: Michelle L. Haynie, Ph.D.

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There are many evolutionary mechanisms that influence the process of speciation, or the creation of new species. One such process is genetic recombination. Hybridization can be argued to be a unique form of genetic recombination, and therefore studies offering insight into the mechanisms influencing hybridization can enhance our understanding of speciation as a whole. Hybridization also can cause shifts in ecological balances of an ecosystem in the form of increasing competition or influencing changes in disease ecology if offspring produced are viable.

The genus *Peromyscus* is invaluable in the study of hybridization as a result of an extensive data set already existing for the evolution, taxonomy, and genetic divergence of the subgenera, species groups, and species included within it. Two such species, *Peromyscus maniculatus* and *P. leucopus* are located within the same subgenus (*Peromyscus*) and separate species groups (*maniculatus* group and *leucopus* group, respectively) that diverged approximately 2.5 million years ago. These two species have extensive ranges across North America and overlap across much of the eastern half of the United States, including Oklahoma.

During a small mammal survey of Four Canyon Preserve (FCP) in western Oklahoma (Ellis County), a unique group of *Peromyscus* individuals were collected that exhibited a distinct sharing of characteristics typically used to distinguish these species and could not be identified as *P. maniculatus* or *P. leucopus*. In this study I investigated hybridization as a potential explanation for this merging of morphological characteristics through the use of 11 nuclear microsatellite loci, restriction fragment length polymorphism (RFLP) analysis of the mitochondrial (mtDNA) cytochrome *b* (*Cyt b*) gene, and skull morphometrics analysis.

A total of 158 samples collected from FCP, across the state of Oklahoma, and further across the range of the two species were examined. An individual was considered to be a putative hybrid (i.e., admixture individual) in three ways: a disagreement between the two species ID methods, RFLP analysis of *Cyt b* and a microsatellite locus (PML08); a disagreement between either species ID method with the complete microsatellite dataset (STRUCTURE) assignment; or being identified as an admixture individual in the STRUCTURE analysis. Skull morphometrics data were inconclusive at this time and further data collection is needed before results can be interpreted from data reported here.

Through these methods, 65 out of 158 specimens examined were identified as admixture individuals, which offers support that these two species might be hybridizing within sympatric areas. Additionally, results offer evidence for new locality records and/or range expansions within the species, suggesting the need for further research into the current distribution of both species across North America.

CHAPTER ONE

INTRODUCTION

The Process of Speciation

Species definitions

How to define and classify species, and even the existence of species, has long been debated. Over the past 60 years, more than 26 species concepts have been proposed based on various criteria for the definition of a species (Simpson 1951; Mayr 1957, 1969; Sokal and Crovello 1970; Mayden 1997; de Queiroz 1998; Wheeler and Meier 2000; Baker and Bradley 2006). The earliest species definitions focused on morphological differences (typological/morphological species concept; Simpson 1951; Mayr 1957), but advances in technology allowed for different criteria to be considered, including cytogenetics, allozymes, and genetics, as well as various combinations of characteristics (i.e., phylogenetic, evolutionary, unified concepts, etc.).

According to the biological species concept, a species is a group of populations that do, or have the potential to, interbreed and are reproductively isolated from other such groups. Groups (populations) can be identified as separate species when reproductive isolation between the groups prevents the production of viable, fertile offspring in areas of sympatry (Mayr 1957, 1970; Sokal and Crovello 1970). The biological species concept is debated for many reasons, including the fact that determining if individuals have the potential to interbreed often is difficult when populations exist across a broad distribution (Mayr 1970; Sokal and Crovello 1970). Hybridization also triggers debate as individuals of closely related species have been known to successfully interbreed and produce viable, fertile offspring (Stebbins 1959).

With the development of technologies that allow for the examination of individuals and populations at a genetic level, the genetic species concept was proposed, which focuses on genetic isolation rather than reproductive isolation when defining species (Simpson 1943; Mayr 1969; Wu 2001; Baker and Bradley 2006). The genetic species concept aids in resolving the conflict of hybridization in that species will be considered separate as long as a specific level of genetic divergence can be detected, even if viable hybrids are known to occur (Baker and Bradley 2006).

These two species concepts focus on traits that are exhibited at a single point in time and fail to address the topic of evolutionary time. The evolutionary species concept takes into account the evolutionary history of a species. Species therefore are defined as any ancestral-descendant sequence of populations, or lineages, that are evolving independently of other lineages (Simpson 1961). Through this concept, populations are interbreeding, forming phyletic lineages, and carrying out specific and unique evolutionary characteristics and roles (Wiley 1978).

Much of the controversy among species concepts is the characteristics by which a species should be defined (e.g., morphological factors of the morphological species concept; reproductive potential of the biological species concept, etc.). The unification of all species concepts, the general lineage concept of species, suggests the issue of speciation should be approached differently (de Queiroz 1998). This method focuses primarily on the foundation on which all species concepts are built – that species evolve through divergence of lineages and the various factors associated with these species will change throughout that process (de Queiroz 1998, 2007, 2011). In this method, species only have to be evolving independently of one another in order to be considered separate.

All other factors (morphological traits, reproductive isolation, genetic uniqueness, etc.) are seen as trait differences that may or may not be acquired throughout the process of divergence, although they are still significant to the issue of species delimitation as it offers evidence of lineage separation (de Queiroz 2007, 2011). The major species concepts discussed here have been summarized in Table 1.1.

Hybridization

There are several natural processes thought to play a significant role in the evolution of new species, one of which is genetic recombination, or the exchange of genetic material between different organisms. The interbreeding of individuals of different species, or hybridization, is therefore a form of genetic recombination. The exact role hybridization plays in the evolution of new species is complicated and not yet fully understood (Stebbins 1959). Arguments can be made both for (Stebbins 1959; Arnold 1992; Grant and Grant 1992; Buerkle et al. 2000; Mallet 2007) and against (Mayr 1963; Wagner 1970; Barton 2013) hybridization playing a significant role in speciation. However, hybridization affects various taxa and populations differently, depending on the rate at which it occurs within each group. While it may be expected between more closely related species with less divergence, the eventual outcome will not always be predictable (Moore 1977). Therefore, to fully understand the role hybridization plays in the evolution of a group, we must first understand the rate at which it is occurring (Stebbins 1959). Hybridization generally results in the production of less fit offspring (Harrison 1986; Griebel et al. 2015) as a result of parental species being better adapted to the environment and outcompeting hybrid offspring (Griebel et al. 2015). On some occasions, hybrid offspring may be better adapted and able to outcompete parental species (Harrison 1986).

In these instances, because hybridization between more genetically distant species creates much higher levels of variation, it may have drastic effects on many aspects of the population including range expansion events (Pfennig et al. 2016) or changes in disease ecology (Leo and Millien 2017). Therefore, studies of hybridization can lead to an understanding of not only the limits of hybridization (i.e., genetic distance of parental species), but also the ecological and evolutionary impacts hybridization may have on a population. In other words, the study of hybridization among species of varying levels of divergence offers the opportunity to evaluate what level of divergence produces complete reproductive isolation and whether or not hybridizing species are on the boundary where natural factors inhibit interbreeding. As more of these investigations are conducted, we might need to reevaluate how we define a species and what impacts the changing world (e.g., climate change, land alteration, etc.) has on the rate of hybridization, and therefore speciation.

More important than the frequency of hybridization is the contribution of hybrids to the gene pool. This means that we also need to understand how hybrids contribute to future generations, whether they are sterile, fertile, or have reduced fertility, and if a pattern of introgression exists (Stebbins 1959; Loschiavo et al. 2007). It is hypothesized that hybridization results in two possible outcomes. The first is an increased potential for more rapid adaptation, enabling establishment of populations in unoccupied habitats, and the speeding up of speciation if the recombination of genes creates a phenotype that is viable and fertile enough to enable introgression and the merging of the two species (Stebbins 1959; Moore 1977). Second, if hybridization of two species is only occurring within a hybrid zone that is significantly smaller than the full extent of the ranges of each

parent species, then divergence might not be significantly affected and no new species will be formed (Barton 2013).

There are multiple factors that contribute to an individual choosing a heterospecific mate, particularly in disturbed or edge habitats (Mayr 1942; Anderson 1948; Rosenthal 2013). For example, environmental disturbances might lead to an inability for conspecifics to send or receive sexual communication signals, resulting in an individual choosing a heterospecific mate. Another possibility is any change in the cost / benefit balance as a result of an imbalance in various ecological processes, for example conspecific mates being rare, therefore making a heterospecific mating opportunity more beneficial than no mating opportunity (Willis et al. 2011), or an increased risk of predation when searching for conspecifics (Willis et al. 2012) resulting in the choice of a low risk heterospecific mate being more beneficial to the individual. Under some ecological conditions, hybrid offspring see an increase in fitness, as seen in the spadefoot toad (Pfennig 2007; Rosenthal 2013). Finally, maturation or developmental learning might also play a role in hybridization as younger individuals, if faced with locations where conspecifics are rare, may gain a preference for heterospecifics in choosing a mate (Verzijden et al. 2012; Rosenthal 2013).

When two species' ranges overlap and the production of hybrids occurs between species, the area of overlap is referred to as a hybrid zone (Short 1969; Barton and Hewitt 1985; Hewitt 1988). These zones can vary geographically from covering a relatively narrow area of less than one kilometer, to being extensive and covering continuous areas of hundreds of kilometers, to creating a mosaic of disjointed hybrid zones (Hewitt 1988; Arnold 1992). Overlapping species within hybrid zones are said to either be in primary

contact, meaning the species have existed in overlapping ranges historically, or secondary contact where the species were historically allopatric and experienced range alterations which introduced previously unseen opportunities to interbreed (Mayr 1963; Hewitt 1988; Harrison 1993). It can be difficult to determine whether a population should be categorized as experiencing primary or secondary contact (Mayr 1963; Woodruff 1973; Endler 1982).

Four models have been proposed in an attempt to describe hybrid zones, each with distinct criteria, benefits, and restrictions (Table 1.2; Moore 1977). The ephemeral model suggests that hybridization will ultimately result in either complete speciation (i.e., reproductive isolation) or the introgression of hybrids back with parental species in a relatively short-lived manner. This hypothesis is based on two concepts, first the concept of mutually adapted gene complexes and second that gene flow is responsible for the continuity of independent species (Moore 1977). Because an individual's phenotype is the result of a combination of several genetic loci and adaptations to certain environmental conditions result from complex interactions between genotypes and the environment, hybridization has the potential to create phenotypes resulting in non-viable or non-fertile offspring. Natural selection will ultimately select against individuals prone to hybridization, a form of reinforcement, resulting in the lineage being completely separated into distinct species. On the contrary, if viable and fertile offspring are produced, they will have the potential of backcrossing with the parental species and might result in the merging of the two species (Moore 1977). This model fails to address those hybrid zones that are long-lived and stable (Moore 1977).

The dynamic equilibrium model works to address more stable hybrid zones,

although it suggests they are restricted as a result of less fit offspring being produced through hybridization. The resulting less-fit offspring means introgression with parental species would be limited by selection; however, this selection pressure would only exist within the narrow hybridization zone and not within the main portions of the parental species' populations. As a result, the narrow hybrid zone stabilizes and can persist long-term as individuals continue to migrate into the hybrid zone from parental ranges and hybridize (Bigelow 1965; Moore 1977; Gay et al. 2008). This model does not address stable hybrid zones that lack parental populations that continuously contribute to the gene pool of the hybrid zones (Moore 1977).

In some instances, hybridization results in phenotypes that are more fit than the parental phenotypes (Short 1972; Littlejohn and Watson 1973; Moore 1977). This phenomenon is addressed by the hybrid superiority model and offers an explanation for stable, long-lived hybrid zones (Moore 1977). This superiority of hybrids might only occur within restricted ranges or ecotones that are unique from the natural range of either parental species (Littlejohn and Watson 1973; Moore 1977). This model remains consistent with the structure of many stable hybrid zones and suggests that hybrid populations are able to persist as a result of relaxed competition from parental species within these unique environments (Moore 1977).

Lastly, the tension zone model refers to areas in which hybridization continues as a result of dispersal and selection against hybrids remaining balanced (Key 1968; Moore 1977; Gay et al. 2008). These zones are independent of local environmental conditions and are able to shift (Key 1968; Barton and Hewitt 1985; Gay et al. 2008). This movement can be influenced by multiple forces including aspects related to individual

fitness, population structure, and the genetic structure of populations (Barton and Hewitt 1985). This model, like the dynamic equilibrium model, suggests hybrid individuals are less fit than the parental species, thus resulting in selection against hybrids and the ultimate reduction of introgression with parental species (Hewitt 1975; Nichols 1989). Tension zones are most likely to form in areas of overlap between the parental species where one, or both, species exhibits a lower population density and may persist long-term within these narrow zones of contact (Nichols 1989).

Evolutionary History of Peromyscus

Peromyscus (deermice) is a large, diverse genus of cricetid rodents. Historically, many New World rodents of Family Cricetidae were classified under the genus *Mus* of Family Muridae (Linnaeus 1758; Thomas 1896; King 1968; Bedford and Hoekstra 2015). However, we now know that Cricetidae and Muridae diverged approximately 25 million years ago (Ma; Steppan et al. 2004). The genus *Peromyscus* originated approximately 8 Ma (Platt et al. 2015), and the name was first employed in the mid-19th century to describe only a few species (Gloger 1841; Audubon and Bachman 1854). The genus grew quickly, however, and by the turn of the 20th century, *Peromyscus* had a total of 42 species and 143 different forms officially described (Osgood 1909).

Peromyscus belongs to the subfamily Neotominae, which consists of 16 genera and over 120 species including pygmy mice (*Baiomys*), singing mice (*Scotinomys*), deermice and similar species (*Habromys, Isthmomys, Megadontomys, Neotomodon, Onychomys, Osgoodomys, Peromyscus,* and *Podomys*), harvest mice (*Reithrodontomys*), wood rats (*Hodomys*, *Nelsonia*, *Neotoma*, and *Xenomys*), and the golden mouse

(*Ochrotomys*; Carleton 1980; Hall 1981; Musser and Carleton 2005). The phylogenetic relationships and evolutionary history of many cricetid rodents, specifically those of subfamily Neotominae, remain unclear as a result of challenges associated with studying this group. For example, this group underwent a rapid radiation event during the late Miocene, exhibits a great deal of morphological similarity among certain species, and has a paucity of phylogenetically informative characters (Reeder and Bradley 2004; Reeder et al. 2006; Fabre et al. 2012).

Several studies have attempted to establish a consensus on the number of tribes found within the subfamily Neotominae and to which tribe each genus should be classified, with limited success (Hooper and Musser 1964; Carleton 1980; Bradley et al. 2004b; Reeder and Bradley 2004; Reeder et al. 2006; Miller and Engstrom 2008, among others). Although most studies recognize four or five tribes within Neotominae, a consensus has yet to be made as to which groups should be considered tribes or which genera should be included within a tribe (Carleton 1980; Bradley et al. 2004b; Reeder and Bradley 2004; Musser and Carleton 2005; Reeder et al. 2006; Miller and Engstrom 2008). Three tribes commonly are agreed upon: Baiomyini (*Baiomys* and *Scotinomys*), Neotomini (*Hodomys, Neotoma,* and *Xenomys*), and Ochrotomyini (*Ochrotomys*; Musser and Carleton 2005; Reeder et al. 2006; Miller and Engstrom 2008). Some studies suggested that *Ochrotomys* should be included within Peromyscini, although this placement was not supported by Bayesian probability values (Bradley et al. 2004b; Reeder and Bradley 2004). Classification of Tylomini (*Nyctomys, Tylomys, Otonyctomys*, and *Ototylomys*) varies between studies, with some elevating this group into a valid subfamily (Tylomyinae; Carleton 1980; Bradley et al. 2004b; Bradley and Reeder 2004;

Reeder et al. 2006) and others considering these genera to be outliers to Neotominae (Musser and Carleton 2005; Miller and Engstrom 2008). The classification of the remaining groups, including *Peromyscus* (and allied genera) and *Reithrodontomys*, is difficult due to a lack of understanding of the phylogenetic relationships within them. Some studies suggest *Peromyscus* and allied genera + *Reithrodontomys* should be combined into a single tribe – Peromyscini (Hooper and Musser 1964; Reeder et al. 2006). Musser and Carleton (2005) agreed on this classification, although they used the name Reithrodontomyini to represent this group instead. Other studies suggest these groups be split between Peromyscini (*Peromyscus* and allies) and Reithrodontomyini (*Reithrodontomys* + *Isthmomys*; Miller and Engstrom 2008). Further studies including more taxa will need to be conducted in order to resolve these questions.

The genus *Peromyscus*, with over 50 species and 200 subspecies, is one of the most speciose and well-studied genera of North American mammals (King 1968; Carleton 1989; Kirkland and Layne 1989; Musser and Carleton 2005). *Peromyscus* species are among the most adaptable of all North American mammals and occupy nearly every terrestrial habitat ranging from the Atlantic to the Pacific Ocean and from as far north as Alaska south to Central America (King 1968; Kirkland and Layne 1989). For these reasons, *Peromyscus* species have served as model organisms to answer questions across a wide range of disciplines that have been summarized in Table 1.3. A summary of research also can be found in King (1968) and Kirkland and Layne (1989). Such exhaustive research on the genus has led to *Peromyscus* being referred to as the *Drosophila* of North American mammalogy (Kirkland and Layne 1989; Dewey and Dawson 2001). Despite this extensive level of research, a lot remains to be determined

about the phylogenetic relationships within this genus. Several revisions and suggestions have been made to the genus in the form of elevating groups to the generic level, classifying species into subgenera and species groups, and identifying new and separate species (Osgood 1909; Hooper and Musser 1964; Hooper 1968; Avise et al. 1974, 1979; Carleton 1989; Bradley et al. 2004a, 2004b, 2007, 2016; Musser and Carleton 2005; Platt et al. 2015; Greenbaum et al. 2017; among others).

Osgood (1909) offered the first comprehensive classification of *Peromyscus* and noted six subgenera (*Baiomys*, *Haplomylomys*, *Megadontomys*, *Ochrotomys*, *Peromyscus*, and *Podomys*). Two of these subgenera were later elevated to generic rank, *Baiomys* (Miller 1912) and *Ochrotomys* (Hooper 1958), and three new subgenera were proposed (*Habromys*, *Isthmomys*, and *Osgoodomys*; Hooper and Musser 1964). Therefore, Hooper (1968) included seven subgenera within *Peromyscus* in his classification revision. However, Carleton (1980) argued that *Megadontomys, Podomys, Habromys, Isthmomys,* and *Osgoodomys* should all be elevated to generic status as well, and Carleton (1989) officially rewrote the classification of *Peromyscus* to include only the subgenera *Haplomylomys* and *Peromyscus*. The most recent comprehensive revisions of *Peromyscus* classification retain Carleton's (1989) taxonomy (Musser and Carleton 1993, 2005). Today, classification within the genus remains a topic of intense study and debate, largely due to the regular addition of new taxa, the presence of cryptic species, and conflicting phylogenies from various studies (Black 1935; Schmidly 1973; Riddle et al. 2000; Bradley et al. 2004a; 2004b, 2014, 2015). *Peromyscus* has been divided into multiple species groups since Osgood's first revision of the genus; and as more species have been added and genetic analyses have become available, additional species groups

have been added. For the most part, species groups consist of genetically related species. However, there is much debate as to what group each species belongs.

Hybridization is known to occur naturally within these proposed species groups (McCarley 1954; Kirkland and Layne 1989) as well as between groups (Maddock and Dawson 1974; Leo and Millien 2017). Additionally, artificial insemination has been used to develop hybrid individuals between several *Peromyscus* species (Maddock and Dawson 1974). Studying reproduction among and between these genetic groups offers the opportunity to quantify genetic divergence and compare this divergence to the success of genetic introgression, thus offering an understanding of the role of genetic distance versus genetic similarity in hybridization. Therefore, *Peromyscus* serves as a model for hybridization studies (Kirkland and Layne 1989).

Project Goals

There are eight *Peromyscus* species (subgenus *Peromyscus*) in Oklahoma, including *P. leucopus* (white-footed deermouse; leucopus species group) and *P. maniculatus* (North American deermouse; maniculatus species group; Caire et al. 1989). These two species share similar habitat preferences, with some distinct preferences for specific seeds and microhabitats, and their ranges overlap nearly statewide across Oklahoma (Fig. 1.1; Lackey et al. 1985; Caire et al. 1989). *Peromyscus leucopus* can be found across Oklahoma and typically is associated with brushy or woody habitats. Similarly, *P. maniculatus* also can be found statewide, although they are more common in grassland habitats (Lackey et al. 1985; Caire et al. 1989).

During a survey of the mammals of Four Canyons Preserve (FCP) in western Oklahoma, a group of *Peromyscus* individuals could not be identified as either *P. leucopus* or *P. maniculatus* using morphological features typically used to distinguish the species because they exhibited a mixture of characteristics belonging to both species (Caire et al. 2010). Characteristics such as tail coloration, lengths of the tail and ear, and weight can be used to identify individuals to species, although the most reliable external features across large spatial distributions have been found to be the length and coloration of the tail (Choate 1973; Lackey et al. 1985; Caire et al. 1989; Stephens et al. 2014). *Peromyscus leucopus* are known to have a tail that is not distinctly bicolored and is rarely as long as the body (range = 56-92 mm, average = 74.8 mm), and an average weight of 19.8 g. *Peromyscus maniculatus* is known to have a distinctly bicolored tailed ranging from 66-103 mm (average = 87.1 mm), and an average weight of 17.4 g (Caire et al. 1989; Stephens et al. 2014). In sympatric areas, individuals tend to fall into an intermediate range for these characteristics, making them nearly impossible to properly identify using morphometric data (Kamler et al. 1998). Therefore, in areas of overlap misidentifications can occur in high percentages (Kamler et al. 1998; Brueso et al. 1999).

To date, only five studies in the published literature have investigated hybridization between these two species. Laboratory attempts to hybridize *P. leucopus* and *P. maniculatus* have been unsuccessful (Dice 1933). Artificial insemination successfully produced some fertilization and embryo implantation events, although they did not survive longer than a few days (Maddock and Dawson 1974). Haines (1983) successfully produced one *P. leucopus* × *P. maniculatus* hybrid offspring. The outcome of this offspring is unclear; it was known to survive long enough to be weaned, but

survival beyond that point was not recorded. Finally, after climate change resulted in the expansion of *P. leucopus* north into *P. maniculatus* range in Quebec, Leo and Millien (2017) and Garcia-Elfring et al. (2017) genotyped wild caught individuals and found evidence of hybridization between the species in this new area of overlap.

Because *P. leucopus* and *P. maniculatus* are both classified under the subgenus *Peromyscus* and are in different species groups (the leucopus and maniculatus species groups, respectively), which are considered to be sister species groups, studying the ability of these two species to hybridize offers the opportunity to better understand factors contributing to the ability to hybridize, including genetic divergence.

This project aimed to 1) identify specimens collected by Caire et al. (2010) at FCP as either *P. leucopus* or *P. maniculatus* using morphological data, microsatellite genetic analyses, and mitochondrial DNA (mtDNA) analyses; and 2) determine if hybridization is occurring naturally in this population.

Introduction to Methods

Combinations of microsatellite and mitochondrial DNA analyses were used in this study. Microsatellites are DNA sequences that are one to six base pairs in length, tandemly repeated, located throughout the nuclear genome, and typically the most variable sequences in a genome (Ellegren 2004; Chistiakov et al. 2006). They are biparentally inherited, and during the DNA replication process, mutations occur in microsatellites that change the length of alleles passed on, making them useful in the identification of distinct genetic clusters and admixed individuals. The mitochondrial genome of *Peromyscus* is approximately 16,000 base pairs (bp) long and serves as a good

marker for determining evolutionary history and species identification for numerous reasons, including that it is maternally inherited, is less conserved than nuclear DNA, does not undergo genetic recombination and is therefore passed through generations intact, and its evolutionary rate presumably is clock-like as a result of an absence of positive selection occurring on mutations (Avise et al. 1987; Mortiz et al. 1987). Therefore, mtDNA has been widely used in genetic studies (Harrison 1989; Cronin et al. 1991; Bradley et al. 2004a, 2004b), as well as to discriminate among *Peromyscus* species (Tessier et al. 2004; Leo and Millien 2017). Restriction enzymes can be used to cut DNA in specific locations that differ between species. Using restriction enzymes on mitochondrial DNA, for example the use of *BamHI* on the mitochondrial cytochrome *b* (*Cyt b*) gene as done in this study, can serve as a means of species identification in the form of species-specific banding patterns (Minarovic et al. 2010).

In addition to genetic analyses, skull morphometrics were analyzed for individuals of the Four Canyon population. Because *P. leucopus* and *P. maniculatus* share similar ecological niches, competition has led to evolutionary divergence and character displacement in the shape of the skull between the species (Millien et al. 2017), and several morphological skull measurements, when combined, can be used to distinguish the species, although it must be noted that the use of single measurements alone in distinguishing these species is not always 100% effective (Choate 1973; Feldhamer et al. 1983; Rich et al. 1996; Stephens et al. 2014). However, the use of genetic techniques in combination with morphological data is common practice in identifying species and arguments have been made in support of the continued use of morphological measurements in conjunction with genetic analyses for species

identification (Katoh and Tokimura 2001; Will and Rubinoff 2004; Mattiucci et al. 2014; Abbas et al. 2016).

Figure 1.1.–Distribution map of *Peromyscus maniculatus* and *P. leucopus* showing the area of range overlap. The black point indicates the location of Four Canyon Preserve in western Oklahoma (Ellis County).

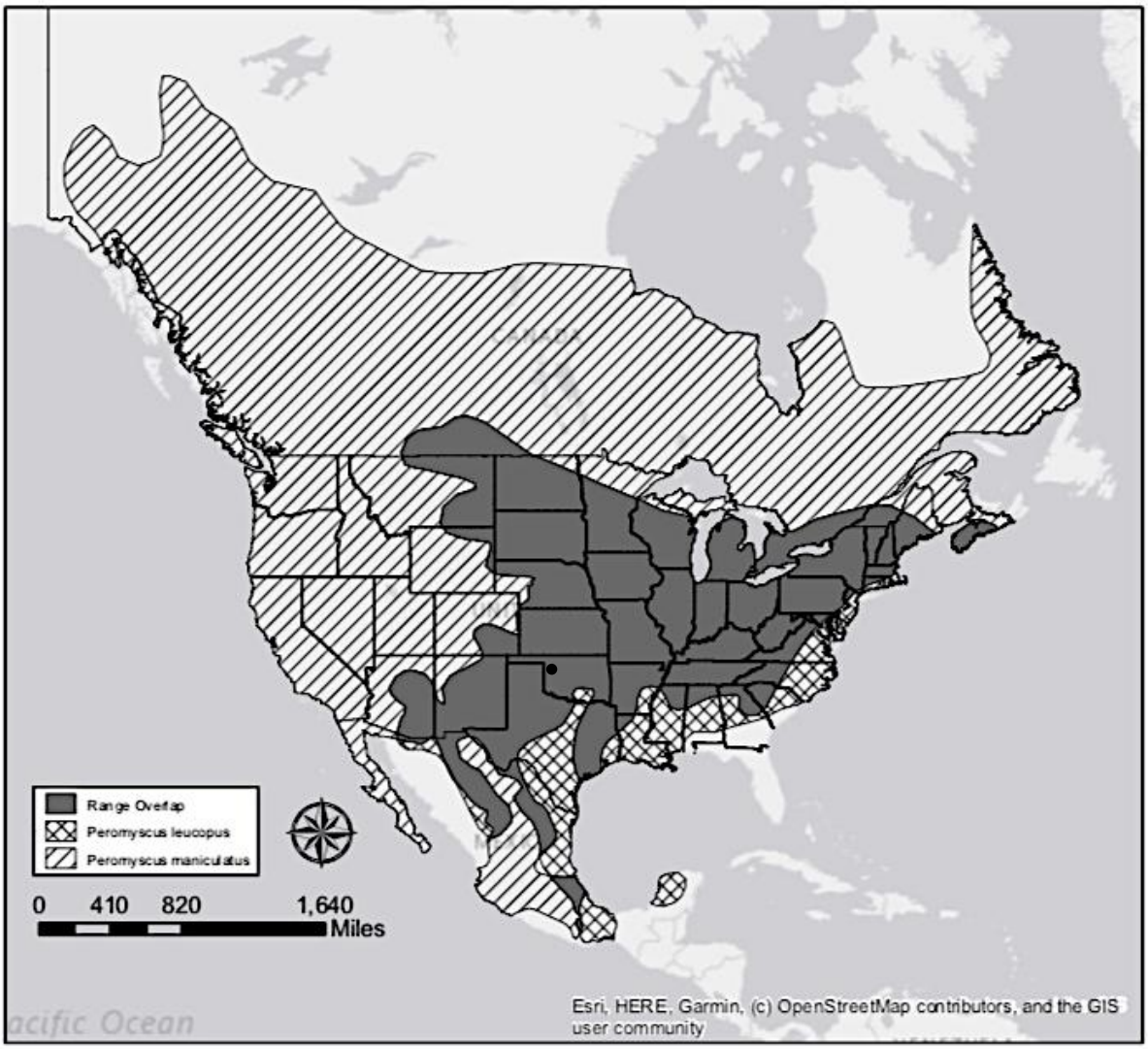

Table 1.1.–Brief description of major species concepts.

Concept	Summary
Biological species concept	A group of individuals or populations that do, or have the potential to, interbreed and are reproductively isolated from other groups. Reproductive isolation is the defining characteristic.
Morphological species concept	A group of individuals identified based on unique identifying morphological characteristics, which are the defining characteristics.
Genetic species concept	Aids in resolving the conflict of hybridization in that species are considered distinct based on the detection of genetic distance, which is the defining characteristic.
Evolutionary species concept	Each species is a distinct lineage evolving separately from other lineages. Evolutionary history is the defining characteristic.
General lineage species concept	Proposes all species concepts define species as evolutionary lineages, and the differences addressed by each concept are a product of said lineage. Therefore, all species concepts are complementary rather than contradictory.

Table 1.2.–Brief description of hybrid zone models.

Model	Summary
Ephemeral Model	Hybridization will result in either complete speciation or introgression of hybrids back with parental species.
Dynamic Equilibrium Model	Hybridization will result in a stable hybrid zone as a result of hybrids being less-fit, thus relaxing long term selection pressure for individuals migrating into the hybrid zone from the parental populations. This model only applies to those hybrid zones with adjacent parental populations that can continuously contribute to the gene pool of the hybrid zone.
Hybrid Superiority Model	Hybrid zones are long-lived and stable as a result of the resulting hybrid phenotype being more fit than the parental phenotypes. May only occur within restricted ranges or ecotones unique from the natural range of either parental species.
Tension Zone Model	Hybrid zones continue as a result of dispersal and selection against hybrids remaining balanced. Hybrid zones are independent of local environmental conditions and are able to shift over time.

Table 1.3.–List of disciplines that have used *Peromyscus spp.* as model species. Representative citations are provided for each discipline.

Discipline	Citations
Allozymes	Zimmerman et al. 1975, 1978; Avise et al. 1979
Behavior	Bester-Meredith et al. 2017; Hu and Hoekstra 2017
Biogeography	Sullivan et al. 1997; Riddle et al. 2000; Carleton and Lawlor 2005
Chromosomal Evolution	Committee for Standardization of Chromosomes of Peromyscus 1977, 1994; Robbins and Baker 1981; Rogers et al. 1984; Stangl and Baker 1984; Vieira-da-Silva et al. 2015; Smalec et al. 2019
Developmental Biology	Vrana et al. 2013
Ecology	Kaufman and Kaufman 1989; Owen 1989; Danielson and Hubbard 2000
Genetics	Shorter et al. 2012
Human Impacts on Populations	Harris et al. 2016
Immunology	Pyter et al. 2005; Martin et al. 2007
Natural Variation	Bedford and Hoekstra 2015
Parasitology	Munger and Karasov 1989; Schmidt et al. 1999; Schwanz 2006, 2008
Rodent-borne diseases	Childs et al. 1994; Botten et al. 2002; Bunikis et al. 2004; Madhav et al. 2007
Speciation	Greenbaum et al. 1978; Zimmerman et al. 1978; Conroy and Cook 1999; Fitzpatrick and Turelli 2006

CHAPTER TWO

INTRODUCTION

The genus *Peromyscus* is one of the largest and most speciose groups of small mammals, being represented in almost every habitat across North America. This diversity has led to *Peromyscus* becoming notable as a model system across a variety of disciplines, including evolutionary biology and genetics (King 1968; Carleton 1989; Kirkland and Lane 1989; Musser and Carleton 2005). Species within this genus are organized into multiple phylogenetically related species groups (i.e., the *eremicus* species group, *boylii* species group, etc.), although it has been argued that these phylogenetic relationships should be better understood if this classification scheme is to continue to be used (Bradley et al. 2007). Hybridization (both naturally occurring and laboratory induced) is known to occur between species within the same species group (McCarley 1954; Kirkland and Layne 1989), as well as between species across different species groups (Maddock and Dawson 1974; Leo and Millien 2017). Because hybridization might lead to inviable offspring (Harrison 1986), many factors have evolved that prohibit hybridization between species (i.e., reproductive barriers, timing of mating, genetic distance, etc.), and understanding how and when these factors are successful or not successful in preventing hybridization is essential for biologists if they are to understand the evolutionary history of a group. Therefore, studying hybridization within and among species groups of *Peromyscus* (i.e., species of varying genetic divergence) will aid in understanding the role of hybridization in evolution, the factors that lead to hybridization in natural populations, as well as the effects it has on natural breeding populations.

Peromyscus maniculatus (North American deermouse) and *P. leucopus* (white-footed deermouse) are classified into separate species groups (*maniculatus* group and *leucopus* group, respectively) estimated to have diverged approximately 2.5 million years ago (Ma; Platt et al. 2015). Both natural and laboratory-induced hybridization of these species with other species has been documented, although natural hybridization generally occurs at low rates (McCarley 1954; Maddock and Dawson 1974; Wolfe and Lindzey 1977). However, hybridization between these two species is not well known, and barriers preventing hybridization are thought to exist. Laboratory attempts at hybridization have either failed or survival of offspring was unknown (Dice 1933; Maddock and Dawson 1974; Haines 1983). Only one occurrence of natural hybridization between *P. maniculatus* and *P. leucopus* has been documented. This occurred at low rates within a wild population in Quebec, Canada, following a range expansion of *P. leucopus* northward into the range of *P. maniculatus* (Garcia-Elfring et al. 2017; Leo and Millien 2017). Although the range overlap between these species covers much of the eastern half of the United States, no other examples of natural hybridization have been reported.

During a small mammal survey of Four Canyon Preserve (FCP) in western Oklahoma (Ellis Co.), experienced surveyors noted that a select group of individuals displayed a distinct mixture of identifying characteristics of both *P. maniculatus* and *P. leucopus*, making morphological identification of these individuals difficult. For example, individuals would have the tail length and coloration of *P. maniculatus* but the foot length of *P. leucopus*. One potential explanation for this convergence of morphological characteristics is that hybridization between these species might be occurring within this population. I investigated this phenomenon with two objectives:

first, using nuclear and mitochondrial DNA to genetically identify FCP samples to species; and second, using nuclear and mitochondrial DNA and morphometrics to determine if hybrids were present.

METHODS

Sample Collection and DNA Extraction

Peromyscus leucopus and *P. maniculatus* specimens were collected from FCP during a mammal survey (Caire et al. 2010). Thirteen individuals were prepared as museum skin and skeleton specimens and the remaining 64 were preserved as alcoholic specimens. Additionally, tissues were collected from all samples (n = 77), and specimens were deposited into the University of Central Oklahoma Natural History Museum (UCONHM). Samples of pure *P. maniculatus* and *P. leucopus* were obtained through specimen and tissue loans from Oklahoma State University Collection of Vertebrates (OSUCOV; n = 60) and the Natural Science and Research Laboratory at the Museum of Texas Tech University (TTU NSRL; n = 21) to serve as control samples for comparison (Appendix 2.1). DNA was extracted from liver samples using DNeasy Blood and Tissue Extraction kits (Qiagen).

Genetic Analyses

Microsatellites

Genetic analyses followed the protocol of Leo and Millien (2017). A genetic profile was developed for each sample collected from FCP, as well as control specimens from OSUCOV and TTU NSRL, using 11 microsatellite primers, one of which, PML08, amplifies only in *P. maniculatus* and therefore serves as a species ID marker (Schmidt

1999; Chirhart et al. 2000; Table 2.1). Samples were amplified via the polymerase chain reaction (PCR; Table 2.2) using the GoTaq® Flexi DNA Polymerase kit (Promega). Reactions contained at least 100 ng of DNA, 1.5-2.5 μL of 25 mM $MgCl_2$, 2.5 μL of 5X Green GoTaq® Flexi Buffer (except PML11, which required 4 μL of buffer), 2.5 μL of 4 mM dNTPs (Bullseye dNTP Set; MidSci™), 1 μL of fluorescent labeled forward primer, 1 μL of unlabeled reverse primer, 0.2 μL 5U/μL GoTaq®, and water to bring the final reaction volume to 25 μL. Reaction conditions consisted of an initial 2 min denaturation at 94°C followed by 30 cycles at 94°C for 60 sec; annealing temperature, which ranged between 50-58°C across all loci (Table 2.2) for 90 sec; 72°C for 90 sec; and a final elongation step at 72°C for 10 min. Amplicons were diluted 1:9 with diH_2O and 0.5 μL of diluted sample was mixed with 9.25 μL Hi-Di™ Formamide (Applied Biosystems) and 0.25 μL of GeneScan™ 500 ROX™ size standard (Applied Biosystems). Samples were analyzed using an ABI3500 Genetic Analyzer. Primers were multiplexed in pairs based on dye color and amplicon size (Table 2.3). Genotypes were visualized, edited, and scored using GeneMapper Software 5 (ThermoFisher Scientific).

MICROCHECKER v2.2.1 (van Oosterhout et al. 2004) was used to determine the presence of null alleles, large-allele dropout, and stutter-induced typing errors for each microsatellite locus. FSTAT 2.9.3.2 (Goudet 1995, 2001) was used to check for linkage disequilibrium, deviations from Hardy-Weinberg Equilibrium (HWE), and to estimate genetic differentiation between groups. CERVUS 3.0.7 (Marshall et al. 1998; Kalinowski et al. 2007) was used to estimate values of observed and expected heterozygosity as well as polymorphic information content (PIC) values.

The number of genetic clusters represented in the data was determined using STRUCTURE 2.3.4 (Pritchard et al. 2000) with parameters set at: 100,000 burnin, 1,000,000 generations of sampling, K = 1-5 (K = number of putative clusters), five iterations per K, and ancestry model and allele frequencies left at default. A separate STRUCTURE analysis was run leaving out PML08 (species ID microsatellite) and each locus for which problematic allele calls existed to ensure inclusion of these loci would not affect results. Results from STRUCTURE were uploaded into Structure Harvester (Earl and vonHoldt 2012) to establish the best supported K value following the methods of Evanno et al. (2005).

The Cluster Matching and Permutation Program (CLUMPP) version 1.1.2 (Jakobsson and Rosenberg 2007) was used to average all STRUCTURE iterations for each value of K. The *distruct* program (Rosenberg 2004) was used to produce and edit a bar plot. Genetic profiles of samples collected on FCP were compared to those of parental species samples, as well as mitochondrial DNA results and PML08 amplification, to serve as a means of species identification, and therefore were used to address my first objective. Genetic profiles also addressed the second objective in that individuals were identified as admixture if STRUCTURE analyses resulted in an estimated membership within the most-likely cluster of less than 80%.

Mitochondrial DNA

Samples were identified to species using PCR restriction fragment length polymorphism (RFLP) analysis of the complete cytochrome *b* (*Cyt b*) gene, 1,140 bp. The *Cyt b* gene was amplified via PCR using LGL 765 forward (Bickham et al. 1995) and LGL 766 reverse (Bickham et al. 2004) primers. Reactions contained 2 μL of 25 mM

$MgCl_2$, 2.5 μL of 5X Green GoTaq® Flexi Buffer, 2.5 μL of 4 mM dNTPs, 1 μL of LGL765 forward primer, 1 μL of LGL766 reverse primer, 0.2 μL 5U/μL GoTaq®, and water to bring the final reaction volume to 25 μL. Reaction conditions consisted of an initial 2 min denaturation at 94°C; followed by 32 cycles of 94°C for 45 sec, annealing temperature of 52°C for 40 sec, and 72°C for 150 sec; and a final elongation step of 72°C for 10 min. The restriction enzyme *BamHI* (New England Biolabs) was used to cut the *Cyt b* amplicons at specific locations that differed between the two species. The restriction enzyme reaction was set up by mixing 1 μg of amplified DNA with 1 μL of *BamHI* restriction enzyme, 5 μL 1X NEBuffer, and water to bring the final reaction volume to 50 μL. This mixture was incubated at 37°C for one hour and run on a 2% agarose gel for 55 min at 120 V. Amplicons of *P. maniculatus* were cut to produce bands at approximately 1,000 bp and 140 bp, whereas amplicons of *P. leucopus* were cut to produce four bands between 100 and 400 bp in length. This data was used to address the first objective of species identification. The data also addressed objective two as it was used to identify admixed individuals if results of *Cyt b* or PML08 species identification did not match each other or the microsatellite genetic cluster generated from nuclear DNA analyses.

Morphological Analyses

Because *P. leucopus* and *P. maniculatus* share similar ecological niches, competition has led to evolutionary divergence and character displacement in the shape of the skull between species (Millien et al. 2017), and several morphological skull measurements, when combined, can be used to distinguish the species (Choate 1973). To classify individuals to species using morphometric data, I followed the protocol of Brant

and Lee (2006). A total of 60 skulls of known *P. leucopus* and *P. maniculatus* were obtained from OSUCOV (30 skulls for each species; 15 male and 15 female). These samples were collected from areas in which the range of *P. leucopus* and *P. maniculatus* overlap and were genetically confirmed using the protocols described above prior to morphometric analyses. Any individuals from this group that were genetically identified as an admixture individual were removed from the control group and included with admixture individuals from FCP. Skull morphometrics of known samples were compared to suspected hybrid (admixture) individuals from FCP. Only undamaged, adult skulls were used in the analyses. The skull was removed from FCP individuals preserved in alcohol that were genetically identified as an admixture. Because there is no way to discriminate species based on measurements of a single trait (Choate 1973; Choate et al. 1979), I measured 18 morphological traits of the skull and body (Table 2.4). A Discriminant Function Analysis (DFA) was conducted to identify unknown individuals (Brant and Lee 2006). If a skull was found to have intermediate characteristics, it was considered additional support that the individual was a hybrid.

RESULTS

Genetic Analyses

Microsatellites

A lower observed rate of heterozygosity (*HObs*) compared to expected (*HExp*) was detected for all loci (mean *HObs* = 0.715, mean *HExp* = 0.914), with a mean PIC value of 0.904 and mean number of alleles per locus of 24.36 (Table 2.5). All loci were found to be in HWE (adjusted *p* value = 0.00455) as well as in linkage equilibrium

(adjusted *p* value = 0.0009). All loci except PLGT15 and PLGT58 had a higher rate of homozygosity than expected, and therefore may have null alleles present. No large allele drop out was detected at any locus, but errors due to stuttering were possible in PLGT66. To ensure no user error in scoring or errors due to stutter, all samples were scored twice before additional analyses were run.

All runs, including those in which loci (e.g., PML08) were removed, showed the same results. Therefore, all further analyses and comparisons are based on the run containing all 11 loci. Results from STRUCTURE and Structure Harvester indicated two genetically distinct clusters (K = 2; Fig. 2.1) when all samples were included. These clusters, however, were determined to have little genetic differentiation, with an F_{ST} value of 0.040. Any individuals with less than 80% inclusion with one cluster were considered to be admixture. A total of 26 admixture individuals were detected using microsatellite analysis alone, 10 samples collected from FCP, 13 samples obtained from OSUCOV, and three samples obtained from TTU NSRL. Species identification using PML08 resulted in 30 FCP samples being identified as *P. maniculatus* and 47 samples as *P. leucopus.* Forty samples obtained from OSUCOV were identified as *P. maniculatus* and 20 as *P. leucopus*. Finally, 18 of the 21 samples obtained from TTU NSRL were identified as *P. maniculatus* and the remaining three as *P. leucopus*. A summary of all microsatellite results can be found in Appendix 2.1.

RFLP analysis

Of all 158 samples, 156 (98.7%) were successfully identified using distinct banding patterns resulting from the RFLP analysis (Fig. 2.2). Twenty-three samples from FCP were identified as *P. maniculatus*, and the remaining 54 samples were identified as

P. leucopus. Thirty-four samples obtained from OSUCOV were identified as *P. maniculatus* and the remaining 26 samples were identified as *P. leucopus*. Finally, 13 samples obtained from TTU NSRL were identified as *P. maniculatus* and six samples as *P. leucopus*. The remaining two samples obtained from TTU NRSL produced inconclusive banding patterns and could not be identified. A summary of all RFLP results can be found in Appendix 2.1.

Morphometrics

MANOVA analyses showed sexual dimorphism to be significant within this sample set ($p = 0.02351$), and therefore suggested that the two sexes should be analyzed separately. A within-sex MANOVA did not find significant differences between the species ($p = 0.5134$ for males and $p = 0.5773$ for females). Based on the available sample size and the lack of detectable differences between the two species, conducting further analyses was deemed unnecessary at this time.

DISCUSSION

Genetic Analyses

Having high levels of heterozygosity indicates a higher level of genetic diversity (i.e., population fitness; Reed and Frankham 2003). Analyses showed a high PIC value (0.904) indicating high genetic diversity within each locus. Observed heterozygosity ranged from 0.382 to 0.918 (mean *HObs* = 0.715) and was less than expected heterozygosity, which ranged from 0.842 to 0.959 (mean *HExp* = 0.914) for all loci used in this study. The lowest heterozygosity occurred in PML08 (*HObs* = 0.382), PML09 (*HObs* = 0.462), and PLGATA70 (*HObs* = 0.561). Despite having low heterozygosity,

values here were higher than detected in previous studies using these loci (Schmidt 1999; Chirhart et al. 2000). Additionally, the average number of alleles per locus was considerably higher across loci, except PLGT15, PLGT58, and PLGT66, which were comparable to previous studies, and PLGATA70 which was considerably lower than previous studies (Schmidt 1999; Chirhart et al. 2000). Results here support the indication that these microsatellite loci act as good markers for population studies of these species.

Species identification

A summary of results of all genetic analysis can be found in Appendix 2.1. Two methods of species identification were used in this study. First, an RFLP analysis produced banding patterns unique to each species as a result of different cut sites from the *BamHI* enzyme (Fig. 2.2), which successfully identified 156 of 158 individuals. RFLP analyses were inconclusive for two samples obtained from TTU. This method resulted in the identification of 23 samples from FCP as *P. maniculatus* and 54 as *P. leucopus*, 34 samples from OSU as *P. maniculatus* and 26 as *P. leucopus*, and 13 TTU samples as *P. maniculatus* and six as *P. leucopus*. Second, the microsatellite locus PML08 was used for species identification because it only amplifies in *P. maniculatus.* This method resulted in the identification of 30 FCP samples as *P. maniculatus* and 47 as *P. leucopus*, 40 OSU samples as *P. maniculatus* and 20 as *P. leucopus*, and 18 TTU samples as *P. maniculatus* and three as *P. leucopus*. It is important to consider a few cautionary notes. First, RFLP analysis using the enzyme *BamHI* was not 100% effective as some individuals produced inconsistent or unusual banding patterns. I recommend that alternative restriction endonucleases are assessed for their utility in PCR-RFLP analyses for future research. PML08 results should be approached with caution as well. This locus

only amplifies in *P. maniculatus*, and a lack of amplification could be the result of a failed PCR, due to null alleles or other mutations, rather than a sample being *P. leucopus*. It also is possible that non-specific binding could produce peaks falsely identified as real peaks, which would result in wrongly identifying a sample as *P. maniculatus*. Although, samples were amplified and analyzed for PML08 three times to avoid these errors, results should be interpreted with a note of caution. In the future, a more reliable species identification method should be considered. STRUCTURE analyses using all microsatellite loci detected two distinct clusters corresponding to the two species; 85 individuals were identified as *P. leucopus* and 47 as *P. maniculatus*.

For samples genetically determined to be *P. leucopus* or *P. maniculatus*, species identifications made through genetic analyses were compared to the museum identifications. Of the 70 samples genetically identified as either *P. leucopus* or *P. maniculatus* with no evidence of admixture, 38 (41%) matched the museum identification. An additional 23 individuals were identified as *Peromyscus spp.* by the museum, of which 14 could be identified to species using genetic methods. Although the genetic techniques used presented some need for cautionary interpretation, the high percentage of misidentifications demonstrates the need for more reliable means of identifying these species both in the lab as well as in the field.

Hybridization

The results of the species identification methods were compared and any samples with disagreeing results were considered to be putative admixture individuals. A total of 24 samples were considered admixture individuals using this method, 14 samples from FCP, eight from OSU, and two from TTU (Table 2.6). It is possible that the issues

discussed above could lead to a disagreement between these species ID methods and should thus be considered with caution.

STRUCTURE results indicated two clusters that were genetically distinct from one another (Fig. 2.1). Additionally, 26 admixture individuals, 10 from FCP, 13 from OSU, and three from TTU were detected. Results of the clustering analyses also were compared with results of both RFLP analysis and PML08 species identification, and any conflicting identifications were considered to be putative admixture individuals. This comparison resulted in an additional 15 samples being considered admixture individuals, 5 from each sample set (Table 2.6).

In summary, a sample was identified as a putative admixture individual through a disagreement between the species ID markers (*Cyt b* and PML08), a disagreement of either species ID marker with the STRUCTURE cluster grouping, or being identified as an admixture individual in the STRUCTURE analysis. Based on these genetic analyses, a total of 65 samples (41%) were considered to be putative admixture individuals across the entire data set (Table 2.6), including samples obtained to serve as parental species controls. RFLP analysis of *Cyt b* for species identification of admixture individuals suggests that bidirectional introgression is occurring if these admixture individuals are deemed to be true hybrids after further analyses. When available, skulls of admixture individuals were included in the morphometrics analysis discussed below.

Assuming hybridization is occurring, comparing identification results allows me to interpret crossbreeding patterns. *Cyt b*, being maternally inherited, indicates maternal parentage whereas microsatellites (i.e., PML08 ID and STRUCTURE clustering) are biparentally inherited and indicate contributions from both sexes. For example, *Cyt b*

agreeing with STRUCTURE clustering but disagreeing with PML08 identification suggests hybridization based on the species ID contradiction. In this case, the results of the clustering analysis suggest that a majority of the genetic material is from one species. This scenario was seen in samples UCONHM334 and UCONHM335 which were identified as *P. leucopus* based on *Cyt b* and STRUCTURE clustering, but *P. maniculatus* based on PML08 identification (Appendix 2.1). This would be the result of introgression back to the parental *P. leucopus* population.

Those samples with a *P. maniculatus Cyt b* and PML08 ID, but a *P. leucopus* STRUCTURE clustering analysis (e.g., UCONHM448 and UCONHM505; Appendix 2.1) are indicative of an F_3 or greater hybrid that has introgressed back to the *P. leucopus* population. This results in an individual with a majority of *P. leucopus* nuclear DNA, a *P. maniculatus* allele for PML08, and mtDNA having been continually passed down from a *P. maniculatus* relative. Samples with a *P. maniculatus Cyt b* ID, and a *P. leucopus* PML08 ID and STRUCUTRE clustering (e.g., UCONHM331 and UCONHM485; Appendix 2.1) also are indicative of introgression back with *P. leucopus* populations, having lost the *P. maniculatus* PML08 allele while retaining the *P. maniculatus* maternal lineage. Interestingly, no evidence of introgression to the *P. maniculatus* population was obtained in this study.

Interbreeding between hybrids is indicated by samples TK27127 and OSUCOV13947 (Appendix 2.1) which show individuals fully clustering with *P. maniculatus* while having a *P. leucopus* ID through the use of PML08. The only way for a majority of the nuclear DNA to be *P. maniculatus* while completely lacking a PML08 allele would be for hybrid individuals to have interbred, as introgression with the *P.*

maniculatus populations would be indicated by the presence of a *P. maniculatus* PML08 allele. Comparison of *Cyt b* assignments of these two samples (TK27127 being *P. leucopus* and OSUCOV13947 being *P. maniculatus*) suggests that maternal inheritance does not affect the ability of hybrids to interbreed with other hybrids.

The varying *Cyt b* assignments of these samples shows that maternal heritage does not affect fertility, and therefore it can be interpreted that the cross of both male *P. leucopus* with female *P. maniculatus* as well as female *P. leucopus* with male *P. maniculatus* can produce fertile offspring. These results suggest that if hybridization is occurring at this rate within this population, offspring produced are viable, fertile, and capable of introgression within the parental population of *P. leucopus* as well as interbreeding with other hybrid individuals.

One consideration that must be made when interpreting these results is the possibility of individuals marked as admixture through microsatellite analysis being obtained as a result of incomplete lineage sorting (ILS). In species that are more recently diverged, not enough evolutionary time has passed for all alleles to coalesce within groups. Therefore, when conducting analyses with shared ancestral alleles across populations, it will give the appearance that alleles are being shared between the genetically distinct groups through hybridization when, in reality, they were passed into both groups from a common ancestor. Further work needs to be conducted to determine the origin of such alleles before individuals can be considered true hybrids. Incomplete lineage sorting may also pose an issue when interpreting results of the microsatellite locus PML08 because amplification within a *P. leucopus* sample could be argued to be the amplification of a previously unknown allele that was inherited from a common

ancestor rather than being unique to *P. maniculatus*. Further study is needed to identify if ILS applies to this system, and I recommend future studies use a more conclusive species identification method, genomics, and / or sequencing techniques to avoid these issues in the future.

However, support for hybridization can be inferred from my results as well as the results of Leo and Millien (2017). Samples obtained from OSU were collected in areas where the ranges of the parental species overlap, and therefore ILS or hybridization are both possible explanations for the detection of admixtures within this sample set. For ILS to be a legitimate explanation for the detection of admixture individuals, one would expect to see admixture at similar rates in analyses across the entire range of both species. In the study of hybridization in Quebec, Canada using a similar set of microsatellites loci (Leo and Millien 2017), results did not support the explanation of ILS between these species as few admixture individuals were detected within their sample set, although it is possible they simply were not collected. This is unlikely, however, as they analyzed a sample set of $n = 153$, which is similar in size to my study ($n = 158$). Therefore, if ILS were occurring within these loci, it would likely be detected within those populations as well. They also detected individuals that were genetically assigned to approximately 50% of each genetic cluster (suggesting an F_1 hybrid) and approximately 75% to one genetic cluster (suggesting an F_2 hybrid). This supports crossbreeding of hybrids with individuals of the parental species. Some individuals determined to be admixture in my study suggest individuals are F_1 or F_2, which suggests these individuals are true hybrids that are reproductively viable and able to crossbreed with the parental species. For additional

support for hybridization and not ILS, Garcia-Elfring et al. (2017) also found hybridization between these two species using genomics techniques.

Additionally, my study offers support of hybridization through the control samples obtained from TTU NSRL. These samples were mostly collected from regions in which the current known ranges of the parental species are not thought to overlap. However, studies have suggested that the range of *P. leucopus* is changing in response to climate change, habitat alterations, etc. (Roy-Dufresne et al. 2013). Among the samples obtained from TTU, those that were identified as admixtures were within a reasonably close distance to areas of overlap, whereas those collected from further away grouped completely to the *P. maniculatus* cluster (Fig. 2.3). If ILS were occurring, one would expect to find shared alleles in samples where the ranges do not overlap. If evidence of range expansion is found, then that provides further support for the presence of admixture individuals representing hybridization events. Because these areas have not historically overlapped, new hybridization events might be occurring as a result of a lack of barriers having evolved within these populations to prevent interbreeding. If that is the case, then range expansions might result in the detection of more hybridization events in the future. These results suggest further study is needed to update the current ranges of the species as well as to determine if hybridization is occurring across the range of overlap or if it is isolated only to the edges of overlapping areas.

Morphometrics Analyses

When available (i.e., for FCP and OSU specimens), skull measurements for admixture individuals were compared to those genetically identified as a parental specimens to determine if morphological features could be used to identify parental

versus admixture samples. Skulls were not available for any TTU specimens or for FCP samples UCONHM331, UCONHM475, and UCONHM496. Additionally, OSUCOV specimens OSUCOV13917, OSUCOV13937, and OSUCOV13947 were determined to be juveniles and were omitted from morphometrics analyses. The unexpected detection of 27 admixture individuals and three juveniles within the OSUCOV sample set meant those skulls could not be used as species controls for morphometrics analyses. The OSUCOV individuals identified as admixture were included within the group of unknowns instead. When available, skulls of FCP samples identified as *P. leucopus* and *P. maniculatus*, with no evidence of admixture, were included as control samples (6 *P. leucopus* and 2 *P. maniculatus*). Results of the MANOVA showed sexual dimorphism to be an issue within this dataset, and therefore the sexes had to be analyzed separately. As a result of this sexual dimorphism, and the removal of juvenile and admixture individuals, the control group size for each species dropped from 30 (15 males and 15 females for each species) to 24 for *P. maniculatus* (9 males, 15 females) and 16 for *P. leucopus* (6 males, 10 females). The MANOVA results of the two datasets (males, females) showed these measurements were unable to distinguish between the two species, possibly a result of having too small of a sample set within each control group. Therefore, further data must be collected to assess whether morphological features can be used to identify admixture individuals.

CONCLUSIONS

Although there are questions that remain to be answered, my results suggest these species might be hybridizing within multiple natural populations across areas of overlap.

This pattern is shown by the detection of admixed individuals from FCP (39%), other areas of Oklahoma (i.e., samples obtained from OSUCOV, 43%), as well as in areas not previously known to be sympatric (i.e., TTU NSRL sample set, 48%). These results offer support to the phenomenon of natural hybridization between *P. leucopus* and *P. maniculatus* also found by Leo and Millien (2017).

Additionally, species identification of TTU NSRL samples that were captured in regions where these species are not known to overlap suggests new locality data or range expansions for these species, possibly in response to climate change and / or land use changes as seen in Quebec, Canada (Roy-Dufresne et al. 2013). Considering most range maps available for these two species are based on data published in Hall (1981), there is a need to update the current ranges of these species, to evaluate the potential for further expansion of these species, and to discern what expansions might mean for existing ecosystems. Additionally, further investigation is needed to determine if there are environmental or ecological factors contributing to the rate of hybridization, whether it is isolated to the edges of sympatric areas or if it exists across the range, and the effects it may have on these populations and the species as a whole.

Hybridization of these two species specifically is an important area of study because both species play significant roles in zoonotic disease transmissions including hantavirus, Lyme disease and other tick-borne diseases, etc. (Giardina et al. 2000). Hybridization between these two species could introduce new traits promoting ecological dominance as well as changes in the ability of successful offspring to serve as hosts and transmit diseases (Arnold 2004). Hybridization of host species, therefore, is an area of research interest as it poses a potential threat to humans through increased efficiency of

these zoonotic diseases. These species also are two of the most widespread species within the genus *Peromyscus,* and both hold the potential to greatly alter existing ecosystems and possibly outcompete local species that may be less able to adapt to introduction. Additionally, continuation of these types of studies to determine when and why these species hybridize can aid in understanding effects of climate change and habitat disturbances on natural ecosystems as a result of species range expansions influencing new hybridization events.

There are some cautionary notes associated with my results that should be taken into consideration for future studies. First, the possibility of ILS being mistaken for natural hybridization could be avoided in future studies through the use of genomics. Microsatellites used in this study, though good markers for studies of each species independently, may not be useful in studies of hybridization due to the possibility of ILS. However, my results and those of Leo and Millien (2017) and Garcia-Elfring (2017) do not suggest ILS as an appropriate explanation for the admixture individuals detected. Finally, my morphometric results were inconclusive as a result of a small control sample set, and no inferences could be made regarding the usefulness of these measurements in identifying hybrid individuals. Data for additional control skulls should be collected before any meaningful analyses can be done.

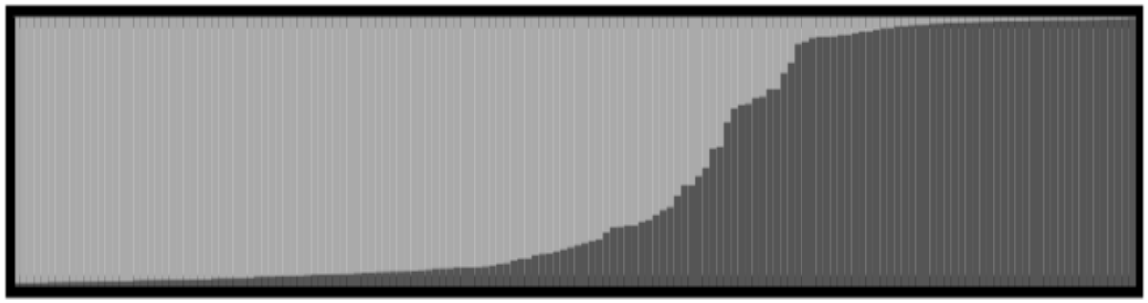

Figure 2.1.–Bar plot of clustering results from the STRUCTURE analysis including all samples used in this study. Samples included those collected from Four Canyon Preserve (obtained from the University of Central Oklahoma Natural History Museum; UCONHM), obtained from Oklahoma State University Collection of Vertebrates (OSUCOV), and obtained from Texas Tech University Natural Science and Research Laboratory (TTU NSRL; *n* = 158). Each bar represents an individual. Light grey corresponds to the *Peromyscus leucopus* cluster and dark grey corresponds to the *P. maniculatus* cluster. Individuals are ordered in decreasing proportion of assignment to the *P. leucopus* cluster.

Figure 2.2.–Example of gel electrophoresis results for PCR-RFLP of the mitochondrial cytochrome *b* gene digested using the restriction enzyme *BamHI*. Lane one contains an EZ Load™ Molecular Mass Standard (50μg/ml; Bio-Rad). Samples were identified as *Peromyscus leucopus* if there were four bands between 140 and 550 bp in length (lanes 2-5) and as *P. maniculatus* if it produced bands of 1,000 bp and 140 bp in length, although the second band produced is faint (lanes 6-9).

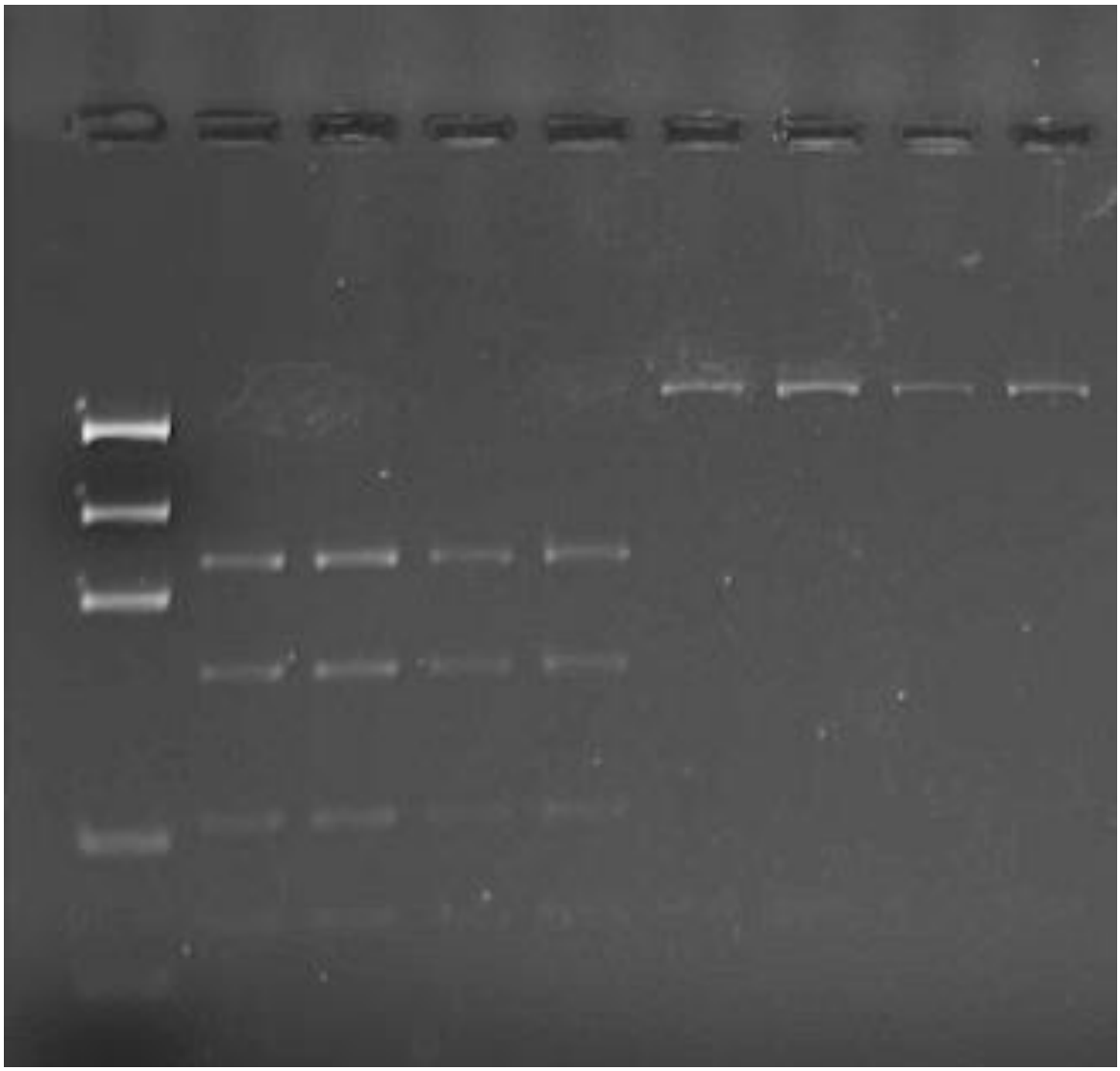

Figure 2.3.–Range map showing locations of the Texas Tech University (TTU) samples analyzed in this study. Admixture individuals were detected in allopatric populations throughout the range of *Peromyscus maniculatus*. No samples were identified as *P. leucopus*, and therefore *P. leucopus* is not represented.

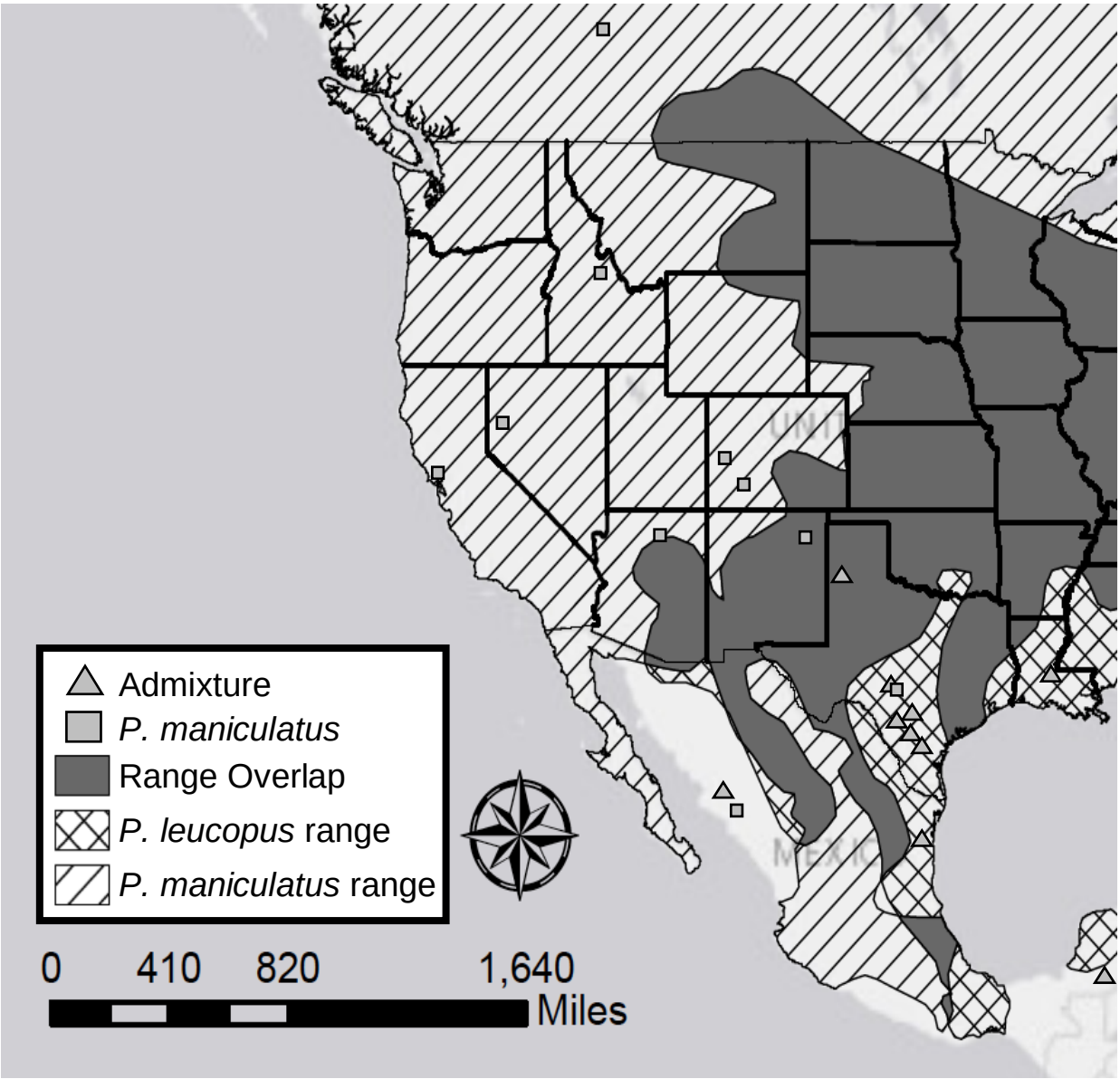

Table 2.1.–Molecular markers used in this study. Primer names, sequences (F = forward, R = reverse), size in base pairs (bp), and source are listed for each pair. All sequences listed are microsatellite primers with the exception of LGL765/LGL766 which is a mitochondrial cytochrome *b* primer set.

Locus	Primer Sequence	Product Size	Citation
PLGT15	F: GATCAAGTCTCACTATGTAG R: GACCTCCACAAATACACTGT	256	Schmidt 1999
PLGT58	F: GATCTTGTGAACACGCTTCT R: TTGATGGCTCTGGAGAGGCT	164	Schmidt 1999
PLGT66	F: CTCTGTCTGCCACACATGCT R: GTGCCATCACAGATGTGACA	147	Schmidt 1999
PLGATA70	F: CTTGGTATGCATCGCCATCT R: TAATCTCTGTAGCTTCATGT	224	Schmidt 1999
PML01	F: CATTCAAGACCTGGCTTTTT R: TGGGTTTCATCAGTGCTTCT	145–187	Chirhart et al. 2000
PML02	F: GTACCAGGCATGAACATAGT R: GAATAATTTTCCGCTGTGT	195–245	Chirhart et al. 2000
PML03	F: GCCATTAGTCTATGTGACAG R: GCGATGTACCCAGAAAT	221–261	Chirhart et al. 2000
PML06	F: CAGGGCTGTAGAGGGAGAAC R: ACTGGAGCAGAGGCATTTG	126–176	Chirhart et al. 2000
PML08	F: AATGGCTCAGTCCTCTTCC R: GGGTGCTATCAACCTTGTTC	211–251	Chirhart et al. 2000
PML09	F: GAATCCATACACCCATGC R: TTGCTTTTCGTCAAGTTTT	190–258	Chirhart et al. 2000
PML11	F: ACCCCCGAGTGCTGAGATT R: TTTGCTGCTTTCCCCAGAGA	218–254	Chirhart et al. 2000
LGL765	F: GAAAAACCAYCGTTGTWATTCAACT	1,140	Bickham et al. 1995
LGL766	R: GTTTAATTAGAATYTYAGCTTTGGG		Bickham et al. 2004

Table 2.2.–PCR conditions for molecular markers used in this study. The numbers in parentheses for PML08 represent the number of cycles for each temperature. For those loci with multiple temperatures listed, different temperatures were found to be optimal for different samples.

Locus	$MgCl_2$ (µL)	Annealing Temperature (ºC)
PLGT15	2	57
PLGT58	1.5	55
PLGT66	1.5	55
PLGATA70	2/2.5	57, 58, or 62
PML01	2	56 or 57
PML02	2	56 or 57
PML03	1.5	50 or 52
PML06	2	58 or 60
PML08	2.5	58(10)/56(10)/54(15)
PML09	1.5	52, 56, 57, or 58
PML11	2	56
LGL765/LGL766	2	56

Table 2.3.–Primer multiplex pairs used on the genetic analyzer for this study. Pairs were determined based on color first and then on size as necessary. PML08 was not included in a multiplex pair.

Primer	Size	Color	Multiplex Pair
PLGT15	~256	Green	PLGATA70
PLGT58	~164	Green	PML03
PLGT66	~147	Blue	PML11
PLGATA70	~224	Blue	PLGT15
PML01	145-187	Blue	PML02
PML02	195-245	Green	PML01
PML03	221-261	Blue	PLGT58
PML06	126-176	Blue	PML09
PML08	211-251	Blue	N/A
PML09	190-258	Green	PML06
PML11	218-254	Blue	PLGT66

Table 2.4.–Morphometric measurements used in this study. Skull measurements were taken using digital calipers and were recorded to the nearest hundredth of a millimeter.

Morphometrics Measurements
Length of Skull
Zygomatic Breadth
Breadth of Braincase
Interorbital Breadth
Breadth of Rostrum
Length of Rostrum
Height of Braincase
Breadth of Zygomatic Plate
Maxillary Toothrow Length
Height of First Molar
Width of Incisor
Width of First Molar
Length of Diastema
Length of Auditory Bullae
Total Length
Tail Length
Hind Foot Length
Ear Length

Table 2.5.–CERVUS results for all *Peromyscus spp.* analyzed in this study (*n* = 158) showing observed heterozygosity (*HObs*), expected heterozygosity (*HExp*), polymorphic information content (PIC), and number of alleles per locus.

Locus	*HObs*	*HExp*	PIC	Number of Alleles
PLGT15	0.867	0.905	0.895	21
PLGT58	0.918	0.929	0.921	22
PLGT66	0.829	0.918	0.909	20
PLGATA70	0.561	0.842	0.822	18
PML01	0.752	0.904	0.893	24
PML02	0.699	0.899	0.887	18
PML03	0.880	0.940	0.934	31
PML06	0.764	0.919	0.910	38
PML08	0.382	0.903	0.890	20
PML09	0.462	0.959	0.948	27
PML11	0.752	0.938	0.931	29
Average	0.715	0.914	0.904	24.36

Table 2.6.–Number of samples identified as admixture using each identification method for each sample set.

Method	Total	FCP	OSU COV	TTU NSRL
1) Spp. ID Disagreement	24	14	8	2
2) Spp. ID/STRUCTURE Disagreement	15	5	5	5
3) STRUCTURE ID	26	10	13	3
4) OVERALL TOTAL	65	29	26	10

Appendix 2.1.–Comprehensive list of genetic analysis results for all samples included in this project. 'L' stands for *Peromyscus leucopus* and 'M' stands for *P. maniculatus*. UCONHM = University of Central Oklahoma Natural History Museum (samples collected from Four Canyon Preserve), OSUCOV = Oklahoma State University Collection of Vertebrates, TK = Texas Tech University Natural Science Research Lab (Tissue Collection number). The Museum ID was provided by the granting museum and was based on field identification and verification of the Curator or Collections Manager (if possible). *Cyt b* represents the results of the PCR-RFLP analysis of the mitochondrial cytochrome *b* gene. Two individuals (marked as N/A) could not be identified using this method. PML08 is a microsatellite marker that only amplifies in *P. maniculatus*. STRUCTURE clustering results were based on analyses of all 11 microsatellite markers, including PML08. Admixture individuals were identified as individuals that had an estimated membership within the most-likely cluster of less than 80%.

Museum	Museum #	Date Collected	Country	State	County	Museum ID	*Cyt b*	PML08	STRUCTURE
OSUCOV	13910	28-Jul-05	USA	OK	Kiowa	L	L	L	L
OSUCOV	13912	22-May-06	USA	OK	Kiowa	L	L	L	L
UCONHM	328	4-Jun-05	USA	OK	Ellis	L	L	L	L
UCONHM	329	4-Jun-05	USA	OK	Ellis	L	L	L	L
UCONHM	336	4-Jun-05	USA	OK	Ellis	L	L	L	L
UCONHM	486	4-Dec-05	USA	OK	Ellis	L	L	L	L
UCONHM	487	4-Dec-05	USA	OK	Ellis	L	L	L	L
UCONHM	490	4-Dec-05	USA	OK	Ellis	L	L	L	L
UCONHM	491	4-Dec-05	USA	OK	Ellis	L	L	L	L
UCONHM	492	4-Dec-05	USA	OK	Ellis	L	L	L	L
UCONHM	495	4-Dec-05	USA	OK	Ellis	L	L	L	L
OSUCOV	13918	15-Apr-15	USA	OK	Kiowa	M	L	L	L
OSUCOV	13921	28-Jul-06	USA	OK	Kiowa	M	L	L	L
OSUCOV	13926	15-Mar-06	USA	OK	Roger Mills	M	L	L	L
OSUCOV	13927	15-Mar-06	USA	OK	Roger Mills	M	L	L	L

Museum	Museum #	Date Collected	Country	State	County	Museum ID	*Cyt b*	PML08	STRUCTURE
OSUCOV	13928	24-Jun-05	USA	OK	Roger Mills	M	L	L	L
OSUCOV	13929	25-Jun-05	USA	OK	Roger Mills	M	L	L	L
OSUCOV	13930	16-Mar-06	USA	OK	Roger Mills	M	L	L	L
OSUCOV	13931	16-Jul-07	USA	OK	Roger Mills	M	L	L	L
OSUCOV	13937	5-Aug-06	USA	OK	Texas	M	L	L	L
UCONHM	333	4-Jun-05	USA	OK	Ellis	M	L	L	L
UCONHM	346	4-Jun-05	USA	OK	Ellis	M	L	L	L
UCONHM	347	4-Jun-05	USA	OK	Ellis	M	L	L	L
UCONHM	348	4-Jun-05	USA	OK	Ellis	M	L	L	L
UCONHM	471	4-Dec-05	USA	OK	Ellis	M	L	L	L
UCONHM	472	4-Dec-05	USA	OK	Ellis	M	L	L	L
UCONHM	473	4-Dec-05	USA	OK	Ellis	M	L	L	L
UCONHM	474	4-Dec-05	USA	OK	Ellis	M	L	L	L
UCONHM	476	4-Dec-05	USA	OK	Ellis	M	L	L	L
UCONHM	477	4-Dec-05	USA	OK	Ellis	M	L	L	L
UCONHM	497	4-Dec-05	USA	OK	Ellis	M	L	L	L
UCONHM	498	4-Dec-05	USA	OK	Ellis	M	L	L	L
UCONHM	499	4-Dec-05	USA	OK	Ellis	M	L	L	L
UCONHM	845	12-Mar-06	USA	OK	Ellis	M	L	L	L
UCONHM	846	12-Mar-06	USA	OK	Ellis	M	L	L	L
UCONHM	851	12-Mar-06	USA	OK	Ellis	M	L	L	L
UCONHM	349	4-Jun-05	USA	OK	Ellis	*Peromyscus spp.*	L	L	L

Museum	Museum #	Date Collected	Country	State	County	Museum ID	*Cyt b*	PML08	STRUCTURE
UCONHM	350	4-Jun-05	USA	OK	Ellis	*Peromyscus spp.*	L	L	L
UCONHM	351	4-Jun-05	USA	OK	Ellis	*Peromyscus spp.*	L	L	L
UCONHM	352	4-Jun-05	USA	OK	Ellis	*Peromyscus spp.*	L	L	L
UCONHM	365	4-Jun-05	USA	OK	Ellis	*Peromyscus spp.*	L	L	L
UCONHM	368	4-Jun-05	USA	OK	Ellis	*Peromyscus spp.*	L	L	L
UCONHM	369	4-Jun-05	USA	OK	Ellis	*Peromyscus spp.*	L	L	L
UCONHM	388	9-Jul-05	USA	OK	Ellis	*Peromyscus spp.*	L	L	L
UCONHM	390	9-Jul-05	USA	OK	Ellis	*Peromyscus spp.*	L	L	L
UCONHM	391	9-Jul-05	USA	OK	Ellis	*Peromyscus spp.*	L	L	L
UCONHM	398	9-Jul-05	USA	OK	Ellis	*Peromyscus spp.*	L	L	L
UCONHM	431	10-Jul-05	USA	OK	Ellis	*Peromyscus spp.*	L	L	L
OSUCOV	13917	7-May-07	USA	OK	Kiowa	M	M	M	M
OSUCOV	13933	18-Jun-07	USA	OK	Texas	M	M	M	M
OSUCOV	13934	18-Jun-08	USA	OK	Texas	M	M	M	M
OSUCOV	13935	18-Jun-08	USA	OK	Texas	M	M	M	M
OSUCOV	13941	15-Mar-05	USA	OK	Texas	M	M	M	M
OSUCOV	13942	16-Mar-05	USA	OK	Texas	M	M	M	M
OSUCOV	13944	15-Mar-05	USA	OK	Texas	M	M	M	M
OSUCOV	13945	19-Jun-07	USA	OK	Texas	M	M	M	M
TK	22441	30-Jul-83	USA	NM	San Miguel de Allende	M	M	M	M
TK	24156	18-Aug-87	USA	ID	Custer	M	M	M	M
TK	27502	25-Aug-84	USA	WY	Sweetwater	M	M	M	M

Museum	Museum #	Date Collected	Country	State	County	Museum ID	*Cyt b*	PML08	STRUCTURE
TK	28074	13-Oct-84	USA	CO	Montrose	M	M	M	M
TK	52288	5-Jun-96	Canada	Alberta	Red Deer	M	M	M	M
TK	72331	2-Jul-97	Mexico	Durango	---	M	M	M	M
TK	119205	29-Oct-01	USA	AZ	Navajo	M	M	M	M
TK	123185	24-Sep-02	---	---	---	M	M	M	M
UCONHM	330	4-Jun-05	USA	OK	Ellis	M	M	M	M
UCONHM	371	4-Jun-05	USA	OK	Ellis	M	M	M	M
UCONHM	372	4-Jun-05	USA	OK	Ellis	M	M	M	M
UCONHM	382	9-Jul-05	USA	OK	Ellis	M	M	M	M
UCONHM	420	10-Jul-05	USA	OK	Ellis	M	M	M	M
UCONHM	422	10-Jul-05	USA	OK	Ellis	M	M	M	M
UCONHM	511	4-Dec-05	USA	OK	Ellis	M	M	M	M
UCONHM	512	4-Dec-05	USA	OK	Ellis	M	M	M	M
UCONHM	520	4-Dec-05	USA	OK	Ellis	M	M	M	M
OSUCOV	13888	5-May-07	USA	OK	Kiowa	L	M	M	M
OSUCOV	13889	5-May-07	USA	OK	Kiowa	L	M	M	M
OSUCOV	13890	5-May-07	USA	OK	Kiowa	L	M	M	M
OSUCOV	13891	5-Jun-05	USA	OK	Kiowa	L	M	M	M
OSUCOV	13892	5-Jun-05	USA	OK	Kiowa	L	M	M	M
OSUCOV	13893	29-Jun-05	USA	OK	Kiowa	L	M	M	M
OSUCOV	13897	15-Apr-08	USA	OK	Kiowa	L	M	M	M
OSUCOV	13898	15-Apr-09	USA	OK	Kiowa	L	M	M	M

Museum	Museum #	Date Collected	Country	State	County	Museum ID	*Cyt b*	PML08	STRUCTURE
OSUCOV	13899	15-Apr-10	USA	OK	Kiowa	L	M	M	M
OSUCOV	13901	15-Apr-12	USA	OK	Kiowa	L	M	M	M
OSUCOV	13902	15-Apr-13	USA	OK	Kiowa	L	M	M	M
OSUCOV	13906	14-Apr-08	USA	OK	Kiowa	L	M	M	M
OSUCOV	13907	14-Apr-09	USA	OK	Kiowa	L	M	M	M
OSUCOV	13915	23-May-06	USA	OK	Kiowa	L	M	M	M
OSUCOV	13946	6-May-07	USA	OK	Kiowa	L	M	M	M
TK	136583	20-May-07	USA	TX	Real	L	M	M	M
UCONHM	389	9-Jul-05	USA	OK	Ellis	*Peromyscus spp.*	M	M	M
UCONHM	396	9-Jul-05	USA	OK	Ellis	*Peromyscus spp.*	M	M	M
TK	25417	17-Jun-84	USA	CA	Napa	M	N/A	M	M
TK	47704	28-Dec-94	USA	NV	Washoe	M	N/A	M	M
OSUCOV	13895	14-Apr-07	USA	OK	Kiowa	L	L	L	Admixture
OSUCOV	13903	15-Apr-14	USA	OK	Kiowa	L	L	M	Admixture
OSUCOV	13905	14-Apr-15	USA	OK	Kiowa	L	L	L	Admixture
OSUCOV	13908	14-Apr-10	USA	OK	Kiowa	L	M	M	Admixture
OSUCOV	13911	28-Jul-06	USA	OK	Kiowa	L	M	M	Admixture
OSUCOV	13913	24-May-06	USA	OK	Kiowa	L	M	L	Admixture
OSUCOV	13916	23-May-07	USA	OK	Kiowa	L	M	M	Admixture
OSUCOV	13920	14-Apr-11	USA	OK	Kiowa	M	L	L	Admixture
OSUCOV	13923	22-May-06	USA	OK	Kiowa	M	L	L	Admixture
OSUCOV	13932	26-Jun-05	USA	OK	Roger Mills	M	L	L	Admixture

Museum	Museum #	Date Collected	Country	State	County	Museum ID	*Cyt b*	PML08	STRUCTURE
OSUCOV	13936	20-Jun-07	USA	OK	Texas	M	L	L	Admixture
OSUCOV	13939	16-Mar-05	USA	OK	Texas	M	L	L	Admixture
OSUCOV	13943	15-Mar-05	USA	OK	Texas	M	M	M	Admixture
TK	27150	28-Feb-85	Mexico	Quintana Roo	---	L	L	L	Admixture
TK	31607	14-Jun-88	USA	LA	Point Coupee Parish	L	L	M	Admixture
TK	98392	5-Jun-02	USA	TX	Dimmit	L	L	L	Admixture
UCONHM	358	4-Jun-05	USA	OK	Ellis	*Peromyscus spp.*	L	L	Admixture
UCONHM	367	4-Jun-05	USA	OK	Ellis	*Peromyscus spp.*	M	M	Admixture
UCONHM	475	4-Dec-05	USA	OK	Ellis	M	L	L	Admixture
UCONHM	481	4-Dec-05	USA	OK	Ellis	M	M	M	Admixture
UCONHM	482	4-Dec-05	USA	OK	Ellis	M	L	L	Admixture
UCONHM	483	4-Dec-05	USA	OK	Ellis	L	L	L	Admixture
UCONHM	504	4-Dec-05	USA	OK	Ellis	L	L	L	Admixture
UCONHM	513	4-Dec-05	USA	OK	Ellis	M	L	M	Admixture
UCONHM	522	4-Dec-05	USA	OK	Ellis	L	L	L	Admixture
UCONHM	529	4-Dec-05	USA	OK	Ellis	M	M	M	Admixture
OSUCOV	13894	29-Jun-05	USA	OK	Kiowa	L	L	M	L
OSUCOV	13896	15-Apr-07	USA	OK	Kiowa	L	L	M	L
OSUCOV	13904	15-Apr-15	USA	OK	Kiowa	L	L	M	L
OSUCOV	13924	22-May-06	USA	OK	Kiowa	M	L	M	L
OSUCOV	13925	22-May-06	USA	OK	Kiowa	M	L	M	L

Museum	Museum #	Date Collected	Country	State	County	Museum ID	*Cyt b*	PML08	STRUCTURE
OSUCOV	13938	5-Aug-06	USA	OK	Texas	M	L	M	L
OSUCOV	13940	16-Mar-05	USA	OK	Texas	M	L	M	L
TK	11744	20-Nov-81	USA	TX	Jim Wells	L	L	M	L
TK	49734	17-May-96	USA	TX	Kimble	L	L	M	L
UCONHM	332	4-Jun-05	USA	OK	Ellis	M	L	M	L
UCONHM	334	4-Jun-05	USA	OK	Ellis	L	L	M	L
UCONHM	335	4-Jun-05	USA	OK	Ellis	L	L	M	L
UCONHM	359	4-Jun-05	USA	OK	Ellis	*Peromyscus spp.*	L	M	L
UCONHM	392	9-Jul-05	USA	OK	Ellis	*Peromyscus spp.*	L	M	L
UCONHM	393	9-Jul-05	USA	OK	Ellis	*Peromyscus spp.*	L	M	L
UCONHM	397	9-Jul-05	USA	OK	Ellis	*Peromyscus spp.*	L	M	L
UCONHM	484	4-Dec-05	USA	OK	Ellis	L	L	M	L
UCONHM	531	4-Dec-05	USA	OK	Ellis	M	L	M	L
UCONHM	847	12-Mar-06	USA	OK	Ellis	M	L	M	L
UCONHM	331	4-Jun-05	USA	OK	Ellis	L	M	L	L
UCONHM	430	10-Jul-05	USA	OK	Ellis	*Peromyscus spp.*	M	L	L
UCONHM	485	4-Dec-05	USA	OK	Ellis	L	M	L	L
UCONHM	496	4-Dec-05	USA	OK	Ellis	M	M	L	L
OSUCOV	13900	15-Apr-11	USA	OK	Kiowa	L	M	M	L
OSUCOV	13909	14-Apr-11	USA	OK	Kiowa	L	M	M	L
OSUCOV	13914	16-Apr-07	USA	OK	Kiowa	L	M	M	L
OSUCOV	13919	15-Apr-15	USA	OK	Kiowa	M	M	M	L

Museum	Museum #	Date Collected	Country	State	County	Museum ID	*Cyt b*	PML08	STRUCTURE
OSUCOV	13922	16-Apr-07	USA	OK	Kiowa	M	M	M	L
TK	24121	26-Mar-88	USA	TX	Castro	M	M	M	L
TK	72593	12-Aug-97	Mexico	Durango	---	M	M	M	L
TK	90072	14-Mar-00	USA	TX	McMullen	L	M	M	L
TK	98192	7-Jan-02	USA	TX	La Salle	L	M	M	L
UCONHM	366	4-Jun-05	USA	OK	Ellis	*Peromyscus spp.*	M	M	L
UCONHM	429	10-Jul-05	USA	OK	Ellis	*Peromyscus spp.*	M	M	L
UCONHM	488	4-Dec-05	USA	OK	Ellis	L	M	M	L
UCONHM	505	4-Dec-05	USA	OK	Ellis	L	M	M	L
UCONHM	514	4-Dec-05	USA	OK	Ellis	M	M	M	L
TK	27127	8-Aug-84	Mexico	Tamaulipas	---	L	L	L	M
OSUCOV	13947	26-Jun-05	USA	OK	Roger Mills	M	M	L	M

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