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Phylogeography of three heteromyid taxa: Insight on the evolution of a North American arid grassland rodent guild

Sean A. Neiswenter
University of Nevada, Las Vegas

PHYLOGEOGRAPHY OF THREE HETEROMYID TAXA: INSIGHTS ON THE EVOLUTION OF A NORTH AMERICAN ARID GRASSLAND RODENT GUILD

by

Sean Adam Neiswenter

Bachelor of Science
Angelo State University
2002

Master of Science
Angelo State University
2004

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School of Life Sciences

Brett Riddle, Committee Chair

Dave J. Hafner, Committee Member

John Klicka, Committee Member

Dan Thompson, Committee Member

Chad L. Cross, Graduate Faculty Representative

Ronald Smith, Ph. D., Vice President for Research and Graduate Studies
and Dean of the Graduate College

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ABSTRACT

Comparative Phylogeography of Three Heteromyid Taxa: Insights on the Biogeography of North American Arid Grasslands

by

Sean Adam Neiswenter

Dr. Brett R. Riddle, Examination Committee Chair
Professor of Biology
University of Nevada, Las Vegas

Revealing how communities are shaped by abiotic and biotic factors plays a central role in biogeographic and comparative phylogeographic studies. The biogeography of North American arid grasslands is explored using nuclear and mitochondrial DNA from three groups of heteromyid rodents that are broadly sympatric in aridlands across western North America. Phylogenetic and molecular clock analyses are used to estimate the timing of divergences within each group. A general pattern of late Miocene divergence and expansion of lineages in each of the groups that is coincident with the rapid expansion of arid grasslands at the time. The initial divergence is followed by temporally and spatially concordant geographic diversification in recognized biogeographic and physiographic regions that corresponds to major climatic and tectonic events during the Pliocene and Pleistocene. The similarities and differences in the response of each taxon to proposed phylogeographic barriers are discussed.

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CHAPTER 1

INTRODUCTION

Phylogeography is a relatively new field of biology that focuses on documenting patterns of genetic diversity across landscapes and attempts to identify processes that have lead to the current distribution of gene lineages within species or closely related species groups. The timeframe of phylogeographic studies is generally the late Neogene. Phylogeographic studies are increasingly documenting cryptic diversity within species and species groups. Much of the diversity is often attributed to climate change and topography isolating populations. These factors interact through time to isolate populations and ultimately drive speciation and the evolution of new genetic lineages. By comparing multiple co-distributed species it is possible to identify congruencies in the patterns of the geographic distribution of gene lineages. Only by comparing multiple co-distributed taxa, termed comparative phylogeography, can common mechanisms driving biodiversity be sorted from the unique evolutionary histories of single species.

The following three chapters focus on two genera of North American rodents constituting at least 7 species that are sympatric over much of their range. They occur principally in arid grassland and shrubland communities which collectively span the Great Plains in Canada and the United States, east to the Great Basin, and south to the trans-Mexican volcanic belt in central Mexico. The second chapter was published previously and explores the relationships of mitochondrial and nuclear gene lineages in the *Perognathus flavus* species group, a complex of species and subspecies of silky pocket mice. It hypothesizes a late Miocene divergence within the group, followed by Pliocene geographic diversification associated with major biogeographic and climatic

regions in North America, and finally postulates Pleistocene population structuring in refugia within those biogeographic regions. The third chapter focuses on an evolutionarily distinct group within the same genus as the second chapter, the *Perognathus fasciatus* species group. This species group is currently composed of 3 recognized species. The study tests a previously hypothesized Pleistocene divergence between two species in the group and shows that the timing of major divergences are complimentary to those reported in the second chapter. The fourth chapter introduces a new genus, *Dipodomys*, which co-occurs across the ranges of the two *Perognathus* species groups in the preceding chapters. Using molecular dating with independent fossil calibrations it is shown that the mitochondrial divergences within all three groups have similarities in the timing of diversification of phylogroups. There are also independent and concerted responses to biogeographic barriers among the groups. The area relationships remain largely unresolved. In total the following three chapters address the late Neogene evolution of 3 groups of arid grassland rodents and provide a sequence of temporal and spatial historical events that can be tested using a comparative phylogeographic framework.

CHAPTER 2

DIVERSIFICATION OF SILKY POCKET MICE IN THE *PEROGNATHUS FLAVUS* SPECIES GROUP: DIVERSIFICATION IN EMERGING ARID GRASSLANDS IN WESTERN NORTH AMERICA

S. A. Neiswenter and B. R. Riddle

Abstract

We investigated the evolutionary history of a group of silky pocket mice (Heteromyidae: Perognathinae: *Perognathus flavus* species group) composed of the species *P. flavus* and *P. merriami* to determine patterns and postulate causes of geographical diversification across arid grasslands and intermontane basins in western North America. The region represents a topographically complex landscape with a Neogene history of dramatic geological and climatic transformations. Phylogenetic and dating analyses of mitochondrial DNA support an initial split among 4 major lineages during the late Miocene, and this hypothesis receives further support from analysis of a portion of the nuclear *IRBP* gene. Two of these lineages have a restricted geographic distribution in the Chihuahuan Desert, and 2 have distributions ranging across large portions of the Chihuahuan Desert, Colorado Plateau, Great Plains, and Tamaulipan Plain. Within the 2 widespread lineages further geographical diversification likely was concentrated in the Pliocene, which coincided with the origin of several hypothesized geographic barriers. These results are consistent with models of allopatric divergence driven by pre-Pleistocene geological and climatic events, particularly the late Miocene expansion of interior grasslands and Miocene-Pliocene evolution of Basin and Range geomorphology.

Therefore, the biogeographic structure displayed in the *flavus* species group may be predictive for a range of sympatric taxa.

S. A. Neiswenter was first author, contributed significant intellectual input, generated the DNA sequences, analyzed the data, prepared the manuscript for publication, and collected some of the samples in this collaborative work.

Introduction

Ecology, landscape attributes, and climate interact across space and time to shape taxonomic and genetic diversity within and among mammal species (Badgley and Fox 2000; Coblentz and Ritters 2004; Kohn and Fremd 2008; Simpson 1964). Evolutionary and biogeographic patterns resulting from these interactions are likely to be especially pronounced in the North American cordillera, the vast group of mountain ranges interleaved with plateaus and canyons extending from Alaska to Guatemala (Pidwirny 2006). Because of extreme topographic and climatic complexity resulting largely from a long Mesozoic and Cenozoic history of geological activity (English and Johnston 2004), the North American cordillera encompasses one of the most heterogeneous assemblages of habitats and ecoregions on Earth (Commission for Environmental Cooperation 1997). As such, the western regions of North America have long been the subject of extensive ecological and biogeographic analysis, most recently focusing on topics as diverse as latitudinal, elevational, environmental, and regional species diversity gradients (Badgley and Fox 2000; Coblentz and Ritters 2004; Rickart 2001), phylogenetic and phylogeographic architectures (Carstens et al. 2005; Riddle et al., 2000b; Spellman and

Klicka 2006; Spellman et al. 2007), and macroecological patterns and processes (Brown 1995, Davis 2005).

The last few decades have seen an increase in the application of genetic techniques to address biogeographic questions (Riddle et al. 2008), including the explosive rise in popularity of phylogeographic approaches (Avise 2009) to examine geographic histories and processes and elucidate morphologically cryptic evolutionary lineages within and across closely related species (Riddle and Hafner 2004, 2006). A central theme of molecular biogeography and phylogeography has been to examine the roles of landscape structure and geographic isolation in the evolutionary histories of populations and taxa. For example, in western North America investigators have used molecular approaches to explore the influence of alternating cycles of population fragmentation and coalescence in a wide range of taxa, including lepidopterans (DeChaine and Martin 2006; Knowles and Carstens 2007), birds (Spellman and Klicka 2006; Spellman et al. 2007), and mammals (Floyd et al. 2005) across forested, montane "sky islands" during the climatic oscillations of the cooler and wetter Pleistocene glacial periods and the warmer and drier interglacial periods.

In contrast to the sky islands, the intervening expansive deserts and grasslands contain many species that are widely distributed geographically within the Holocene interglacial climatic regime of the most recent 10,000 years. Major genetic breaks in desert and grassland taxa often are associated with the mountains, plateaus, and rivers that create temporally stable and long-term barriers to dispersal. These physical features have been associated causally with geographic genetic architecture in a variety of taxa inhabiting primarily desert, semidesert, or shrub-steppe ecoregions, including reptiles

(Castoe et al. 2007; McGuire et al. 2007; Zamudio et al. 1997), rodents (Lee et al. 1996; Riddle 1995; Riddle et al. 2000a, 2000b, 2000c), amphibians (Jaeger et al. 2007), and spiders (Crews and Hedin 2006).

The rodent family Heteromyidae comprises an almost entirely North American radiation (Hafner et al. 2007), and although heteromyid rodents have served as model organisms for studies ranging from physiological ecology to macroevolution (Genoways and Brown 1993), robust molecular-based phylogenetic hypotheses that span the entire family or particular clades have become available only recently. For example, Alexander and Riddle (2005) and Hafner et al. (2007) clarified relationships and the timing of major diversification events across subfamilies, genera, and species groups of heteromyid rodents. Beginning in the middle Miocene about 27–23 million years ago (mya) a rapid radiation within the Heteromyidae produced distinct bipedal (Dipodomyinae) and quadrupedal (Perognathinae + Heteromyinae) body forms (Hafner et al. 2007). Shortly thereafter an ecological split within the quadrupedal forms occurred between the arid-adapted Perognathinae and tropical Heteromyinae. The Perognathinae diversified further approximately 15 mya into a clade of larger, coarse-haired species (*Chaetodipus*) and a clade of smaller, silky-haired species (*Perognathus*—Hafner et al. 2007). Both Alexander and Riddle (2005) and Hafner et al. (2007) recovered *Perognathus* as a monophyletic genus composed of 4 previously postulated (Williams 1978) species groups – *flavus*, *flavescens*, *longimembris*, and *parvus*.

Divergence in *Perognathus* that produced the modern species groups might have begun as early as the late Miocene (Hafner et al. 2007). Osgood (1900) placed extant species of *Perognathus* into 1 of 3 species groups, but 1 of his groups later was separated

into the *fasciatus* and *flavus* groups by Williams (1978). Williams (1978) further proposed that ancestral *Perognathus* split initially into northern (*parvus* and *fasciatus*) and southern (*flavus* and *longimembris*) clades. Both Alexander and Riddle (2005) and Hafner et al. (2007) supported Williams' hypothesis uniting the *flavus* and *longimembris* species groups as sister clades, but neither of these studies was able to resolve a sister-clade relationship between the *flavescens* and *parvus* species groups with robust statistical support.

The foci of our study are 2 nominal species of silky pocket mice that comprise the *Perognathus flavus* species group, *P. flavus* and *P. merriami* (Fig. 2.1). *Perognathus flavus* (Baird 1855) is a small rodent (body mass 6–10 g) associated with sandy to gravelly soils in a wide range of semiarid and desert grassland habitats, sometimes in association with xeric shrub and woodland species. Although ambient temperatures in these habitats can range seasonally between highs of 40°C and lows of -15°C, *P. flavus* can enter short bouts of torpor at lower temperature extremes to conserve energy (Best and Skupski 1994a). *P. merriami* (Allen 1892) occupies a similar range of habitats (Best and Skupski 1994b), but as understood prior to this study, the distribution of *P. merriami* extended to the east of, but not as far north or south as, that of *P. flavus* (Fig. 2.1). The *flavus* species group is distributed throughout several ecoregions in North America, including the Colorado Plateau shrublands, western short grasslands, central and southern mixed grasslands, Chihuahuan desert, meseta central, central Mexican matorral, and Tamaulipan Mezquital (Fig. 2.1).

The *flavus* species group has been the subject of numerous taxonomic revisions over the last century, primarily revolving around the systematic status of *P. merriami*

(Allen 1892; Lee and Engstrom 1991; Wilson 1973). Recent evidence suggests that external morphology alone is not sufficient to distinguish *P. merriami* from *P. flavus*, but skeletal morphology can be used to assign specimens to nominal species (Brant and Lee 2006; but see Wilson 1973). The 2 species historically have been considered largely allopatric in distribution, and in the few cases where they occur sympatrically, allozyme data indicate that they act as biological species (Lee and Engstrom 1991). However, the 2 most recent of these studies have been restricted geographically to western Texas and eastern New Mexico, and no study to date has addressed the molecular evolution of the *flavus* species group within a modern phylogeographic framework using samples drawn from across its expansive geographic range (Fig. 2.1).

The biogeographic regions over which the *flavus* species group is distributed have experienced extensive geological and climatic variation over the last several million years (Kohn and Fremd 2008). Given the generally deep divergence between species groups within the Heteromyidae (Alexander and Riddle 2005; Hafner et al. 2007), it seems plausible that a certain degree of cryptic divergence, not necessarily coincident with current (and still controversial) taxonomic entities, could be embedded within the *flavus* species group, similar to what has been discovered in other heteromyid rodents using a phylogeographic sampling design and molecular genetic data (e.g., the *C. penicillatus* species group—Lee et al. 1996; Jezkova et al. 2009; the *C. baileyi* species group—Riddle et al. 2000b; the *P. longimembris* species group—McKnight 2005; and *Microdipodops pallidus*—Hafner et al. 2006).

Herein, we address regional genetic structuring of the *flavus* species group within the tapestry of Late Neogene geologic events and climatic shifts. In doing so we provide

a more generalized biogeographic insight into the Late Neogene origins and expansion of the arid grasslands biome in western North America when interpreted in concert with paleontological records and geological evidence (Axelrod 1985; Cerling et al. 1997; Kohn and Fremd 2008; Retallack 1997, 2001; Stromberg 2002, 2005). Given the broad temporal span over which we examine components of biogeographic structure within and among species, we reconstruct phylogenetic histories using sequences from 2 mitochondrial gene regions with different evolutionary rates in mammals – the protein-coding gene cytochrome oxidase III (*COIII*) and a noncoding portion of the control region (*CR*). We then use a fossil-calibrated molecular clock to estimate times of diversification of the major clades within this group. We also use an exon from the nuclear-encoded gene interphotoreceptor retinoid-binding protein (*IRBP*), which, because it is more slowly evolving than the mitochondrial sequences, we would expect to be informative at deeper levels of divergence and therefore of some value in assessing the robustness of the phylogenetic and molecular clock results from the mitochondrial data. Specifically, we examine detailed mtDNA phylogenetic and geographic structure within the *flavus* species group to determine whether the estimated divergence times of major mtDNA lineages are coincident with a Pleistocene or pre-Pleistocene time frame and whether geographically definable monophyletic clades are consistent with divergence associated causally with certain features of landscape and biome evolution in western North America.

Materials and Methods

Sample collection and sequencing

Specimens were gathered from throughout the range of the *flavus* species group (Appendix 1). Those that were field-collected for this study were handled in accordance with the guidelines established by the American Society of Mammalogists (Gannon et al. 2007). Voucher skin and skeleton specimens were deposited in the New Mexico Museum of Natural History (NMMNH) collections. Other specimens included preserved tissues obtained from museum collections or ear clips obtained from private collections (Appendix 1).

We sequenced 644 bp of *COIII* and 440 bp of *CR* from up to 10 individuals per locality for a total of 132 specimens (Appendix 1; Fig. 2.1). To assess the robustness of our mitochondria-based trees, particularly at deeper times where mtDNA can become uninformative due to saturation, we arbitrarily selected representatives from each of the major recovered mtDNA lineages and sequenced 1,133 bp of an exon from the nuclear *IRBP* gene (Jansa and Voss 2000).

DNA was extracted from preserved tissues using DNeasy kits (Qiagen Inc., Germantown, Maryland). PCR conditions for *COIII* and *CR* were 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min, for 30 cycles; and for *IRBP* were 95°C for 30 s, 55°C for 30 s, and 72°C for 45 s, for 38 cycles. Genes were amplified and sequenced using published PCR primers: *COIII*, H8618 and L9232 (Riddle 1995); *CR*, 16007 and 16498 (Kocher et al. 1989; Meyer et al. 1990); IRBP, H651 and 1297D (Jansa and Voss 2000). Internal primers 761E and 878F (Jansa and Voss 2000) also were used for sequencing IRBP. Sequences were run on an ABI 3130 automated sequencer (Applied Biosystems,

Foster City, California), checked for ambiguous base calls in Sequencher 4.8 (Gene Codes Corporation, Ann Arbor, Michigan), and aligned in MEGA 4 (Tamura et al. 2007) with final corrections by eye for alignment integrity. Protein-coding genes were converted to amino acids to ensure no stop codons were present, an indication of a possible nuclear copy of the mitochondrial gene. All sequences were deposited in GenBank under accession numbers GQ469647-GQ469777, GQ470232-GQ470359, and GQ480797-GQ480822.

Phylogenetic analyses

Phylogenetic trees were constructed using a combination of Bayesian and maximum-likelihood (ML) analyses performed separately on each mtDNA sequence, and on a *COIII + CR* combined data set. The *IRBP* sequence was analyzed separately, first, because we believed that much of the genetic signal within the highly conserved *IRBP* would be swamped by the highly variable mtDNA (see Results) should they be combined into a "total evidence" data set, and second, because by combining the 2 we could lose information about the independent history of 1 of the genomes. *Perognathus longimembris*, a member of the sister clade to the *flavus* species group, was used as the outgroup for all phylogenetic analyses. Before combining data sets, we performed a partition homogeneity test in PAUP* (Swofford 2002) to test for significant differences between the mtDNA partitions.

Bayesian analyses were implemented in MrBayes 3.1 (Huelsenbeck and Ronquist 2001) using the model of evolution selected from ModelTest 3.04 (Posada and Crandall 1998). The GTR+G model was chosen for the combined mtDNA data set, and the HKY+G model was chosen for the *IRBP* data set. Each Bayesian analysis was run

multiple times to confirm convergence. Convergence was assumed when no apparent pattern was detected in the log probability plots, the average standard deviation of split frequencies dropped to <0.01, and convergence of harmonic means resulted from independent runs. We performed several runs for each data set with different initial chain temperatures and branch length priors to confirm good mixing and convergence. The final run for the combined mtDNA data set was conducted with temperature = 0.05 and branch length = 50, whereas the *IRBP* data set was run with default values (temperature = 0.2 and branch length = 10). For each analysis we performed 2 independent runs with 4 chains each (1 hot and 3 cold). We ran these analyses for 4,000,000 generations and summarized the last 10,000 trees of each run (20,000 trees total) using a 50% majority rule consensus tree employing the posterior probabilities for clade support. The parameters for the final samples were summarized to ensure they conformed to our assumption of convergence defined above.

Maximum-likelihood (ML) analyses were performed using the program Treefinder (Jobb 2008) and the same models of evolution used in the Bayesian analyses. Node support for the ML analysis was assessed using 100 bootstrap replicates for each of the data sets described above. With the exception of the model of evolution, default values were used in all ML analyses.

Molecular clock estimates

In the molecular clock analyses we included sequences for several heteromyid genera and species outside of the *flavus* species group, including *Dipodomys nelsoni, C. formosus, P. longimembris, P. amplus, P. parvus, P. fasciatus,* and *P. flavescens* (Appendix 4). *COIII* gene sequences for these taxa were downloaded from Genbank, and

IRBP was sequenced as described above. This sampling strategy allowed use of published fossil data for calibration of a node outside of the *flavus* species group (Hafner et al. 2007). We used the same 26 individuals (18 ingroup, 8 outgroup; Appendix 1) for independent *IRBP* and *COIII* molecular clock estimates of the diversification of major lineages in the *flavus* species group. The *CR* gene was not used in the molecular clock estimates because we believe that the high rate of evolution in this sequence would likely lead to greater phylogenetic noise at deeper subfamilial nodes.

Errors associated with the incorrect placement of a fossil in a phylogeny or incorrect estimate of divergence time can have dramatic affects on the outcome of molecular dating (Benton and Ayala 2003). The paucity of fossils diagnosed to an appropriate level of taxonomic resolution, and the ambiguities that surround morphological identification of Perognathinae in the fossil record (Wahlert 1993) preclude use of fossils specific to the *flavus* species group. Therefore, fossil calibration was based on the oldest known fossil that is a taxonomically reliable representative of the subfamily *Perognathinae*, estimated at 20-22 mya (Hafner et al. 2007), from the John Day Formation in Oregon (James 1963). We conservatively placed this fossil basal to the Perognathinae clade that includes the genera *Chaetodipus* + *Perognathus*, as was done by Hafner et al. (2007) because the fossil record does not differentiate between these 2 genera (Wahlert 1993).

Prior to estimating divergence times, we tested our data for clock-like evolution using a likelihood ratio test. ML scores were compared in PAUP* 4.0*b*10 (Swofford 2002) for a tree generated under a molecular clock constraint and one that was unconstrained. A significant difference was found between the constrained and

unconstrained trees for both the *COIII* and *IRBP* data, so methods that relax the assumption of a strict clock were used for our data. We used the uncorrelated lognormal relaxed molecular clock analysis implemented in BEAST 1.4.6 (Drummond and Rambaut 2007). The HKY model for the *COIII* and the HKY+G model for *IRBP* were selected from ModelTest. Because of the large genetic divergence within the *flavus* species group (see Results) and the family-level diversity used in calibrating the molecular clock estimates, we assumed a Yule process for the tree prior. The time to most recent common ancestor (tMRCA) for Perognathinae was calibrated as a normal distribution with a mean ($\pm$ *SD*) of 21 $\pm$ 0.5 mya. This is equivalent to a 95% confidence interval from approximately 20–22 mya, the estimated time of the oldest *Perognathus* fossil (Hafner et al. 2007). Chain lengths were 10,000,000 generations long with sampling every 1,000 generations, and results were summarized after a 10% burn-in.

Results

Phylogenetic analyses

Of 132 individuals sequenced for the *COIII* and *CR* genes, based on resulting phylogenetic trees, 19 were selected to represent major mtDNA clades for the *IRBP* analysis. The *COIII* and *CR* data sets, run separately, resulted in similar topologies and support values with respect to major clades; therefore we report only the combined mtDNA analyses. The nuclear and mitochondrial data differed considerably in their variability, as was expected. Mitochondrial diversity was high with respect to the number of variable sites (367), parsimony informative sites (307), and genetic distance between major clades (5.6% to 19.6% divergence; Table 2.1). Conversely, the *IRBP* data

set displayed low variability (50 variable sites of which 12 were parsimony informative). No insertions/deletions (indels) or stop codons were present in either of the protein-coding data sets, but multiple indels of varying length were noted in the *CR* data set. Sites with indels and missing data were omitted in all phylogenetic analyses.

Four deep clades were recovered in the mtDNA tree (Fig. 2.2). For clarity, and not necessarily in accord with current species-level taxonomy, we refer to each major clade using the following naming system: 1) *merriami* clade, a deep lineage that includes individuals that historically have been considered *P. merriami*, although this clade also includes individuals that currently are not considered part of this species; 2) *flavus* clade, a 2nd deep clade that contains most of the individuals currently recognized as *P. flavus*; 3) Meseta Central Matorral clade, a newly recognized lineage with known distribution restricted to the Meseta Central Matorral ecoregion; and 4) the Southern Chihuahuan Desert clade, also a newly recognized lineage currently known only from the southern Chihuahuan Desert. The Southern Chihuahuan Desert clade forms a trichotomy with the *flavus* and *merriami* clades (Fig. 2.2).

Two of the major clades contain multiple, well-supported phylogroups. A basal near-simultaneous diversification within the *merriami* clade (Fig. 2.3) resulted in 3 extant clades now distributed across 3 distinct geographic areas: southern and central Great Plains; Tamaulipan Mezquital ecoregions; and northern Chihuahuan Desert. Within the *flavus* clade (Fig. 2.4), basal and near-simultaneous divergence events distinguished separate phylogroups from the central Great Plains, the Tehuacan Valley, the southern Chihuahuan Desert south of the Rio Conchos, and the common ancestor to the northern Chihuahuan Desert-Colorado Plateau phylogroups.

Several relationships recovered in the mtDNA analysis (Fig. 2.2) were supported by the *IRBP* analysis (Fig. 2.5), including separation of the *flavus*, *merriami*, Meseta Central Matorral, and Southern Chihuahua Desert clades. The sister-clade relationship between the Meseta Central Matorral clade and the *flavus* clade depicted in the mtDNA tree (Fig. 2.2) is replaced by a sister relationship between the Meseta Central Matorral and Southern Chihuahua Desert clades in the IRBP tree (Fig. 2.5).

Estimates of divergence dates

Fossils always will postdate the origin of the clade to which they belong, so to the degree that taxonomy is correct, fossil calibration of a molecular phylogeny should be considered an underestimate of the age of the clade (Benton and Ayala 2003). Here, we set the *Perognathus* fossil from the John Day formation, with an estimated age of 20-22 mya, at the base of the Perognathinae, which might counter the underestimate bias if this fossil represents a basal taxon of *Perognathus* after its split from *Chaetodipus*. Based on the fossil-calibrated molecular clock analyses for both the *COIII* and *IRBP* data sets, the 4 deep clades recovered within the *flavus* species group (Fig. 2.4) might have begun diverging as early as the late Miocene, although some discrepancy exists between the mitochondrial and nuclear genes (Table 2.2). All estimates had large confidence intervals, but in both data sets the estimates for the time to most recent common ancestor of the *flavus* species group excluded the Pleistocene and placed the divergence time somewhere in the latest Miocene or Pliocene. The estimates are similar for the *merriami* clade, although the confidence interval of the nuclear data set includes the early Pleistocene. All estimates exclude the more extreme glacial cycles that began about 700,000 years ago (Table 2.2).

Discussion

Phylogenetic history of the flavus species group

The *flavus* species group displays a pattern of deep divergences giving rise to 4 clades with very different patterns of geographic distribution. The Meseta Central Matorral and Southern Chihuahua Desert clades appear to be narrowly distributed, whereas the *merriami* and *flavus* lineages are more broadly distributed and genetically diverse (Table 2.1 and Fig. 2.2). Geographic expansion of the *merriami* and *flavus* lineages was accompanied by diversification of several geographically localized phylogroups within each (Figs. 3 and 4).

Despite considerable genetic divergence within the *flavus* species group (5.6% to 19.6%; Table 2.1), only 2 species currently are recognized in the group. Other heteromyid rodents typically show genetic distances between sister species similar to the divergence between phylogroups in our study. For example, Riddle et al. (2000b) measured 10-11% sequence divergence between *Chaetodipus baileyi* and *C. rudinoris* based on combined *COIII* and cytochrome *b* (*Cytb*) gene sequences. McKnight (2005) measured up to 19.8% divergence at the *Cytb* gene between species in the *Perognathus longimembris* species group. Under their proposed genetic species concept Bradley and Baker (2001) suggested that > 11% of sequence divergence at *Cytb* usually indicated species-level distinction in mammals, whereas lower levels of divergence (2-11%) required a more detailed study of the organisms involved to determine species boundaries. Although *COIII* may evolve at a different rate than *Cytb* in small-bodied rodents (Pesole et al. 1999), the initial divergence between the *merriami*, *flavus*, Meseta Central Matorral, and Southern Chihuahua Desert clades (Fig. 2.2), and phylogroup

divergences within the *merriami* and *flavus* lineages (Table 2.1), are certainly large and may signal as many as 4 species-level lineages and several subspecies-level clades within the *flavus* species group. However, use of a genetic yardstick for delimiting species has several potential downfalls (Ferguson 2002), and we recognize the need for a broader range of evidence, including more comprehensive multigene data sets and more detailed geographic sampling within and among populations, before proposing formal taxonomic revisions.

The general phylogenetic and biogeographic agreement between the mtDNA and nuclear DNA data sets in this study provides provisional support for the hypothesis that the 4 main lineages in the *flavus* species group are the result of independent evolutionary histories rather than idiosyncratic transfer of maternally inherited genomes across species boundaries. We found mice with phylogenetically congruent *merriami* mitochondrial and nuclear genomes as far west as the Arizona–New Mexico border, and all 5 individuals sampled for both mitochondrial and nuclear genes in the vicinity of the previously postulated zone of introgression in eastern New Mexico into western Texas (*P. flavus gilvus*—Lee and Engstrom 1991; Wilson 1973;) had congruent genomes. Wilson (1973) suggested the *gilvus* subspecies was likely interbreeding with *P. flavus* to the west and *P. merriami* to the south east. In contrast, our results suggest that Wilson's morphologically and geographically intermediate *gilvus* subspecies, still recognized by Brant and Lee (2006), might simply represent a mixture of sympatric *flavus* and *merriami* specimens.

The geographic distributions of phylogroups within the *merriami* and *flavus* clades (Fig. 2.2) clearly are not consistent with the currently accepted distribution of *P.*

flavus and *P. merriami* (Brant and Lee 2006; Lee and Engstrom 1991; Fig. 2.1). Although our data suggest a general east-west pattern of 2 deeply divergent lineages (the *merriami* and *flavus* clades), the *merriami* clade extends much further to the north (approximately 400 km) and west (approximately 600 km) than previously thought.

Biome evolution and biogeographic history of the *flavus* species group

Retallack (1997, 2001) has marshaled evidence from paleosols, fossils, and stable isotopes to reconstruct 3 stages in the Cenozoic origination and expansion of grasslands in western North America. First, a major cooling and drying event at the Eocene–Oligocene boundary (33.5 mya) led to replacement of dry tropical forests with seasonally dry woodlands and savannas that included bunchgrasses and desert shrub, which themselves might have originated earlier in discontinuous pockets of habitat throughout a tropic and subtropic belt in western North America (Axelrod 1985). Retallack's (1997) model postulates the origination of desert shrub and desert (bunch) grassland prior to sod-forming shortgrass and tallgrass prairie ecosystems. Next, geographic expansion of various arid or semiarid ecosystems, including sod-forming shortgrass prairie, occurred during the early to mid-Miocene (approximately 15 mya) in concert with another large episode of global cooling and drying, possibly associated with the rise of the North American cordillera and the Tibetan Plateau (Raymo and Ruddiman 1992). Finally, tall C4 grasslands, desert shrub, and C4 desert grasslands expanded during the late Miocene (about 5-7 mya) in concert with another episode of global cooling and increased aridity, driven perhaps by either global lowering of atmospheric CO_2 concentrations (Retallack 2001) or increased seasonality of precipitation and fire (Osborne 2008).

Although there is a 4 million-year discrepancy between the divergence estimates based on *COIII* and *IRBP* (Table 2.2), molecular clock analyses of both genes suggest a late Miocene divergence within the *flavus* species group. The discrepancy between the 2 estimates may result from saturation of informative sites in the *COIII* gene or relative scarcity of informative characters in the *IRBP* data set. Current ecological restriction of *flavus* species group members to arid grasslands and xeric shrub habitats (Best and Skupski 1994a, 1994b) is consistent with the hypothesis that the *flavus* species group evolved in the late Miocene (or early Pliocene), coincident with expansion of C4 desert shrub and grasslands. During the middle to late Miocene multiple lineages within the *flavus* species group might have existed in pockets of desert shrub and bunch grassland habitats ranging from the Mexican Plateau northward onto the Great Plains, as evidenced by presence of xeric bunch grassland as far north as South Dakota and sod-forming short grasslands as far north as Nebraska (Retallack 1997). This broad geographic distribution would be consistent with the basal trichotomy involving the *flavus*, *merriami*, and Southern Chihuahuan Desert lineages (Fig. 2.2).

The 2 more geographically restricted lineages, the Meseta Central Matorral and Southern Chihuahuan Desert clades, are distributed narrowly on the Mexican Plateau in northern Mexico and might represent paleoendemics. Presumed paleoendemics restricted to the Mexican Plateau are known in other animal and plant taxa. For example, the ocotillo species *Fouquieria shrevei*, a sister lineage to the widespread warm desert species *F. splendens* (Schultheis and Baldwin 1999), is restricted to gypsum soils within the Bolson de Mapimi. The xantusiid lizards *Xantusia bolsonae*, *X. extorris*, and *X. sanchenzi* form a deeply divergent clade (also including *X. gilberti* from the Cape Region

of the Baja California Peninsula) restricted to rock and plant habitats within the Bolson de Mapimi or trans-Mexican Volcanic Belt (Sinclair et al. 2004). Within crotaphytid lizards, the basal species *Crotaphytus antiquus* (McGuire et al. 2007) is restricted to rock outcrops in southern Coahuila. Our data do not exclude the possibility that additional geographically restricted lineages remain cryptically embedded within the *P. flavus* species group, although recovering them will require a better sampling of the Mexican Plateau and southward into the trans-Mexican Volcanic Belt.

The *flavus* and *merriami* clades likely reached their maximum distributions in the early Pliocene, which was the driest part of the North American Tertiary (Axelrod 1985). Increased seasonality during this period and wildfires resulting from extended dry seasons would have facilitated expansion of grasslands at the expense of woodlands in lowland areas (Keeley and Rundel 2005). Geographic diversification within the *flavus* species group coincident with development of major biogeographic regions in North America probably began in the early to middle Pliocene. Although our molecular clock analyses provide only rough time estimates, it seems clear that diversification within the *merriami* and *flavus* clades occurred prior to the large glacial–interglacial cycles of the Pleistocene (Table 2.2).

Divergence within the *merriami* clade

The 3 phylogroups recovered within the *merriami* lineage (Fig. 2.3) appear to have diverged coincidentally during the Pliocene (Table 2.2). Currently, these 3 phylogroups appear to be distributed largely allopatrically, although additional geographic sampling could reveal overlap between the Great Plains and northern Chihuahuan Desert phylogroups in the trans-Pecos of western Texas along the Rio

Grande corridor (Fig. 2.3). Although our current sampling in the Tamaulipan Mezquital ecoregion is restricted to 2 populations in south Texas, we suspect that the Tamaulipan Mezquital phylogroup extends south into the Mexican states of Tamaulipas and Nuevo Leon along the Tamaulipan Plain east of the Sierra Madre Oriental. The northern limit of this clade is consistent with the Balcones Escarpment along the Edwards Plateau, which may be a barrier to northward movement (Fig. 2.3).

The pattern of divergence between *merriami* populations in the northern Chihuahuan Desert and populations to the east of the trans-Pecos (Tamaulipan Mezquital and Great Plains phylogroups) is consistent with the culmination of faulting and beginning of epeiric uplift along the Rio Grande Valley (Axelrod and Bailey 1976; McMillan et al. 2002; Morgan et al. 1986). Geological activity along the Rio Grande resulted in the closing of the savanna corridor that once connected populations across this region and could have had a significant effect on many species distributed across this region. For example, the grasshopper mouse, *Onychomys leucogaster longipes*, a taxon distributed within the Tamaulipan Mezquital ecoregion, represents a basal splitting of mtDNA lineages within the widely distributed grassland species *O. leucogaster* (Riddle and Honeycutt 1990). A general vicariant event across the Sierra Madre Oriental might have influenced divergence between the woodrats *Neotoma leucodon* (Chihuahuan Desert) and *N. micropus* (Tamaulipan Mezquital and Great Plains—Edwards et al. 2001; Matocq et al. 2007), the kangaroo rats *Dipodomys ordii* (Chihuahuan Desert and elsewhere) and *Dipodomys compactus* (Tamaulipan Mezquital—Alexander and Riddle 2005; Hafner et al. 2007), and the aforementioned collared lizards, *C. antiquus* (Chihuahuan Desert) and *C. reticulatus* (Tamaulipan Mezquital—McGuire et al. 2007).

Current geographic restriction of the northern Chihuahuan Desert phylogroup of the *merriami* clade to northernmost portions of the Chihuahuan ecoregion, with a southern extension into xeric grasslands along the western flank of the Sierra Madre Oriental, could indicate a stronger affiliation with grassland rather than xeric shrubland habitats in the *merriami* lineage relative to the *flavus* lineage. For example, this phylogroup is geographically (and probably ecologically) overlapping with the Mexican prairie dog (*Cynomys mexicanus*), a known denizen of xeric grassland habitats (Scott-Morales et al. 2004). An ecological difference of this sort between the *merriami* and *flavus* lineages also is supported by the newly revealed distributional differences between these 2 lineages on the Great Plains (Figs. 3 and 4), with apparent west–east geographic separation congruent with the transition between the more xeric Western Short Grasslands and the more mesic Central and Southern Mixed Grasslands ecoregions.

With 1 exception, haplotypes within each of the 3 *merriami* phylogroups coalesce to common ancestry at ≤2% divergence. This pattern is indicative of recent decreases in population size, perhaps caused by isolation in refugia during late-Pleistocene glacial cycles. The 1 exception occurs within the northern Chihuahuan Desert phylogroup, which shows a coalescence of 2 nested phylogroups at about 5.5% divergence. Although these nested phylogroups appear to be distributed generally west and east of the Rio Grande River (Fig. 2.3), presence of the eastern group south into Coahuila (Fig. 2.1, locality 13) suggests that the river itself has not acted as a continuous barrier leading to isolation and diversification.

Divergence within the *flavus* clade

Basal divergence of the *flavus* clade into 3 geographically distinct phylogroups suggests a history of sustained widespread distribution, albeit with substantial isolation of populations within areas of suitable habitat. Distribution of the *flavus* phylogroups appears to be associated with the widespread Pliocene distribution of xeric scrub and bunch grasslands through the Mexican Plateau and trans-Mexican Volcanic Belt regions and sod-forming short grasslands across the Great Plains (Retallack 1997),

Divergence of *flavus* phylogroups within the Chihuahuan Desert coincides with a previously postulated barrier across the Rio Conchos. For example, this river appears to be associated with current distributional limits between the woodrat species *Neotoma albigula* and *N. leucodon* (Edwards et al. 2001) and between western and eastern phylogroups of the cactus mouse *Peromyscus eremicus* (Riddle et al. 2000a). Our estimated divergence time for separation of the northern and southern Chihuahuan Desert phylogroups suggests a middle- to late-Pliocene split that is temporally congruent with that proposed between *N. albigula* and *N. leucodon* (Edwards et al. 2001).

The Southern Coahuila Filter Barrier, composed of the Rio Nazas, Rio Aguanaval, and western extensions of the Sierra Madre Oriental, is another putative barrier that may be important to the distribution of silky pocket mice. The Southern Chihuahuan Desert clade (Fig. 2.2) is restricted to the area north of the Southern Coahuila Filter Barrier and south of the Rio Conchos, whereas the southern Chihuahuan Desert phylogroup of *flavus* (Fig. 2.4) is distributed across the Southern Coahuila Filter Barrier. That the barrier has no apparent influence on the distribution of the southern Chihuahuan

Desert phylogroup of *flavus* suggests ecological differences between this group and the Southern Chihuahuan Desert clade.

The most recent divergence in the *flavus* clade is between animals of the Southern Rockies/Colorado Plateau phylogroup and those of the northern Chihuahuan Desert phylogroup (Fig. 2.4 and Table 2.1), which may have occurred at the end of the Pliocene or early Pleistocene. The intermontane area occupied by the northern Chihuahuan Desert phylogroup experienced extensive geological uplift and volcanism in the Pliocene and Pleistocene (Raymo and Ruddiman 1992; Sahagian et al. 2002), which physically could have isolated populations north and south of the Mogollon Rim and southern Rockies. However, divergence between these clades also could have resulted from habitat fragmentation during the Pleistocene glacial cycles on a more geologically stable landscape. Current sympatry of haplotypes from both phylogroups along the Rio Grande corridor in central New Mexico (Fig. 2.4) suggests subsequent erosion of the original physical or ecological barrier. If these clades only recently are coming into contact from Pleistocene refugia, these phylogroups could be introgressing along their contact zone.

Implications and future directions

This study clarifies the geographic component of genetic history of the *P. flavus* species group and by doing so opens the door to new directions for research. Research into the influences of Pleistocene glacial cycles on introgressive hybridization will benefit from access to the hierarchical spatiotemporal framework for the *flavus* species group, including elucidation of cryptic evolutionary lineages. The pattern of diversification in the *flavus* species group establishes a baseline hypothesis of diversification for exploring the evolution of sympatric arid grassland and shrubland taxa.

The temporal and spatial pattern described here needs to be explored further, both within the *flavus* species group and across sympatric taxa, using an integrative approach including fossils, geology, and multigene phylogeography and historical biogeography.

Table 2.1**.** Uncorrected pairwise divergence values between major mtDNA clades and phylogroups in the *Perognathus flavus* species group and the outgroup, *P. longimembris*, based on concatenated sequences from a portion of the control region and cytochrome oxidase III mitochondrial genes.

	2	3	4	5	6	7	8	9	10	*P. longimembris*
1. *flavus* Colorado Plateau	0.056	0.084	0.085	0.092	0.169	0.175	0.181	0.177	0.177	0.225
2. *flavus* Northern Chihuahuan Desert		0.092	0.097	0.100	0.169	0.174	0.183	0.175	0.178	0.227
3. *flavus* Southern Chihuahuan Desert			0.097	0.106	0.170	0.178	0.171	0.174	0.176	0.221
4. *flavus* Great Plains				0.106	0.167	0.176	0.175	0.174	0.180	0.223
5. *flavus* Tehuacan Valley					0.178	0.177	0.185	0.176	0.190	0.229
6. Meseta Central Matorral						0.190	0.190	0.180	0.196	0.227
7. *merriami* Tamaulipan Mezquital							0.101	0.116	0.171	0.215
8. *merriami* Great Plains								0.100	0.173	0.217
9. *merriami* Northern Chihuahuan Desert									0.177	0.214
10. Southern Chihuahuan Desert										0.218

Table 2.2**.** Time to most recent common ancestor for select clades in the *P. flavus* species group for the nuclear *IRBP* and mitochondrial *COIII* genes based on fossil-calibrated divergence estimates in BEAST.

Most recent common ancestor	*COIII* mya (95% *CI*)	*IRBP* mya (95% *CI*)
flavus species group	10.4 (8.4-12.5)	6.3 (3.0-10.5)
flavus clade	4.5 (3.4-6.6)	4.5 (2.0-8.0)
merriami clade	4.8 (3.3-6.0)	3.9 (1.5-7.3)

Figure 2.1. Geographic ranges of the 2 species in the *Perognathus flavus* species group redrawn from Hall (1981), with species boundaries estimated from Lee and Engstrom (1991) and Brant and Lee (2006). Light shading reflects the geographic range of *P. flavus* and dark shading the range of *P. merriami*. Locality numbers as per Appendix 1. Hatched areas indicate major mountain ranges, and dashed polygons show approximate location of ecoregions from http://www.nationalgeographic.com/wildworld/terrestrial.html. The area labeled Great Plains includes the Western Short Grasslands and Central and Southern Mixed Grasslands ecoregions. The area labeled Central Matorral includes the Meseta Central Matorral and Central Mexican Matorral ecoregions.

Figure 2.2. Majority rule consensus tree (50%) generated from Bayesian analysis of concatenated mtDNA sequences from representatives of the *Perognathus flavus* species group, and map showing the distribution of each clade. Hatched areas indicate major mountain ranges. Posterior probability values followed by ML bootstrap values are shown for supported nodes. Scale represents expected changes per site from Bayesian analysis.

Figure 2.3. Linearized phylogram of 50% majority rule consensus tree generated from Bayesian analysis of concatenated mtDNA sequences from representatives of the *Perognathus merriami* lineage, and map showing distribution of phylogroups. Hatched areas indicate major mountain ranges. Posterior probability values followed by ML bootstrap values are shown for supported nodes.

Figure 2.4. Linearized phylogram of 50% majority rule consensus tree generated from Bayesian analysis of concatenated mtDNA sequences from representatives of the *Perognathus flavus* clade, and map showing distribution of phylogroups. Hatched areas indicate major mountain ranges. Posterior probability values followed by ML bootstrap values are shown for supported nodes.

Figure 2.5. Cladogram generated from 50% majority rule consensus from Bayesian analysis of nuclear *IRBP* gene sequences from representatives of the *Perognathus flavus* species group. Posterior probability values followed by ML bootstrap values are shown for supported nodes. Numbers at the end of branches are mtDNA haplotype numbers (Appendix 1).

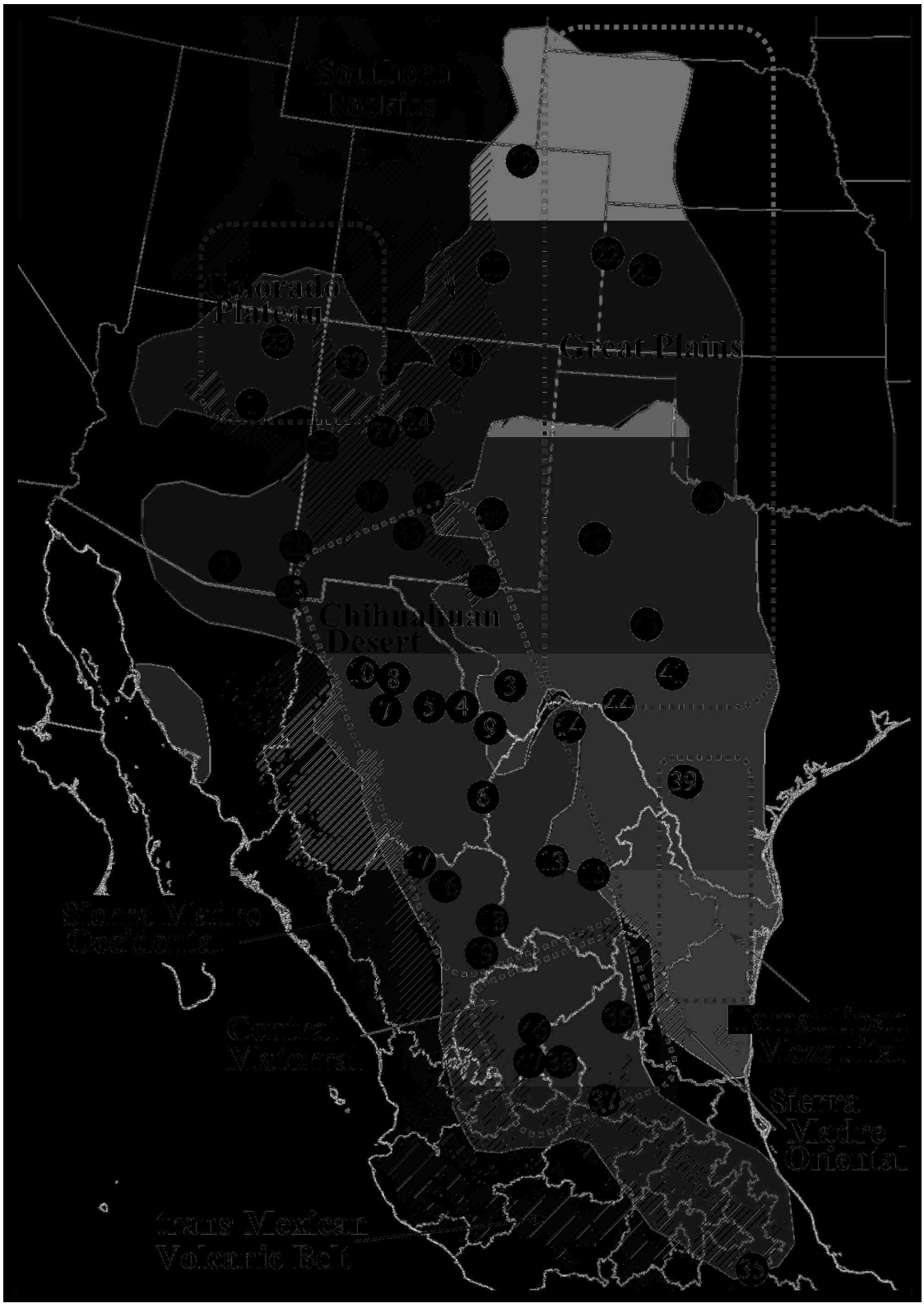
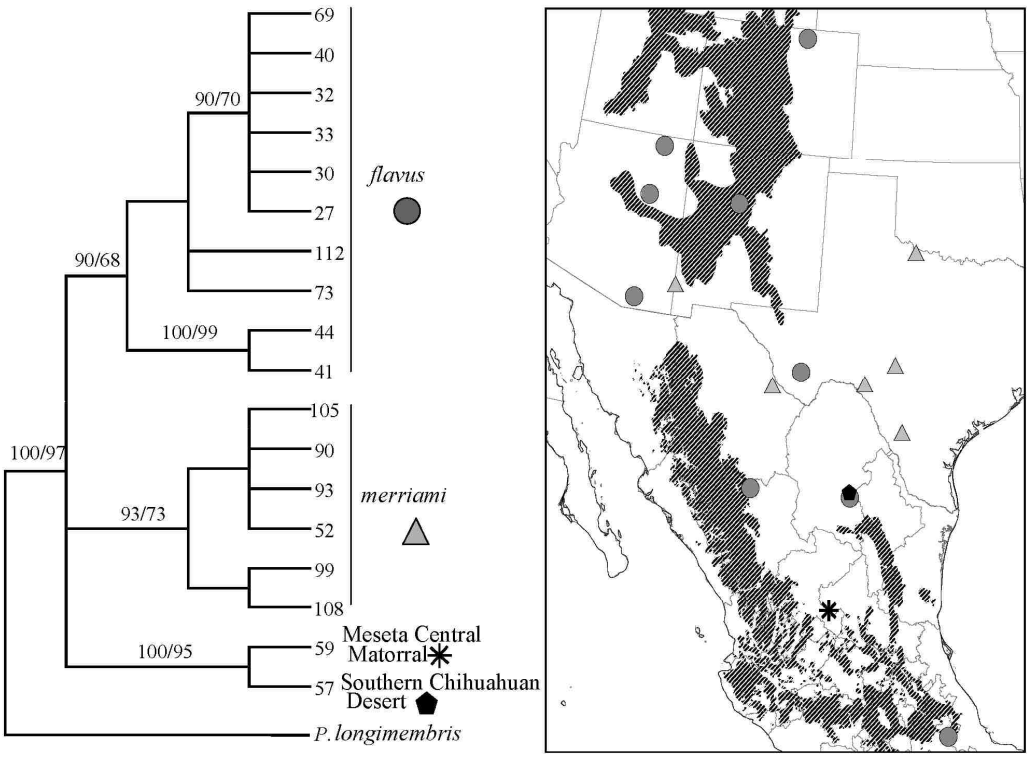

Southern Rockies
Colorado Plateau
Great Plains
Chihuahuan Desert
Sierra Madre Occidental
Sierra Madre Oriental
Trans Mexican Volcanic Belt

Figure 2.1

Figure 2.2

Figure 2.3

Figure 2.4


69
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108
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57
90/70
90/68
100/99
100/97
93/73
100/95
flavus
merriami
Meseta Central
Matorral
Southern Chihuahuan
Desert
P. longimembris

Figure 2.5

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CHAPTER 3

LANDSCAPE AND CLIMATIC EFFECTS ON THE EVOLUTIONARY DIVERSIFICATION OF THE *PEROGNATHUS FASCIATUS* SPECIES GROUP

Abstract

We evaluated evolutionary relationships of taxa within silky pocket mice of the *Perognathus fasciatus* species group, composed of three species *P. fasciatus*, *P. flavescens*, and *P. apache*. These species are distributed throughout the Great Plains, Wyoming Basin, Colorado Plateau, and northern Chihuahuan Desert biogeographic regions in North America. We test a previously postulated hypothesis of Pleistocene species divergence and introgression by analyzing mitochondrial (mtDNA) and amplified fragment length polymorphisms (AFLP). Both mtDNA and AFLP data support several genetic lineages in the *fasciatus* species group that are geographically structured. Molecular clock estimates reject a Pleistocene speciation hypothesis in favor of a deeper, more complex evolutionary history of initial divergence in the Miocene followed by secondary diversification beginning in the mid-Pliocene and progressing through the Pleistocene. Results support recognition of an additional species within the group. Temporal and spatial congruence between the mtDNA clades in the *fasciatus* species group and other co-distributed species of *Perognathus* appear to support an hypothesis of concerted diversification throughout the Chihuahuan Desert, Colorado Plateau, and Great Plains.

Introduction

Understanding the geography of genetic variation across closely-related species and among populations within species is a central focus of phylogeography (Avise 2009). Many phylogeographic studies have focused on diversification driven by the geological and climatic dynamics associated with the North American cordillera. This system of mountain ranges and intervening basins spans the western half of North America from Alaska to southern Mexico, which is one of the most topographically and climatically diverse areas of the world. These studies have invoked both climate change associated with Pleistocene glacial cycles and geological transformations throughout the Neogene, a time period that includes the Miocene and Pliocene epochs, as drivers of diversification in taxa ranging from invertebrates (Crews and Hedin 2006; Knowles and Carstens 2007; DeChaine and Martin 2010) to birds (Spellman et al. 2007; Hull et al. 2010) and mammals (Carstens et al. 2005; Riddle et al. 2000 a,b,c; Galbreath et al. 2010). Mountain building throughout the Cenozoic molded a landscape that interacted with global cooling and drying trends to facilitate a remarkable change in mammalian diversity (Blois and Hadley 2009). Heteromyid rodents appear to be a good example of this trend, having diversified in the newly emerging and rapidly expanding arid biomes of western North America (Riddle 1995; Riddle 2000 a,b,c; Alexander and Riddle 2007; Hafner et al. 2007; Neiswenter and Riddle 2010), This diversification chronicles a transformation from more mesic subtropical to shrub-steppe, arid grassland, and ultimately desert biomes (Axelrod 1985; Retallack 1997, 2001).

The focus of this study is on the phylogeographic diversification of the *Perognathus fasciatus* species group of silky pocket mice, which is distributed

throughout the North American arid grasslands (Fig. 3.1), and is one of four phylogenetically well-delineated species groups (along with the *parvus, longimembris,* and *flavus* groups) that comprise the genus *Perognathus*. Osgood (1900), in his taxonomic revision of pocket mice, grouped the species of *Perognathus* into species groups primarily for convenience, although he recognized the close relationships of the species within each group. Williams (1978a) supported the close evolutionary relationships within Osgood's species groups based on karyotype diploid number and separated the *flavus* species group from the *fasciatus* species group (*sensu* Osgood 1900). Osgood suggested a close relationship between the *flavus* and *longimembris* species groups, a view upheld by recent mitochondrial DNA evidence (Alexander and Riddle 2005; Hafner et al. 2007). Williams (1978a) further suggested a close relationship between the *fasciatus* and *parvus* species groups, but this relationship is yet to be robustly supported in recent studies.

The *fasciatus* species group currently includes at least two recognized species of pocket mice, *P. fasciatus* and *P. flavescens*. Although Williams (1978b) subsumed *P. apache* under *P. flavescens,* proposing that detailed morphological and karyotypic data did not support the recognition of two species, Hoffmeister (1986) continued to recognize *P. apache* as a separate species, suggesting that Williams' (1978b) data were not conclusive. For clarity, we follow Hoffmeister in referring to populations within the intermountain basins of the North American cordillera as *P. apache,* and populations east of the Front Range of the Rocky Mountains in the central and southern Great Plains as *P. flavescens* (Fig 1), except when noted.

Williams (1978b) proposed that the ancestral species giving rise to the extant *fasciatus* species group was distributed across the northern and central Great Plains during the penultimate Pleistocene interglacial period. Under this model, as climates cooled during the latest glacial period, this species tracked habitats to the south, where the range was fragmented across the trans-Pecos region of west Texas. He envisioned this region as a transition zone: to the east, *P. fasciatus* originated in the higher elevation habitats of the Edwards Plateau of central Texas; and to the west, *P. flavescens* (including *apache*) originated in the Chihuahuan Desert of northern Chihuahua and southern New Mexico. As climates warmed again, *P. fasciatus* tracked expanding shrub and grassland habitats into the northern Great Plains, while *P. flavescens* remained in the northern Chihuahuan Desert, later also expanding northward onto the Colorado Plateau and into the Great Plains, less far to the north as *P. fasciatus*. This model predicts a Late Pleistocene diversification between *P. flavescens* and *P. fasciatus*. Furthermore, the model predicts little genetic diversity between populations of *P. flavescens* in the Great Plains and *P. apache* in the intermountain basins given the predicted recent coalescence of these lineages and predicted introgression near their contact zone (Williams 1978b).

An alternative hypothesis for biotic diversification across western North American arid lands is gaining support, in part from recent studies of a variety of heteromyid taxa. This hypothesis portrays a deeper history of geographic evolution in western North American arid lands, with diversification of species groups rooted in the Late Miocene, and species diversification continuing through the Pliocene and the Pleistocene (e.g. Riddle et al. 2002a,b,c; McKnight 2005; Hafner et al. 2007,2008; Jezkova et al. 2009). A study of particular relevance to developing plausible hypotheses

for geographic evolution of the *fasciatus* species group traced geographic evolution of the *flavus* species group using molecular clock analyses with both mitochondrial and nuclear data (Neiswenter and Riddle 2010). That study proposed a causal association between the diversification of basal lineages within the *flavus* species group and the initial development and expansion of arid grasslands. The authors identified several geographically distinct and reciprocally monophyletic phylogroups that were hypothesized to have arisen by vicariance between the Chihuahuan Desert, Great Plains, and Colorado Plateau biogeographic regions. This distribution and set of postulated biogeographic events include a broad area of sympatry between the *fasciatus* and *flavus* species groups (Fig. 3.2), which provides rationale for developing a hypothesis for divergence within the *fasciatus* species group. First, we postulate that basal species group divergence began in the Late Miocene coincident with the initial rapid spread of arid grasslands (Retallack 2001) and associated biota (see Neiswenter and Riddle 2010). Second, we hypothesize a later bout of diversification as lineages comprising separate geographic isolates on the Colorado Plateau (including the southern Rocky Mountains), and in the northern Chihuahuan Desert and Great Plains began diverging during the Pliocene or possibly early Pleistocene.

We test the above hypotheses, which outline specific spatial and temporal relationships within the *fasciatus* species group, within a modern molecular phylogeographic framework. We use mitochondrial DNA (mtDNA) and amplified fragment length polymorphisms (AFLP) to infer the distribution of evolutionary lineages across the topographically complex shrub-steppe and grassland communities over which the group is distributed (Fig. 3.1). Specifically we develop mitochondrial phylogenetic

hypotheses, estimate the timing of major divergences, and correlate the lineages with geologic and climatic events. We use a molecular clock to estimate divergence times for the major lineages within the *fasciatus* species group and evaluate the Pleistocene timeframe of diversification between *P. fasciatus* and *P. flavescens* postulated by Williams (1978b). We also evaluate the extent to which mtDNA provides evidence of sex-biased gene flow between populations of *P. apache* and *P. flavescens* by testing for concordance between the mtDNA and AFLP data. Finally, we compare the evolution of two arid grassland rodent groups that co-occur across several biogeographic regions, the *fasciatus* and *flavus* species groups (Fig. 3.2), to evaluate whether diversification in each group may have been influenced by a similar suite of historical geologic or climatic events.

Materials and Methods

Specimens were sampled throughout the range of the *fasciatus* species group (Fig 1). Samples field collected for this study were handled according to standards set forth by the American Society of Mammalogists (Gannon et al. 2009). Voucher skins and skeletons were collected when possible; however, in several instances samples where provided from other researchers or state permits allowed only the collection of non-destructive ear clips. Sampling was supplemented with tissues loaned from various natural history museums and personal collections (Appendix 2).

Sequencing

We sequenced the protein coding cytochrome oxidase III mitochondrial gene (COIII) from 1-5 individuals per locality. The primers, 8618 and 9323 (Riddle 1995), and

in some cases a primer specific to samples of *P. apache* (Table 3.1), were used for PCR amplification using the following protocol: 95°C for 1 min, 55°C for 1 min, and 70°C for 1 min repeated 30 times. Sequencing was performed in one of two ways. Several of the mtDNA sequences included in the analyses originated in Nickle (1994) and were sequenced using the protocol of Allard et al. (1991). All others were sequenced on an ABI 3130 (Applied Biosystems, Foster City, California) following the manufacturer's protocol. These sequences were checked for arbitrary base calls in SEQUENCHER 4.8 (Gene Codes Corporation, Ann Arbor, Michigan). Sequences were aligned using MEGA4 (Tamura et al. 2007).

Phylogenetic trees were constructed using Bayesian analysis in MRBAYES (ver. 3.1.2, Huelsenbeck and Ronquest 2001). We performed several initial runs for each dataset with different chain temperatures and branch length priors to confirm good mixing and convergence. The final analysis was run with a temperature of 0.05 and branch lengths set to 10. The GTR+G model was chosen based on likelihood ratio test results from MODELTEST (Posada and Crandall 1998). For each analysis we performed 2 independent runs with four chains each (one hot and 3 cold), ran analyses for 4,000,000 generations, and summarized the last 10,000 trees of each run (20,000 trees total) using a 50% majority rule consensus tree employing the posterior probabilities for clade support.

Maximum likelihood (ML) analyses were performed using the program TREEFINDER (Jobb 2008) and the GTR+G model of evolution. Node support for the ML analysis was assessed using 100 bootstrap replicates. With the exception of the model of evolution, default values were used in all ML analyses.

In the molecular clock analyses we included COIII sequences downloaded from GenBank for several heteromyid genera and species outside of the *fasciatus* species group, including: *Dipodomys merriami, Chaetodipus bailyei, C. formosus, C. hispidus, C. eremicus, P. longimembris, P. parvus, P. flavus,* and *P. merriami* (Appendix 4). We used published fossil data (see Hafner et al. 2007 for summary) to calibrate the molecular clock at a node outside of the *fasciatus* species group. We selected a representative from each of the mtDNA clades (see Results) and conducted a relaxed uncorrelated log-normal molecular clock analysis in BEAST v.1.4.7 (Drummond and Rambaut 2007). Fossil calibration was based on the oldest know fossil that is a taxonomically reliable representative of the subfamily Perognathinae, estimated at 20-22 mya (James 1963; Hafner et al. 2007). We conservatively placed this date at the base of the clade that includes *Chaetodipus* + *Perognathus,* as was done by Hafner et al. (2007), because the fossil record does not distinguish between these two genera. We used the HKY+I+G model and constrained the Perognathinae and the *fasciatus* species group clades to be monophyletic. Several runs were conducted to ensure stationarity. The final analysis was 10 million generations sampling every 1,000 generations and results were summarized after a 10% burn-in in TRACER v1.4 and FIGTREE v1.1.2.

Fragment analyses

We followed the protocol designed by Vos (1995) for AFLP amplification. Briefly, total genomic DNA was restricted using EcoRI and MSEI enzymes and known sequences were ligated to the restriction cut sites (Table 3.1). Restriction-ligated DNA fragments were amplified first in a pre-selective amplification and then used for subsequent selective amplifications using combinations of fluorescently labeled primers

for the EcoRI primer and unlabeled MSEI primers (Table 3.1). Selective amplifications were sequenced at the genomics facility at the University of Nevada Reno on an ABI 3430 (Applied Biosystems, Foster City, California). Selective amplification reactions were conducted twice to confirm the recovery of identical peak profiles. AFLP profiles were scored automatically in GeneMapper (Applied Biosystems, Foster City, California) using a peak height threshold of 100 for peaks between 100 and 500 base pairs (bp). Final calls were confirmed by eye and only unambiguous peaks were used in final analyses. Selective primers screened but not used are available from the authors.

To assess potential gene flow between *P. apache* and *P. flavescens* we used the clustering program Structure 2.2.3 (Falush et al. 2007). We first ran exploratory analyses for a range of genetic groups (*k*), burn-in, and chain length. Initial runs were used to confirm good mixing and stationarity, and to determine appropriate burn-in and chain length. We followed the guidelines in the user's manual for determining *k* qualitatively. Exploratory results suggested that there was strong evidence for population structuring in the AFLP dataset that was qualitatively similar to the mtDNA results.

We used the following methodology to assess nuclear gene flow between mtDNA populations of *P. flavescens* and *P. apache*. We a priori assigned each individual of either *P. flavescens* or *P. apache* to 1 of 3 populations based on the results of the mtDNA analyses (see Results) to test for introgression of the nuclear genome among the mtDNA clades. Final analyses were run using prior population information (USEPOPINFO = 1) to identify possible hybrids. The RECESSIVEALLELES option was set to 1, burn-in was 10^5, and chain lengths were 10^6 generations. To determine the extent of introgression we defined two additional parameters, the probability that an

individual is an immigrant (*v*) or has an immigrant ancestor in the last *G* generations. We set GENSBACK = 2 to assess immigration back to an individual's grandparents (2 generations) and ran the analyses for multiple values of *v* (MIGRPRIOR = 0.01, 0.05, and 0.1) to cover a range of plausible migration scenarios (Pritchard et al. 2000).

Results

Sequencing analyses

Seventy nine samples from the *Perognathus fasciatus* species group from 29 general localities were sequenced for COIII. The resulting alignment consisted of 576 basepairs (bp) of which 178 sites were parsimony informative. No gaps, insertions, or deletions were detected. Sequences are deposited in GenBank.

Results of the Bayesian and ML phylogenetic analyses were congruent and supported four major lineages (Fig. 3.3) with considerable uncorrected pair-wise percent divergence between them (Table 3.2). The basal node of the tree corresponds to approximately 18% divergence between *P. fasciatus* and the rest of the group. Two of the major clades correspond with *P. apache.* The *apache North* clade is distributed in the northern Colorado Plateau north of the San Juan River in Utah and the *apache South* clade is distributed in the southern Colorado Plateau/northern Chihuahuan Desert. The two *apache* clades do not collectively form a single monophyletic clade; rather, the fourth major clade is sister to the *apache* South clade and corresponds with the distribution of *P. flavescens*.

Three of the major clades have further structuring within them. The *fasciatus* clade contains three well-supported subclades: one currently restricted to the Wyoming

Basin, a second from the front range of the Rocky Mountains, and the third widely distributed across the northern Great Plains and Wyoming Basin (Fig. 3.4a). The *apache North* clade is further divided north and south of the Colorado River in eastern Utah, and the *apache South* clade is divided east and west of the Chuska Mountains along the northern Arizona-New Mexico border (Fig. 3.4b).

Under the fossil-calibrated molecular clock the divergence between *P. fasciatus* and the ancestor to the other taxa in the group is estimated to have occurred in the late Miocene, approximately 7.4 mya (95% highest posterior density interval (HDP) = 4.8-9.9 mya; Fig. 3.5). The mean time to most recent common ancestor (tMRCA) for the *apache*/*flavescens* clade is 3.6 mya (95% HDP = 2.3-5.0 mya) and the divergence between the *flavescens* clade and *apache South* is estimated at 2.5 mya (95% HDP = 1.5-3.5 mya). Within the major clades further diversification is estimated to have occurred during the middle Pleistocene. The estimated mean mutation rate under the fossil calibrated clock is $0.034 \pm 2.5 \times 10^{-4}$ substitutions/site/million years and the likelihood estimate is -4684.78 ± 0.1.

Fragment analyses

AFLP profiles for two primer combinations were developed for 68 individuals from the *fasciatus* species group. Some individuals were not included because DNA or tissue was no longer available for some of the samples obtained from Nickle (1995). A total of 189 variable sites were scored for the Mse+AGC primer with an average of 59 alleles present per individual. The Mse+ATC primer resulted in a total of 157 variable sites being scored with an average of 53 alleles present per individual. Exploratory

analyses suggested there were 4 groups (mean ln likelihood = -9560), with membership corresponding closely to the 4 major mtDNA clades reported above (Fig. 3.3)

The results of the gene flow analysis suggest a few individuals may have had immigrant ancestry in the past 2 generations (Table 3.3). With the highest probability of migration, $v = 0.1$, 3 individuals show a high probability of having an immigrant grandparent and 1 individual is not strongly assigned to any group. When migration is assumed to be low, $v = 0.01$, only 2 of these individuals were shown to have a high probability of immigrant ancestry. All other individuals were assigned to their respective mtDNA clades with a probability > 0.9 in all analyses.

Discussion

Phylogeography of the *Perognathus fasciatus* species group

The biogeographic hypothesis (and resulting taxonomy) suggested by Williams (1978b), largely based on morphology, underestimates the evolutionary diversity within the *fasciatus* species group that is revealed by the molecular sequence divergence. We identified four major mtDNA clades within the *fasciatus* species group that are also recovered using nuclear DNA. Additionally, within the four mtDNA clades there is further geographic and genetic structuring. Using the fossil calibrated molecular clock, diversification within the *fasciatus* species group began during the latter half of the Miocene and continued into the Pleistocene (Fig. 3.3).

Fossil and molecular evidence suggest massive alteration of mammalian diversity throughout the Cenozoic (Webb 1977; Riddle 1995; Kohn and Fremd 2008; Blois and Hadley 2009). Widespread changes in the distribution of arid biomes, including the

expansion of arid grasslands and shrublands throughout the western North American lowlands, occurred in concert with several bouts of global cooling and drying (Axelrod 1978; Retallack 1997, 2001). Retallack (1997) used paleosols, stable isotopes, and fossil evidence to develop a model of the evolution of the grassland biomes which depicts 3 stages of successive drying and cooling beginning with the shift from dry tropical forest to savannas with desert shrub and bunchgrasses around the Eocene-Oligocene boundary. The second stage includes the expansion of sod-forming short-grass prairies during the mid-Miocene (15 mya). The latest bout of cooling and increased aridity, beginning around the late Miocene (5-7 mya), involved the expansion of C4 grasslands and desert scrub.

Molecular clock analyses suggest the major lineages of the *fasciatus* species group initially began diverging during the third climatic episode of the late Miocene, coincident with expanding arid grasslands throughout North America. The timing of diversification and habitat use (Williams 1978b; Manning and Jones 1988; Monk and Jones 1996) of the species in the *fasciatus* species group is consistent with an hypothesis outlined for the *flavus* species group (Neiswenter and Riddle 2010), which is co-distributed with the *fasciatus* species group across parts of the Colorado Plateau, northern Chihuahuan Desert, and Great Plains (Fig. 3.2). The expansion of the *fasciatus* species group throughout the northern latitudes of North America may have began in the late Miocene as the ancestor to the group followed the expanding arid adapted C4 grasslands. This expansion was likely caused by the decrease in carbon dioxide concentrations (Retallack 2001), increased seasonality of precipitation and wildfires (Osborne 2008), or some combination of these and possibly other factors (Kohn and Fremd 2008). The initial

divergence within the *fasciatus* species group may have arisen as northern and southern isolates in the Wyoming Basin/Northern Great Plains and Colorado Plateau/Southern Great Plains. *Perognathus fasciatus* is found only in higher elevation grasslands in the southern portions of its geographic distribution along the Front Range of the Rocky Mountains, suggesting it is adapted to cooler climates compared with *P. flavescens* or *P. apache*.

Following the north-south split within the *fasciatus* species group regional diversification continued throughout the Pliocene and Pleistocene. The *apache North* clade is estimated to have diverged during the middle Pliocene, coincident with extensive geological uplift and volcanism in the Colorado Plateau region throughout the Pliocene (Raymo and Ruddiman 1992; Sahagian et al. 2002). The molecular clock analysis points to a late Pliocene/Pleistocene time for the most recent common ancestor between the *flavescens* clade and *apache South* clade across the southern Rocky Mountains (Fig. 3.5). A similar estimated timing of the Great Plains divergence within the co-distributed *flavus* species group (*merriami* Chihuahuan desert vs. Great Plains, 3.3-6.0 mya) may represent the response of these taxa to a common event; the culmination of faulting and beginning of epeiric uplift along the Rio Grande Rift which resulted in the closing of a savanna corridor that connected populations to the east and west (Axelrod and Bailey 1976; McMillan et al. 2002; Morgan et al. 1986). In conjunction with the geologic activity, the late Pliocene transition (ca. 2.7 – 3.2 mya) marks a distinct period of cooling in the Northern Hemisphere (Sosdian and Rosenthal 2009) that may have promoted regional adaptations of the local biota to the changing environmental conditions within each of these biogeographic regions, further contributing to diversification of the associated

biota. These results are consistent with the hypothesis of Neiswenter and Riddle (2010) that a large portion of the phylogeographic diversity in these arid grassland species is a result of lineage diversification from geologic and climatic phenomena prior to the major glacial cycles of the Pleistocene, laying the foundation for within-region population structuring beginning in the mid-Pleistocene.

Several of the major clades have further geographic structuring that is consistent with a scenario of persistence of discrete lineages in separate Pleistocene refugia. Each of the major clades has further structuring that is estimated to have begun in the mid-Pleistocene. The mid-Pleistocene transition, ~1.2 – 0.7 mya, is marked by Milankovich cycles shifting from the dominant 41ky obliquity cycles of the early Pleistocene to longer more extreme 100ky cycles (Sosdian and Rosenthal 2009). The longer more extreme cycles may have further isolated populations of each of the clades in the *fasciatus* species group as they shifted their distribution tracking their preferred habitat in response to the ever-changing climate. In North America, much of the northern Great Plains was covered by glaciers during the colder climate cycles so the habitable area available to *P. fasciatus* was likely reduced to areas along the Front Range of the Rocky Mountains and within the Wyoming Basin which could have served as refugia during the Pleistocene, resulting in the current genetic structure recovered within this clade (Fig. 3.4a). The Front Range and Wyoming Basin may have served as refugia for other co-distributed lowland taxa, such as grasshopper mice (*Onychomys leucogaster*; Riddle and Honeycutt 1990; 1993), as these regions could have been buffered from severe climate changes due to their topographic complexity. Additionally, within both *apache South* and *apache North* there are clades whose diversification may be explained by the persistence of

multiple Pleistocene refugia throughout the basins of the Colorado Plateau (Fig. 3.4b). Because the Great Plains is topographically less complex than the intermountain basins it is plausible that there was only a single refugium available to the *flavescens* clade during the Pleistocene, which would explain the lack of similar substructure within this clade (Fig. 3.3). A population genetic approach with more detailed sampling of individuals and genes for each of these species and other sympatric taxa is necessary to evaluate further details for presence, size, and locations of each of these postulated refugia.

Nuclear introgression

Our nuclear data support the mtDNA groupings and lend credibility to the evolutionary history documented with the maternally inherited mtDNA, although a small number of individuals were identified as having immigrant ancestry. Using nuclear data all but 4 individuals were assigned to their respective mtDNA clades with high probability in the STRUCTURE analysis, even when assuming the highest migration rate. Williams (1978b) suggested that *P. flavescens* and *P. apache* were probably introgressing across the trans-Pecos region of west Texas and southeast New Mexico, an area where both species are fairly uncommon. There is some indication of local introgression at each of the contact zones between the 3 mtDNA clades that comprise *P. apache* and *P. flavescens* under the assumed migration probabilities. To the extent that the range of migration priors used in these analyses reflect the true range of dispersal probability in these species we can identify the probability of nuclear immigration among the mtDNA clades identified. For example, 2 individuals (NMMNH 3259 and NMMNH 3258) with *flavescens* mtDNA located near the trans-Pecos and southern Rocky Mountains at localities 13 and 15 (Fig. 3.1, Appendix 2) have a high probability of

having an immigrant grandparent, particularly under the higher migration scenarios. Additionally, 1 of the individuals with *apache North* mtDNA (MSB 76895; Appendix 2) is predicted to have an *apache* South nuclear DNA component. This individual is from near the San Juan River (locality 25; Fig. 3.1, Appendix 2) in Utah, close to the probable contact zone between *apache North* and *apache South* mtDNA clades. One of the samples with *apache North* mtDNA (LVT 9907) shows a possible immigration ancestry with *flavescens* but only under the highest migration prior. This sample is not near a contact zone with *flavescens* populations making an introgression hypothesis less likely. Furthermore, the individual does not have a high probability of coming from any population under the highest migration prior. This individual may retain some ancestral polymorphisms and/or homoplasies that make it more difficult to assign to its respective mtDNA population under a high migration scenario.

If nuclear introgression is occurring between mtDNA populations, it is geographically limited and uncommon. *Perognathus* are relatively small rodents that likely have restricted dispersal abilities (Williams 1978b; Manning and Jones 1988; Monk and Jones 1996) making the lower value assumed for the migration prior (0.01) a more likely representation of the true value. Only two individuals are predicted to have immigrant ancestry under this assumption, although only 3 individuals have a high probability of immigrant ancestry under the highest migration prior. Regardless, in each instance the introgression of nuclear DNA to mtDNA populations is predicted to have occurred at least 2 generations ago (i.e., no F1 hybrids were found). Moreover, the same localities have other individuals that are not predicted to have immigrant ancestry. There are also other localities in the same general area of the localities with predicted

immigrants and within Williams' (1978b) proposed area of introgression (e.g., locality 20) with multiple individuals that have no immigrant ancestry. In light of this, we propose that the congruent mtDNA and AFLP groups are maintaining their genetic distinctness but suggest further investigation in each of the potential hybrid areas to better describe the nature and extent of the contact zones.

Implications for *Perognathus* systematics

Morphologically-based taxonomy may not be appropriate for delineating species diversity in *Perognathus*. We identified at least 4 genetic lineages that likely satisfy the requisites of a variety of species concepts, for example the Genetic Species Concept (as it applies to mammals; Baker and Bradley 2002) and Genealogical Concordance Concept (Avise and Ball 1990). Species designation was not an objective of this study and we acknowledge that a more detailed analysis of clade boundaries with the inclusion of all recognized subspecies is necessary to fully evaluate the specific status of members in this group. However, several studies to date have shown that current taxonomy (largely based on skeletal morphology) grossly underestimates the molecular diversity within the genus *Perognathus* (McKnight 1995; Alexander and Riddle 2005; Neiswenter and Riddle 2010). Species of *Perognathus* exhibit extensive plasticity in external morphology that can be causally or directly linked to environmental conditions experienced by the individual. This is evidenced by extreme intraspecific color variation depending on the color of substrate on which an individual is found (e.g., *P. apache melanotis*), as well as skeletal variation associated with climatic conditions. Williams (1978b) showed morphological variation tended to follow basic ecogeographic rules in *Perognathus*: mice

from colder environments are larger and the variation in relative measurements of auditory bullae and rostral size are associated with wetter environments.

We hypothesize that the apparent introgression between *P. flavescens* and *P. apache* across the trans-Pecos region is due to the convergence of morphology within similar environments: warmer, drier deserts and grasslands in the southern portions of the ranges of both species contain mice that have converged morphologically. This pattern may be more widespread within the genus *Perognathus* than previously recognized, being apparent in different species and in different supposed transition zones. For example, across the same region *P. flavus* and *P. merriami* were thought previously to hybridize (based on intermediate morphology) through *P. m. gilvus* (Wilson 1973), but probably coexist as separate species that are morphologically similar based on levels of molecular divergence (Brant and Lee 2006; Neiswenter and Riddle 2010). Additionally, Osgood (1900) believed *P. callistus* (currently synonymized with *P. fasciatus*) from the Wyoming Basin was intermediate between *P. fasciatus* and *P. apache*, resembling *P. apache* from the Uinta Basin in skull characteristics (presumably due to intermediate climate) but similar in color to *P. fasciatus* from the Great Plains. A reanalysis of Williams' (1978b) morphological data in light of the molecular results reported here is warranted to evaluate this hypothesis

Conclusions and future directions

A general model for the evolution and diversification of a North American arid grasslands biota is beginning to emerge. This study tested hypotheses regarding the diversification of arid grasslands and shrublands across several ecoregions in western North America. Although no formal statistical comparative phylogeographic analysis was

used, we found congruence with previous results from the *flavus* species group that indicate these two independent lineages of small-bodied pocket mice likely followed expanding arid grassland habitat as it emerged in the late Miocene and diversified throughout the Pliocene and Pleistocene across the topographically complex North American cordillera. We recognize the need to include multi-gene datasets and more co-distributed species within a comparative phylogeographic framework within this system, as well as the need for a more detailed sampling and statistical approach to evaluate the Pleistocene refugial hypotheses outlined above. Finally, species limits within the *fasciatus* species group should be reevaluated by incorporating all available evidence from every subspecies along with a denser sampling at each of the clade boundaries.

Table 3.1. Primers used for Amplified Fragment Length Polymorphisms and sequencing. Asterisk denotes 56-FAM dye-labeled primer.

Name	Sequence (5'-3')
ECO-F	CTCGTAGACTGCGTACC
ECO-R	AATGGTACGCAGTCTAC
ECO+C	GACTGCGTACCAATTCC
ECO+CAC*	ACTGCGTACCAATTCCAC
MSE-F	GACGATGAGTCCTGAG
MSE-R	TACTCAGGACTCAT
MSE+A	GATGAGTCCTGAGTAAA
MSE+AGC	GATGAGTCCTGAGTAAAGC
MSE+ATC	GATGAGTCCTGAGTAAATC
9323apache	ACGAATTATACAAACTAGA

Table 3.2. Uncorrected pair-wise differences between mitochondrial clades of the *Perognathus fasciatus* species group.

	apache North	*apache South*	*flavescens*	*parvus*
fasciatus	0.182	0.177	0.163	0.221
apache North		0.112	0.113	0.205
apache South			0.103	0.225
flavescens				0.216

Table 3.3. Results from the STRUCTURE analysis showing the possible source population and ancestry in individuals with less than 0.9 probability of no immigrant ancestry under different migration priors. Individuals are labeled by their museum numbers or other number if no museum voucher is available; see Appendix 2 for more information. No immigrant ancestry is the probability that the nuclear genomic ancestry of the individual is from the same region as the mtDNA from that individual. The other immigrant columns show the probability that the individual has ancestry from the possible immigrant source up to 2 generations ago. Rows do not add to 1 because there is a small probability of ancestry from other populations. Bold numbers are probabilities greater than 0.5.

Individual	mtDNA population	Possible immigrant source	*v*	No immigrant ancestry	Immigrant	Immigrant parent	Immigrant grandparent
NMMNH 3258	*flavescens*	*apache South*	0.01	0.263	0.001	0.001	**0.731**
			0.05	0.019	0.000	0.002	**0.974**
			0.1	0.005	0.000	0.002	**0.989**
NMMNH 3259	*flavescens*	*apache South*	0.01	**0.533**	0.000	0.000	0.453
			0.05	0.104	0.000	0.000	**0.867**
			0.1	0.043	0.000	0.001	**0.928**
MSB 76895	*apache North*	*apache South*	0.01	0.161	0.000	0.008	**0.831**
			0.05	0.025	0.000	0.010	**0.964**
			0.1	0.010	0.000	0.012	**0.978**
LVT 9907	*apache North*	*flavescens*	0.01	**0.916**	0.000	0.000	0.072
			0.05	**0.701**	0.000	0.000	0.234
			0.1	0.409	0.000	0.000	0.496

Figure 3.1. Geographic ranges of species in the *fasciatus* species group following taxonomy of Hoffmeister 1986: Light shaded = *P. fasciatus*, Dark shaded = *P. apache*, Stippled = *P. flavescens*. Lined areas represent major mountain ranges. Black dots show approximate collecting localities of specimens examined. Numbers refer to localities listed in Appendix 2.

Figure 3.2. Distribution of *fasciatus* species group (grey) and *flavus* species group (stippled) showing the area of sympatry in three regions.

Figure 3.3. The distribution of major mtDNA clades and AFLP groups in the *fasciatus* species group are shown in relation to the major mountain chains (lined area on map). The phylogenetic tree at left is the consensus tree from the Bayesian analyses, and is identical in supported topology to the ML tree (not shown). Numbers at nodes represent Bayesian posterior probability and ML bootstrap values respectively. The bar graph shows the results of the exploratory AFLP analysis in STRUCTURE 2.2.3 for k=4. Each bar represents the probability of a single individual belonging to one of the four groups. Color of the bars corresponds to the color of the symbols for each of the four mtDNA clades to show general congruence of mtDNA and AFLP groupings.

Figure 3.4. Phylogenetic results of the mtDNA Baysian analysis showing further geographic structuring within A) *P. fasciatus* in the Wyoming Basin and northern Great Plains and B) the two *apache* clades in relation to the Colorado and San Juan rivers on

the Colorado Plateau. Numbers at nodes are posterior probabilities and maximum likelihood bootstrap support, respectively. Lined areas represent major mountain ranges.

Figure 3.5. Cronogram from relaxed molecular clock analyses showing molecular dating of major lineages in the *fasciatus* species group. Numbers at nodes are median values in millions of years and dark bars represent 95% intervals. Time scale is estimated for visual purposes.

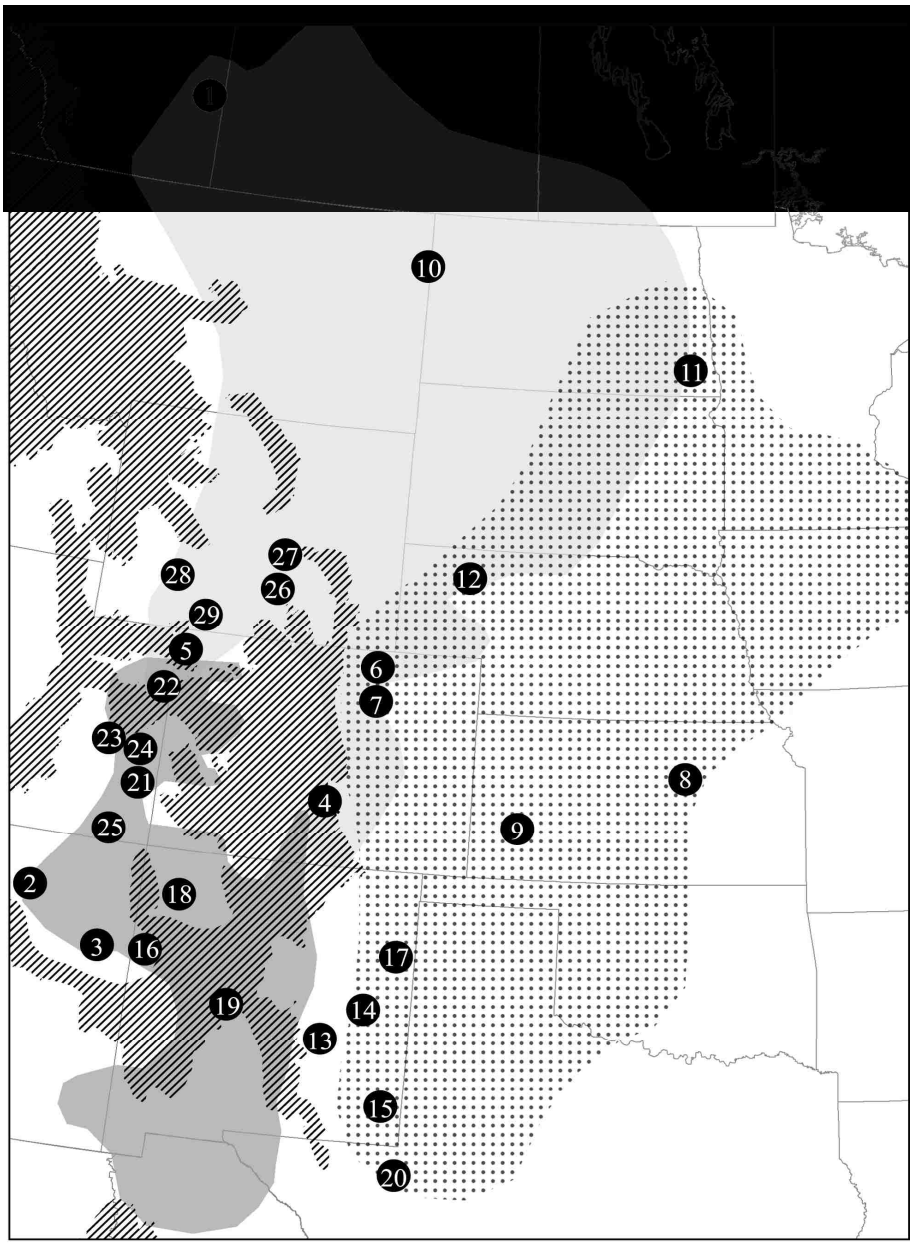

Figure 3.1.

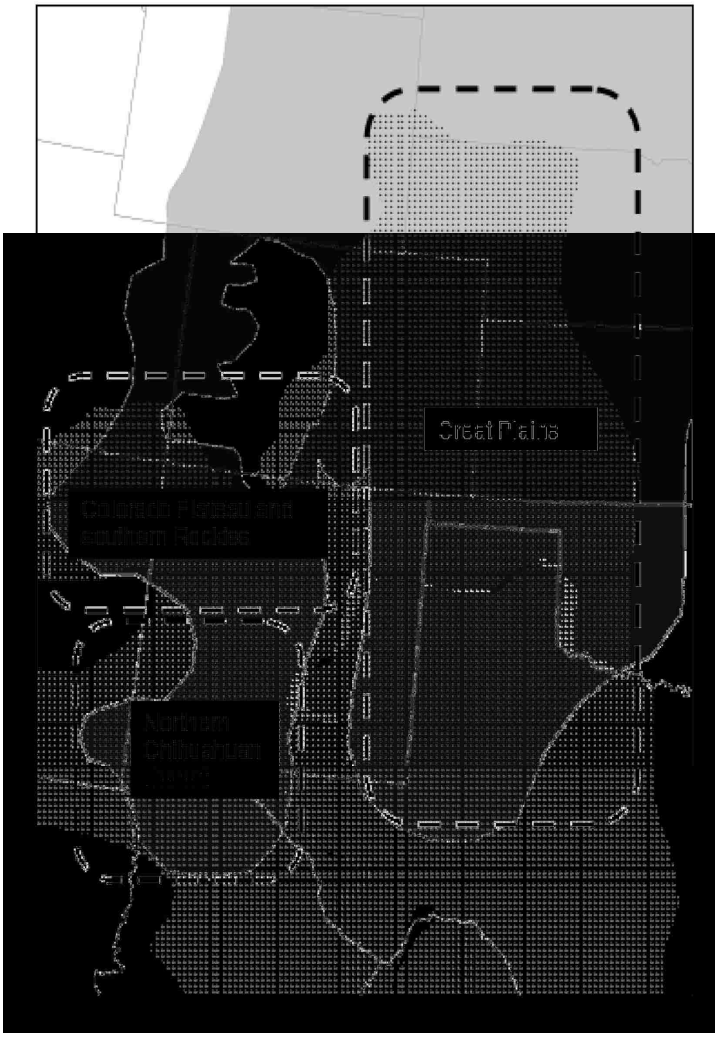

Figure 3.2

Figure 3.3

Figure 3.4

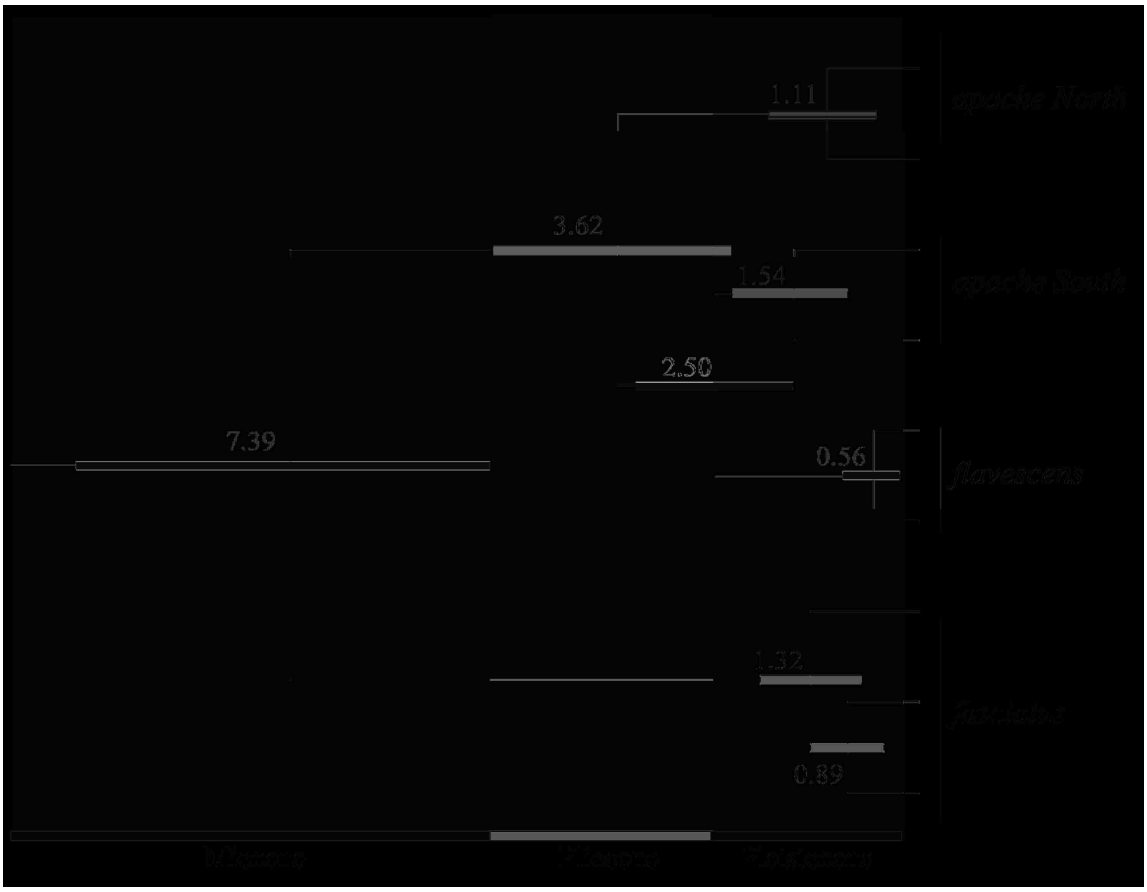
1.11
apache North
3.62
1.54
apache South
2.50
7.39
0.56
flavescens
1.32
fasciatus
0.89

Figure 3.5

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CHAPTER 4

PHYLOGEOGRAPHY OF ORD'S KANGAROO RAT (*DIPODOMYS ORDII*) IN WESTERN NORTH AMERICA.

Abstract

Dipodomys ordii (Ord's Kangaroo Rat) is distributed in western North american arid grasslands throughout the Chihuahuan Desert, Colorado Plateau, Great Basin, and Great Plains. The phylogeographic history of *D. ordii* is examined using Bayesian and maximum likelihood phylogenetic analyses of the cytochrome oxidase III mitochondrial gene. A fossil calibrated relaxed molecular clock along with the credible intervals is used to apply a range of dates to each of the major nodes in the phylogeny. The divergence between *D. ordii* and the closest know sister species *D. compactus*, which is limited in geographic range to the Tamaulipan Mezquital in southern Texas, is estimated to have occurred in the late Miocene or possibly the early Pliocene. There are several phylogroups, geographically distinct monophyletic clades, that occur in recognized biogeographic regions including, the Great Basin, Colorado Plateau, Chihuahuan Desert, Central Matorral,and Great Plains. The timing of the divergence among phylogroups is estimated to have begun in the Pliocene and continued through the Pleistocene. The diversification of *D. ordii* is similar to the sympatric *Perognathus* species groups with respect to timing of divergence and geographic distribution of mtDNA clades when compared, although concerted and independent responses to geographic barriers and climatic events are revealed.

Introduction

Comparative phylogeography seeks to understand how historical processes, such as climate change and geological events, have shaped regional biodiversity by inferring signatures of concordant phylogenetic divergence across co-distributed species (Bermingham and Moritz 1998; Avise 2000; Zink 2002). Comparative phylogeographic analyses can provide a robust understanding of the effects of broad scale historical events on communities and ecosystems as a whole by going beyond the individualistic nature of single species phylogenies (Riddle et al. 2000; Arbogast and Kenagy 2001; Riddle and Hafner 2006; Castoe et al. 2009). Identifying a single mechanism for the shared diversification of co-distributed taxa can be difficult because climate change and geological processes tend to occur over long periods of time and are often not mutually exclusive (Molnar and England 1990; McMillan et al. 2006; Kohn and Fremd 2008; Hoorn et al. 2010). Major geological events can redirect global weather patterns (e.g Molnar et al. 2010), while on a local scale, rainshadows and seasonality of precipitation can influence local fauna which in turn can impact fluvial processes (McMillan et al 2002). To compound the issue further, species diversity in a given area may reflect responses to both factors acting at different points in time (Hoorn et al 2010). These processes should have the greatest effect on biotic diversity in areas of high topographic relief during times of rapid climate change, where complex landscapes and changing climates together promote divergence of lineages (Badgley 2010).

One of the most topographically complex regions in the world is a massive assortment of mountain ranges and basins spanning the western half of North America, collectively known as the North American Cordillera (NAC). The history of geophysical

and climatic changes in the NAC throughout the Cenozoic is complex and while research in this area offers little consensus on the timing of geologic events, some general conclusions can be ascertained (Wilson and Pitts 2010). The Laramide orogeny finalized in the early Cenozoic (55mya) and gave rise to the block uplifts and fold-and-thrust belts that are currently known as the Rocky Mountains, Sierra Madre Oriental, and areas surrounding the Colorado Plateau. During the late Miocene a second bout of uplift may have occurred, beginning 15 mya with most of the uplift less than 7 million years old. The second bout of uplift has been considered responsible for the aridification of the NAC (reviewed in Wilson and Pitts 2010) although the nature and extent of the uplift is still actively debated (Molnar and England 1990; Hay et al. 2002; McMillan et al 2006). Since the end of the Miocene, incision, rifting, and localized geologic events continued to shape the topography of the Rocky Mountains and Colorado Plateau (Axelrod and Bailey 1976; Morgan et al. 1986; McMillan et al. 2002; McMillan et al. 2006; Wilson and Pitts 2010).

Along with the tectonics of the Cenozoic, the global climate was cooling. The overall gradual cooling was punctuated by periods of rapid climate change. Some notable cooling events occurred at the Oligocene/Eocene boundary (34 mya) and again in the late Miocene (beginning 14 mya) coincident with the second uplift of the NAC. Near the end of the Pliocene rapid cooling between 3.2-2.7 mya marks the transition from an unglaciated to glaciated Northern Hemisphere and increased 41ky obliquity cycles (Sosdian and Rosenthal 2009). During the mid-Pleistocene 1.2-0.7 mya the Milankovitch cycles shifted from 41ky to 100ky larger amplitude cycles and marked the beginning of

the extreme glacial cycles that would characterize the Northern Hemisphere to the present day (Sosdian and Rosenthal 2009).

The NAC harbors an impressive amount of mammalian biodiversity (Kays and Wilson 2009) which can be directly or indirectly associated with the complex climate and topographic changes the area experienced throughout the Cenozoic. Climate change and orogeny has been causally associated with the diversification of numerous mammalian lineages including ungulates, carnivores, primates, and rodents (Webb 1983; MacFadden 1997; Kohn and Fremd 2008; Blois and Hadley 2009; Badgley 2010, but see Alroy et al. 2000 for an alternative view). The Miocene was a particularly active time in the evolution of many rodent lineages in North America (Riddle 1995; Alexander and Riddle 2007; Hafner et al. 2007; Finarelli and Badgley 2010). Comparative phylogeographic analyses of rodent fauna in the North American desert southwest have associated much of the assembly of these communities with the aridification and provinciality of lowland desert regions due to the final uplift of mountains and general cooling during the Pliocene and Pleistocene (Riddle et al. 2000; Riddle and Hafner 2006). A full understanding of the geographic evolution of biota across an area as diverse as the NAC will necessarily involve a combination of tectonic and climatic factors, and perhaps other factors, acting alone and in concert, through time.

The Heteromyidae is a family of rodents which contains four genera that are endemic to North America (Hall 1981) and one that is found throughout Central and northern South America. The Heteromyidae probably evolved in North America at the beginning of the Neogene (Alexander and Riddle 2005, Hafner et al. 2007) and have since become the most diverse lineage of rodents in the lowlands of western North

America. They occupy a wide variety of mid to low elevation ecosystems including desert, grassland, and shrub-steppe habitat (Genoways and Brown 1993). Many of the species and species groups are wide spread and co-distributed across several biogeographic regions, making the family ideal for addressing North American aridlands diversification.

The central goal of this study is to examine the phylogeographic history of *D. ordii* by documenting the spatial patterns and temporal diversification of mtDNA clades throughout the entire distribution of the species. A secondary objective of this study is to compare and contrast the phylogenetic results of *D. ordii* with previous results on two co-distributed species groups in the genus *Perognathus* (silky-haired pocket mice), the *Perognathus flavus* species group (Neiswenter and Riddle 2010) and the *P. fasciatus* species group (Chapter 3). These taxa inhabit similar ecosystems throughout their respective ranges, including arid grasslands and shrublands with sandy substrate, across a broad geographic distribution (Fig. 1 and 2). The *Perognathus flavus* species group is composed of 2 recognized species that are found throughout arid grasslands from the trans-Mexican volcanic belt east of the Sierra Mandre Occidental to the central Great Plains of Nebraska. The *P. fasciatus* species group is sympatric with the *P. flavus* species group across the southern and central Great Plains and portions of the northern Chihuahuan Desert and southern Colorado Plateau (Fig 2). When in sympatry some evidence suggests that the *P. fasciatus* species group occupies a narrower range of habitats (generally restricted to extremely sandy soils) than the *P. flavus* species group, although they are found in syntopy (Williams 1978, and pers obs). *Dipodomys ordii* occupies the same sandy soil grassland and shrub-steppe habitat and is distributed similar

to the composite distribution of the two *Perognathu*s species groups, but also occurs throughout the Great Basin (Fig. 4.1). Although ecologically similar, the two genera differ with respect to size and locomotor performance, with *Perognathus* being small and quadrupedal (scansorial) and *Dipodomys* being much larger and bipedal (ricochetal).

The *Perognathus flavus* species group displays a high level of cryptic diversity in both mitochondrial and nuclear DNA that has been attributed to climatic and geologic events that occurred since the late Miocene. Neiswenter and Riddle (2010) suggested that the divergence and current widespread distribution of two lineages in the *P. flavus* species group, *merriami* and *flavus*, coincided with the rapid spread of C4 grasses throughout North America near the end of the Miocene (Retallack 2001). Following the geographic expansion, futher diversification within the two lineages began in the Pliocene and continuing into the early Pleistocene. The second bout of diversification occurred in concert with a general global cooling trend and local geologic events which were hypothesized to have isolated lowland populations in major biogeographic regions throughout the NAC. Finally, regional population structuring within biogeographic regions was attributed to the mid-Pleistocene shift to extreme glacial-interglacial cycles isolating populations into refugia.

In the current study, the overall pattern, timing, and distribution of mtDNA haplotypes in *D. ordii* is explored using Bayesian and maximum likelihood phylogenetic methods. Phylogroups, monophyletic clades that are geographically defined, are identified by mapping the geographic location of mtDNA clades from the phylogenetic results. The pattern and timing of diversification within *D. ordii* is discussed in the context of hypothesized phylogeographic barriers from the two species groups of

Perognathus (Fig. 4.1 and Fig 4.2). To test for concordance in timing of major mtDNA genetic lineages between the *Perognathus* species groups and *D. ordii*, fossils specific to each genus are used to calibrate the respective molecular clocks. Concerted and independent responses to phylogeographic barriers are also discussed.

Materials and Methods

Sample collection and sequencing

Ninety six specimens from *D. ordii* were sampled from throughout their range (Appendix 3). Those that were field-collected for this study were handled in accordance with the guidelines established by the American Society of Mammalogists (Gannon et al. 2007). Other specimens included preserved tissues obtained from museum collections or ear clips obtained from private collections.

DNA was extracted from preserved tissues using DNeasy kits (Qiagen Inc., Germantown, Maryland). Genes were amplified and sequenced using published PCR primers, 8618 and 9232 (Riddle 1995) and the following PCR conditions: 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min, for 30 cycles. Sequences were run on an ABI 3130 automated sequencer (Applied Biosystems, Foster City, California), checked for ambiguous base calls in Sequencher 4.8 (Gene Codes Corporation, Ann Arbor, Michigan), and aligned in MEGA 4 (Tamura et al. 2007) with final corrections by eye for alignment integrity. Protein-coding genes were converted to amino acids to ensure no stop codons were present, an indication of a possible nuclear copy of the mitochondrial gene.

Phylogenetic analyses

Detailed methodology for the phylogenetic analyses conducted for the two *Perognathus* species groups can be found elsewhere and are largely similar to those used for *D. ordii* (Neiswenter and Riddle 2010 and Chapter 3). Phylogenetic trees were constructed using a combination of Bayesian and maximum likelihood (ML) analyses. We chose the following taxa as outgroups for phylogenetic analyses to confirm the sister relationship between *D. ordii* and *D. compactus*: *D californicus, D. spectabilis, D. desertii, D. merriami*, *Microdipodops pallidus*, and *M. megacephalus.* Analyses with just ingroup taxa, *D. ordii* and *D. compactus*, were also run to avoid the potential of long-branch attraction from including distantly related taxa (Kolaczkowski and Thornton 2009) and to confirm the relationships and support for phylogroups within *D. ordii*. Bayesian analyses were implemented in MrBayes 3.1 (Huelsenbeck and Ronquist 2001) using the GTR+G model of evolution selected based on likelihood ratio tests from ModelTest 3.04 (Posada and Crandall 1998). We performed several runs with different initial chain temperatures and branch length priors to confirm good mixing and convergence. The final run for the *D. ordii* COIII dataset was conducted with temperature = 0.05 and branch length = 50. For each analysis we performed 2 independent runs with 4 chains each (1 hot and 3 cold). We ran these analyses for 4,000,000 generations and summarized the last 10,000 trees of each run (20,000 trees total) using a 50% majority rule consensus tree employing the posterior probabilities for clade support.

Maximum likelihood analyses were performed using the program Treefinder (Jobb 2008) and the GTR+G model of evolution. Node support for the ML analysis was

assessed using 100 bootstrap replicates. With the exception of the model of evolution, default values were used in all maximum likelihood analyses.

Molecular clock estimates

In the molecular clock analyses we included sequences for several heteromyid species, including *D californicus*, *D. spectabilis*, *D. desertii*, *D. merriami*, *Microdipodops pallidus*, and *M. megacephalus* (Appendix 4). Gene sequences for these taxa were downloaded from Genbank. This sampling strategy allowed use of published fossil data for the *Dipodomys* molecular clock calibration by incorporating diversity from different clades within *Dipodomys* and its sister taxon *Microdipodops* (Hafner et al. 2007). We conservatively calibrated the basal node of the *Dipodomys* clade to between 12.5-15.9 mya, as was done by Hafner et al. (2007).

We used the uncorrelated lognormal relaxed molecular clock analysis implemented in BEAST 1.4.6 to estimate divergence times for major clades (Drummond and Rambaut 2007). The HKY model was selected with assistance of ModelTest. Because of the family-level diversity used in calibrating the molecular clock estimates, we assumed a Yule process for the tree prior. The time to most recent common ancestor (tMRCA) for the Dipodomyinae clade was calibrated as a normal distribution with a mean ($\pm$ *SD*) of 14. $\pm$ 0.9 mya. This is equivalent to a 95% confidence interval from approximately 12.5 – 15.9 mya, the estimated time of the oldest *Dipodomys* fossil (Hafner et al. 2007). Chain lengths were 10,000,000 generations long with sampling every 1,000 generations, and results were summarized after a 10% burn-in.

Results

A total of 96 individuals of *D.ordii* and 1 *D. compactus* from 47 general localities (Appendix 3) were sequenced and used in analyses. The final alignment consisted of 679 base pairs of the COIII gene. No gaps, insertions, deletions, or stop codons where detected.

Results of the maximum likelihood and Bayesian phylogenetic analyses within the *Dipodomys* species provided support for the current species taxonomy with additional structuring within *D. ordii* (Fig. 4.3). *Dipodomys ordii* and *D. compactus* were confirmed as sister taxa so phylogenetic results focus on the analyses involving ingroup taxa only. There was substantial uncorrected pairwise divergence between *D. ordii* and *D. compactus* (Table 4.1). Within *D. ordii* 6 mtDNA phylogroups, geographically structured mtDNA clades, are distributed within biogeographic regions previously identified in the *Perognathus* groups (Neiswenter and Riddle 2010 and Chapter 3) and range in uncorrected pairwise difference froom 0.02-0.053 (Fig. 4.3 and Table 4.1). The *Great Plains* phylogroup is distributed across the northern, central, and southern Great Plains and Wyoming Basin from Canada to eastern New Mexico. Within the intermountain basins, there is a phylogroup distributed within the *Great Basin* and *Colorado Plateau* regions. The Chihuahuan Desert contains two phylogroups, *Northern Chihuahuan Desert* and *Southern Chihuahuan Desert*, distributed roughly north and south of the Rio Conchos. The *Central Matorral* phylogroup is distributed south of the Chihuahuan Desert but extends north east toward the Tamaulipan Mezquital ecoregion in southern Texas.

Three exceptions to the distinct distribution of phylogroup haplotypes within respective regions exist. Haplotypes from the Colorado Plateau phylogroup are found

within the Great Basin at one locality (Fig. 4.3 and Fig. 4.1, locality 24). North of the Rio Conchos, a *Southern Chihuahuan Desert* haplotype occurs at locality 2. Haplotypes from the *Southern Chihuahuan Desert* and *Central Matorral* co-occur at a single locality, also (Fig. 4.3 and Fig. 4.1, locality 3).

The relationships among many of the *D. ordii* phylogroups remain unresolved and collapse to a polytomy at the base of the *D. ordii* clade (Fig. 4.3). The *Great Basin* and *Colorado Plateau* phylogroups are rendered sister to one another but have no support for the relationship from posterior probabilities (<90) and only weak bootstrap support. The two Chihuahuan Desert phylogroups are robustly supported as sister clades with both posterior probabilities (>90) and bootstrap support (>70). The *Central Matorral* phylogroup tends to group sister to the *Northern + Southern Chihuahuan Desert* but there is no support for this relationship.

The initial diversification between *D. ordii* and *D. compactus* is estimated to have begun in the late Miocene (Fig. 4.4), similar to the initial divergence within the 2 *Perognathus* groups. The *D. ordii* phylogroups are estimated to have begun diverging in the late Pliocene and continued throughout the Pleistocene. The overlapping 95% intervals around the median estimated tMRCA between each phylogroup do not allow us to reject the hypothesis that the phylogroups within *D. ordii* all diverged contemporaneously. Within-phylogroup divergence is estimated to have occurred during the late Pleistocene.

Discussion

D. ordii mtDNA phylogeography

Although the species are rather divergent, *D. compactus* was found to be the closest known extant species to *D. ordii* using Bayesian and maximum likelihood analyses (Hafner et al. 2007). Alexander and Riddle (2005) did not find support for a sister relationship between these two taxa in either their parsimony or maximum likelihood analyses but did show robust support with Bayesian posterior probabilities. We did not include all species of *Dipodomys* in our analyses; however, *D. compactus* and *D. ordii* were well resolved as sister taxa in the current study, as well (Fig. 4.3). Furthermore, both Alexander and Riddle (2005) and Hafner et al. (2007) suggest a rather divergent relationship between the two taxa. This may explain the discrepancy in the placement of these taxa on a broad phylogeny. Our analyses confirm the relatively deep relationship and place the divergence between these two taxa in the late Miocene (Fig 4) similar to Hafner et al. (2007).

Following the species level divergence geographic diversification occurred within *D. ordii*. We did not have a detailed geographic sampling of *D. compactus* although given the small range it is likely there is little genetic diversity within the species; the exception perhaps being among the mainland and the three islands forms in the Gulf of Mexico (Baumgardner and Schmidly 1981). In contrast, within the wide spread *D. ordii* there is considerable geographic diversification estimated to have occurred throughout the Pliocene and Pleistocene; the general pattern and timing of which is broadly consistent with the previously hypothesized evolution of co-distributed arid grassland taxa (Neiswenter and Riddle 2010 and Chapter 3).

Regional phylogeographic barriers

The divergence between the Great Plains and intermountain basins in the *P. fasciatus* species group and the *merriami* lineage of the *P. flavus* species group was hypothesized (Neiswenter and Riddle 2010 and Chapter 3) to be due to the closing of the savanna corridor between the Great Plains and northern Chihuahuan Desert as rifting along the Rio Grande progressed northward (Axelrod and Bailey 1976; McMillan et al. 2002; Morgan et al. 1986). Coincident with the rifting, the rapid climatic cooling during the mid-Pliocene transition may have reinforced regional adaptations to the diverging climates in each of the biogeographic regions (chapter 3). There is considerable variation in the median estimated time for this divergence among the different taxa; however, the coincident timing of each of these events cannot be rejected based on overlapping 95% credible intervals from the molecular clock analyses (Fig. 4.4).

The only well supported sister relationship among the phylogroups within *D. ordii* is between the *Northern* and *Southern Chihuahuan Desert*. The Rio Conchos has been hypothesized as the barrier between *Northern* and *Southern Chihuahuan Desert* phylogroups in *P. flavus* (Neiswenter and Riddle 2010), eastern and western phylogroups in *Peromyscus eremicus* (Riddle et al. 2002), and between *Neotoma albigula* and *N. leucodon* (Edwards et al. 2001). The Rio Conchos is the approximate location between the subspecies *D. o. ordii* and *D. o. obscurus* (Baumgardner and Schmidley 1981). Within mtDNA, there is approximately 2% uncorrected pairwise divergence between the *Northern* and *Southern Chiahuahuan Desert* phylogroups across this region providing further support for the subspecific designations; however there is not an exclusive north and south distribution of haplotypes at the Rio Conchos (Fig. 4.3).

The Southern Coahuila Filter Barrier, a geographic barrier composed of rivers, alkali flats, and mountains, has variable effects on numerous mammalian taxa (Peterson 1976). It has been cited as important in defining the distribution of arid grassland taxa such as pocket gophers in the genus *Cratogeomys* (Hafner et al 2007) and a presumed paleoendemic lineage in the *Perognathus flavus* species group, although it has no apparent effect on the distribution of another *flavus* phylogroup (Neiswenter and Riddle 2010). A phylogenetic break roughly coincident with the Southern Coahuila Filter Barrier is evident in *D. ordii* (*Southern Chihuahuan Desert* and *Central Matorral* phylogroups) but there is some overlap between phylogroups across the eastern edge of the barrier which is composed of the Sierra Madre Oriental. This phylogenetic break is coincident with the taxonomic designation of two subspecies, *D. o. obscurus* north of the Rio Nazas and *D. o. palmeri* distributed throughout the Central Matorral region.

Dipodomys ordii may not be as restricted by the proposed phylogeographic barriers as many of the other species that show similar breaks, such as the *Perognathus* species studied here. In addition to the above situations where *D. ordii* mtDNA clades are not distinctly separated into geographic regions, there is overlap between the *Colorado Plateau* and *Great Basin* phylogroups in the Great Basin in northeastern Nevada (Fig.3). In each of the cases, the proposed geographic barrier is a mountain range (Wasatch Range between the Great Basin and Colorado Plateau), river (Rio Conchos), or combination of both (Southern Coahuila Filter Barrier) that is thought to provide an unfavorable habitat matrix through which dispersal is limited. Given its size, ricochetal locomotion, and possibly more general habitat preferences, *D. ordii* may be capable of dispersing across less favorable areas when compared to *Perognathus*. This could explain

the lack of distinct geographic separation of phylogroups in *D. ordii* when compared to *Perognathus*. Alternatively, the localities with haplotypes from two different regions could represent the failure of the mtDNA lineages to completely sort. Nuclear markers and denser sampling at contact zones could differentiate between these hypotheses.

Within *D. ordii*, significant structuring of haplotypes appears to coincide with the mid-Pleistocene transition between 41 ky Milankovich cycles to the longer more extreme 100ky cycles (Sosdian and Rosenthal 2009). There are two clades within the Great Plains phylogroup of *D. ordii*, one distributed in the Wyoming Basin and further north and one in the southern and Central Great Plains (Fig. 4.2). The Wyoming Basin may have served as a Pleistocene refugium for some populations of the Great Plains phylogroup, as was suggested for other arid grassland rodents, including *Perognathus fasciatus* (Chapter 3) and *Onychomys leucogaster* (Riddle and Honeycutt 1990). A much more detailed sampling of each of the phylogroups is needed to fully evaluate the effects of Pleistocene glaciations on the population structure of this species.

Temporal diversification within *D. ordii*

The results presented here are based on a single mtDNA lineage calibrated with a single fossil outside of the group of interest, which together with the uncertainty in the fossil record prevents robust estimates of divergence times and therefore the results should be interpreted with caution. There is however an interesting insight that is worth mentioning and deserves considerably more attention than can be given here. The late Pliocene and predominantly Pleistocene timing of geographic diversification of phylogroups within *D. ordii* appears to have occurred after the geographic diversification in both of the *Perognathus* species groups examined previously (Neiswenter and Riddle

2010 and Chapter 3); This is also reflected in the uncorrected pairwise divergence among *D. ordii* phylogroups (Table 4.1) being considerably less than within either *Perognathus* species group. While *Perognathus* appears to be composed of very old lineages within the North American arid grasslands it would appear *D. ordii* may have entered the ecosystem and diversified later near the end of the Pliocene and predominantly during the Pleistocene. More research is warranted on this subject.

Table. 4.1. Pairwise uncorrected genetic distances between phylogroups of *Dipodomys ordii* and *D. compactus*.

	2	3	4	5	6	*D. compactus*
1. *Northern Chihuahuan Desert*	0.024	0.047	0.052	0.049	0.04	0.156
2. *Southern Chihuahuan Desert*		0.047	0.05	0.047	0.04	0.155
3. *Colorado Plateau*			0.031	0.046	0.045	0.145
4. *Great Basin*				0.052	0.051	0.157
5. *Great Plains*					0.053	0.153
6. *Central Matorral*						0.157

Figure 4.1. Distribution and collection localities of *D. ordii* (dark shading) and *D. compactus* (light shading). Numbers refer to specific localities labeled in Appendix 3. Hatched areas indicate major mountain ranges, and dashed polygons show approximate location of biogeographic regions based on The Nature Conservancy terrestrial ecoregions: http://gis.tnc.org/. The area labeled Great Plains includes the Southern and Central Short Grass Praire, Central Mixed Grass Praire, and Northern Great Plains steppe ecoregions. The area labeled Central Matorral includes the Meseta Central Matorral and Central Mexican Matorral ecoregions. The area labeled Great Basin also includes portions of the Columbian Plateau ecoregion south of the Middle Rocky Mountain-Blue Mountain ecoregion.

Figure 4.2. Geographic ranges of the three taxon groups examined. A) *Dipodomys ordii* and *D. compactus* B) *Perognathus flavus* species group and C) *P. fasciatus* species group. Lined areas are major mountain ranges.

Figure 4.3. Bayesian phylogenetic tree for the cytochrome oxidase III mitochondrial gene of *D. ordii* showing support values for phylogroups. Node support is shown using the posterior probability values and bootstrapped maximum likelihood support, respectively. The distribution of each clade is displayed with respect to major mountain ranges (lined areas) and the Rio Conchos, Rio Grande, and Southern Coahuila Filter Barrier are shown in dark black lines.

Figure 4.4. Chronogram from the BEAST molecular clock analyses. Numbers and thick black bars at nodes are median estimates of divergence times and associated 95% credible intervals of major divergences, respectively. *Perognathus flavus* species group tree was pruned to show only the *merriami* and *flavus* lineages. See text for more details regarding phylogroup regions. Grey highlighted area shows approximate time frame for the rapid expansion of C4 grasses. Geological timescale scale is approximated for reference only.


Rocky Mountains
Wyoming Basin
Great Plains
Great Basin
Colorado Plateau
Chihuahuan Desert
Sierra Madre Occidental
Central Mexican
trans-Mexican Volcanic Belt
Tamaulipan Mezquital
Sierra Madre Oriental

Figure 4.1

Figure 4.2

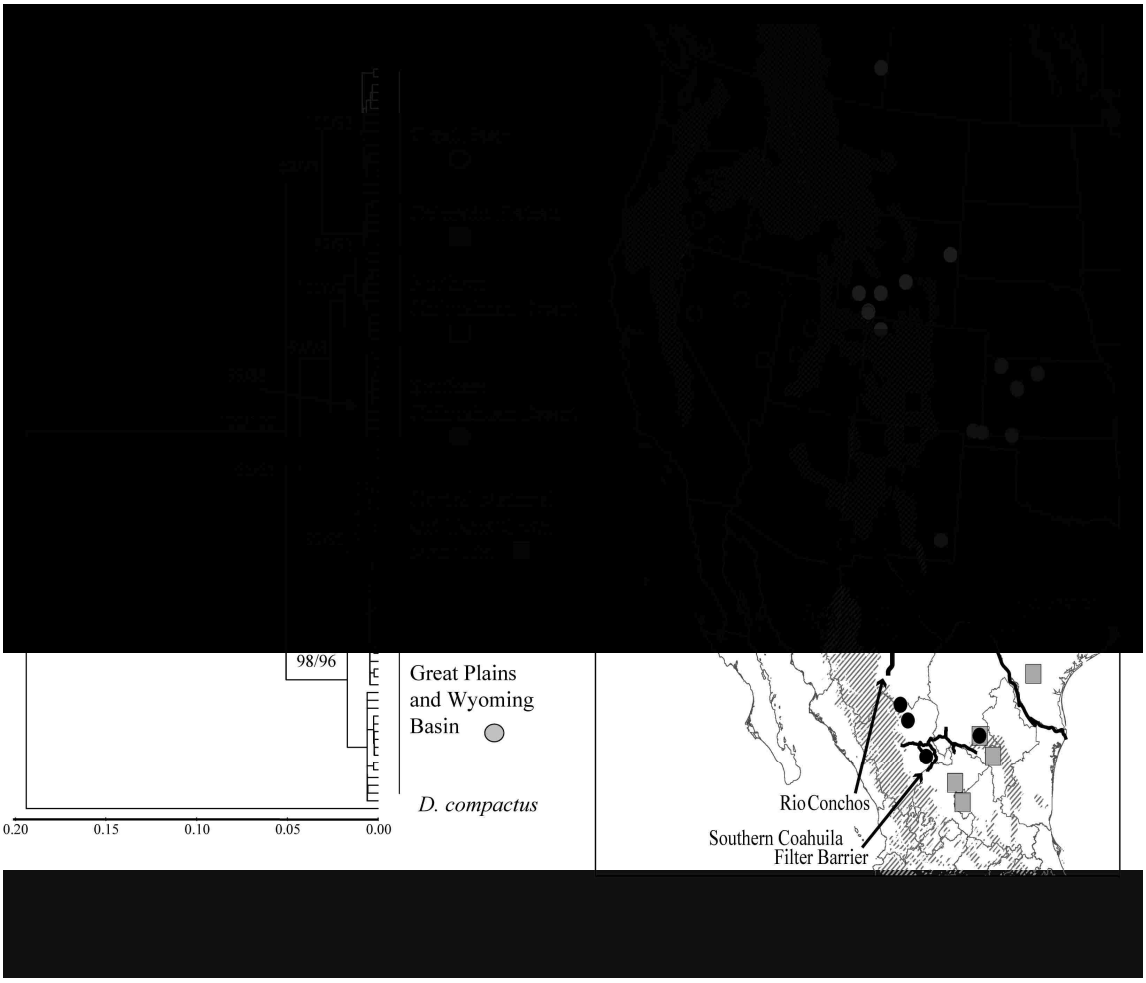
98/96
Great Plains
and Wyoming
Basin
D. compactus
0.20
0.15
0.10
0.05
0.00
Rio Conchos
Southern Coahuila
Filter Barrier

Figure 4.3

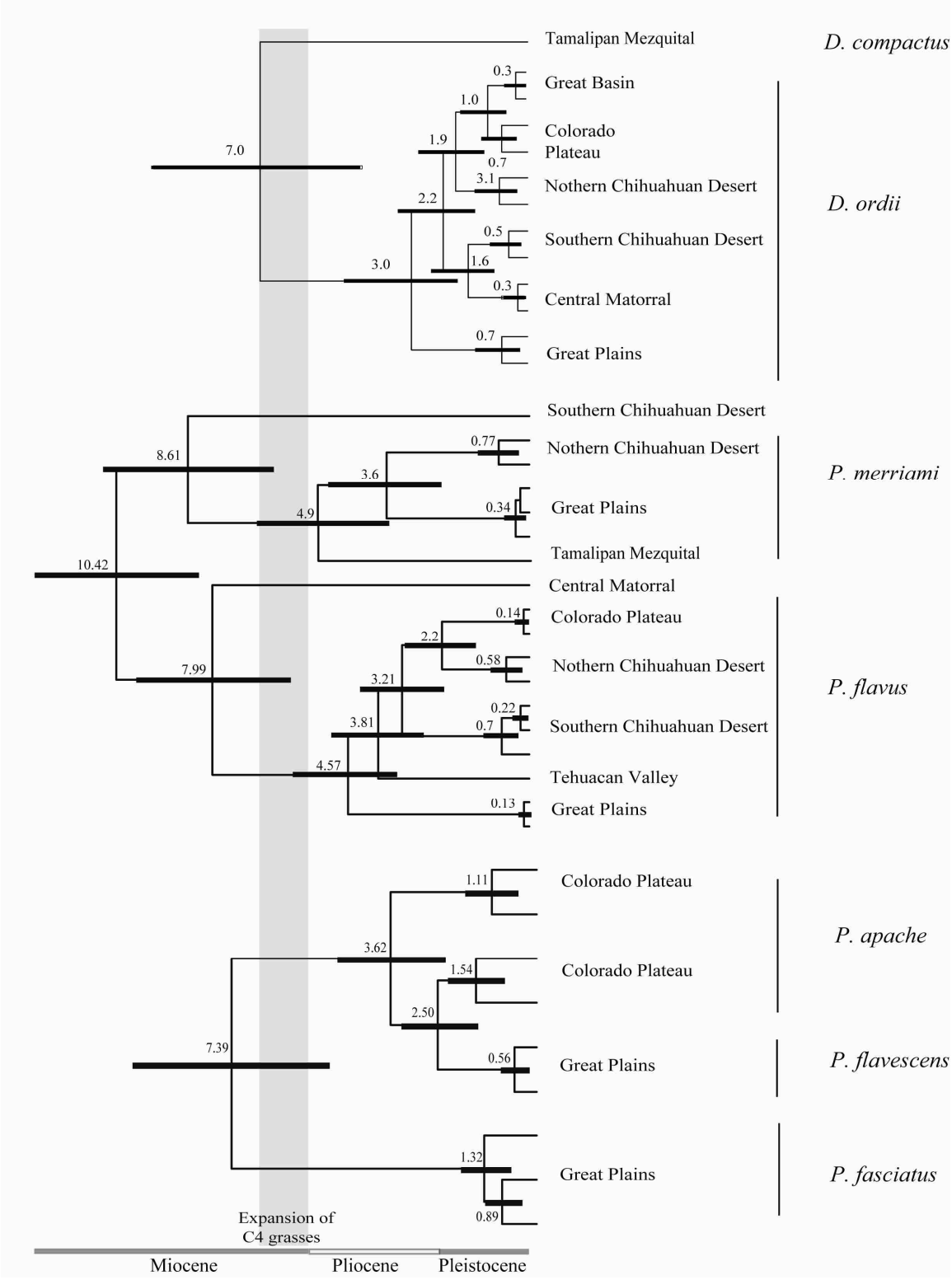
Tamalipan Mezquital
D. compactus
0.3
Great Basin
1.0
Colorado Plateau
1.9
7.0
0.7
3.1
Nothern Chihuahuan Desert
2.2
D. ordii
0.5
Southern Chihuahuan Desert
3.0
1.6
0.3
Central Matorral
0.7
Great Plains
Southern Chihuahuan Desert
0.77
Nothern Chihuahuan Desert
8.61
3.6
P. merriami
0.34
Great Plains
4.9
Tamalipan Mezquital
10.42
Central Matorral
0.14
Colorado Plateau
2.2
0.58
Nothern Chihuahuan Desert
7.99
3.21
P. flavus
0.22
3.81
0.7
Southern Chihuahuan Desert
4.57
Tehuacan Valley
0.13
Great Plains
1.11
Colorado Plateau
P. apache
3.62
1.54
Colorado Plateau
2.50
7.39
0.56
Great Plains
P. flavescens
1.32
Great Plains
P. fasciatus
0.89
Expansion of C4 grasses
Miocene
Pliocene
Pleistocene

Figure 4.4

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APPENDIX 1

SPECIMENS EXAMINED - *PEROGNATHUS FLAVUS* SPECIES GROUP

The 1st column of numbers refers to localities displayed in Fig. 2.1 (some numbers refer to more than 1 specific locality). Voucher acronyms are as follows: ASNHC and ASK, Angelo State Natural History Collection; CNMA, Universidad Nacional Autonoma de Mexico; DTZM and ZM, Denver Museum of Nature and Science; LSUMZ and M, Louisiana State University Museum of Natural Science; LVT, University of Nevada, Las Vegas tissue collection; MHP, Sternberg Museum; MSB and NK, Museum of Southwestern Biology; MVZ, Museum of Vertebrate Zoology; NMMNH, New Mexico Museum of Natural History; PF, personal catalogue of P. Stapp; TLB, sample provided by T. L. Best; TTU and TK, the Museum of Texas Tech University; UAMI, Universidad Autonoma Metropolitana-Iztapalapa. PF specimens are represented by ear clips only. Samples marked B in the Clock column represent individuals used from the *COIII* and *IRBP* data sets for divergence estimates in BEAST.

	Locality	Museum number	Other number	Clock
1	Arizona, Navajo County, 2 mi E, 3 mi N Winslow	NMMNH 5722	LVT 9261	
		NMMNH 5733	LVT 9275	
		NMMNH 5734	LVT 9276	
		NMMNH 5735	LVT 9277	B
2	Arizona, Pima County, Sonoita	NMMNH 5805	LVT 9339	B
		NMMNH 5807	LVT 9346	
3	Texas, Brewster County, Elephant Mountain	NMMNH 4619	LVT 6545	
		NMMNH 4626	LVT 6559	B

		NMMNH 4631	LVT 6569	
4	Chihuahua, Mexico, 1mi E El Mesquite	NMMNH 4554	LVT 6422	B
		NMMNH 4470	LVT 6423	
		NMMNH 4555	LVT 6424	
		NMMNH 4471	LVT 6425	
5	Chihuahua, Mexico, 30 km W Coyame	NMMNH 5256	LVT 7584	
		NMMNH 5258	LVT 7585	
		NMMNH 5257	LVT 7586	
		NMMNH 5254	LVT 7587	
		NMMNH 5255	LVT 7588	
6	Chihuahua, Mexico, 35 km SW Hercules	NMMNH 5277	LVT 7627	
7	Chihuahua, Mexico, 4 km SW Parrita	NMMNH 2442	LVT 1050	
		NMMNH 2443	LVT 1051	
		NMMNH 2521	LVT 1052	
		NMMNH 2444	LVT 1053	
		NMMNH 2525	LVT 1054	
		NMMNH 2445	LVT 1055	
		NMMNH 2522	LVT 1056	
		NMMNH 2446	LVT 1057	
		NMMNH 2523	LVT 1058	
		NMMNH 2447	LVT 1059	
		NMMNH 2524	LVT 1060	
8	Chihuahua, Mexico, 6 km E El Sueco	NMMNH 5181	LVT 8726	
9	Chihuahua, Mexico, 6 km NW Manuel Benavides	NMMNH 5211	LVT 7596	
		NMMNH 5263	LVT 7597	
		NMMNH 5212	LVT 7598	
		NMMNH 5264	LVT 7599	

		NMMNH 5213	LVT 7600	
10	Chihuahua, Mexico, 6 mi NW Ricardo Flores Magon	NMMNH 4459	LVT 6401	
		NMMNH 4460	LVT 6403	
11	Colorado, Pueblo County, Fort Carson, Niobrabra	ZM.11527	DTZM 118	
12	Colorado, Ward County, Pawnee National Grass Land	Tissue only	PF 0061	B
		Tissue only	PF 1162	B
		Tissue only	PF 0028	
		Tissue only	PF 1218	
		Tissue only	PF 1331	
		Tissue only	PF 1358	
		Tissue only	PF 1585	
13	Coahuila, Mexico, 1 mi SE Hundido	NMMNH 2586	LVT 1171	
14	Coahuila, Mexico, 2 mi E Agua Nueva	NMMNH 4668	LVT 6634	
15	Coahuila, Mexico, 2 km S Santa Teresa	NMMNH 4684	LVT 6676	
		NMMNH 4685	LVT 6677	B
15	Coahuila, Mexico, Plan de Guadalupe	NMMNH 4674	LVT 6648	B
		NMMNH 4728	LVT 6649	
		NMMNH 4675	LVT 6650	
		NMMNH 4676	LVT 6651	
16	Durango, Mexico, 4 km SSE La Zarca	CNMA 41886		
16	Durango, Mexico, 7 mi NNW La Zarca	NMMNH 2473	LVT 1109	
		NMMNH 2550	LVT 1110	
17	Durango, Mexico, 20 km S, 10 km E Torreon de Canas	NMMNH 3611	LVT 4834	
		NMMNH 3612	LVT 4835	B
18	Durango, Mexico, 5 km NW La Union	NMMNH 5412	LVT 8864	

19	Durango, Mexico, Hidalgo Atotonilco	NMMNH 4580	LVT 6467	
20	New Mexico, Eddy County, 4 mi S, 2 mi W Whites City	NMMNH 4245	LVT 5882	
21	Kansas, Logan County, 2.5 mi S, 7.5 mi W Russell Springs	MHP 37500		
21	Kansas, Scott County, 12 mi N, 1 mi W Scott City	MHP 37501		
22	Kansas, Wallace County, 2.5 mi N, 9.75 mi W Russell Springs	MHP 37502		
23	Arizona, Navajo County, 3 mi S Kayenta	NMMNH 3226	LVT 702	B
24	New Mexico, Bernalillo County, 3 mi N, 5.5 mi W Albuquerque	NMMNH 1907		
24	New Mexico, Bernalillo County, 5.5 mi N, 4.5 mi W Albuquerque	NMMNH 1869		B
25	New Mexico, Catron County, Quemado, Zuni Salt Lake	MSB 88073	NK 78038	
		MSB 88074	NK 78039	
		MSB 88075	NK 78040	
		MSB 87776	NK 78043	
		MSB 88099	NK 78063	
26	New Mexico, Chaves County, Bottomless Lakes State Park	MSB 74134	NK 65570	
26	New Mexico, Chaves County, Bitter Lake NWR, T10S R25E Sec 21	NMMNH 2366		
26	New Mexico, Chaves County, Dexter Fish Hatchery, T13S R26E Sec 16	NMMNH 2368		
27	New Mexico, Cibola County, 4 mi S, 1.5 mi W Correo	NMMNH 3943		
		NMMNH 3944		
		NMMNH 3945		

28	New Mexico, Hidalgo County, 2 mi NW Cloverdale	NMMNH 5810	LVT 9869	
29	New Mexico, Hidalgo County, Doubtful Canyon, 8 mi N, 0.5 mi W Steins	NMMNH 4417	LVT 6162	
		NMMNH 4418	LVT 6163	
		NMMNH 4423	LVT 6168	B
		NMMNH 4424	LVT 6169	
		NMMNH 4425	LVT 6170	
		NMMNH 4428	LVT 6173	
30	New Mexico, Lincoln County, Valley of Fires Camp Ground	MSB 146017	NK 133625	
		MSB 146182	NK 133710	
		MSB 146184	NK 133712	
31	New Mexico, Sandoval County, Placitas Web	MSB 90727	NK 86776	
31	New Mexico, Sandoval County, Star Lake	NMMNH 4161		
32	New Mexico, McKinley County, 15 mi N Crownpoint of Hwy 371	MSB 88136	NK 78017	
		MSB 88137	NK 78018	
32	New Mexico, San Juan County, 57 km S of Farmington	MSB 90210	NK 86410	
		MSB 90214	NK 86414	
		MSB 90215	NK 86415	
		MSB 90218	NK 86418	
		MSB 90221	NK 86421	
33	New Mexico , Otero County, White Sands Missle Range	MSB 85857	NK 40934	
34	New Mexico, Soccoro Co, 10 mi S, 20 mi W San Marcial	NMMNH 3953		
		NMMNH 3954		

		NMMNH 3955		
35	Puebla, Mexico, 1.4 km E San Miguel Zozutla	UAMI 16220		
35	Puebla, Mexico, 3 km S Ciudad Serdan	LSUMZ 36684	M 8609	B
36	San Luis Potosi, Mexico, 3 mi S Matehuala	NMMNH2514	LVT 1198	
37	San Luis Potosi, Mexico, 13 km NE Villa de Reyes	CNMA 43018		
38	San Luis Potosi, Mexico, 10 mi S Villa de Ramos	NMMNH 3705	LVT 4912	
38	San Luis Potosi, Mexico, 10 mi W Salinas	NMMNH 3669	LVT 4889	B
39	Texas, Dimmit County, Chaparral Wildlife Management Area	TTU 98021	TK 98098	
		TTU 98132	TK 98101	
		TTU 98137	TK 98154	B
		TTU 98172	TK 98174	
40	Texas, La Salle County, Chaparral Wildlife Management Area	TTU 80785	TK 84610	
		TTU 80898	TK 84686	
		TTU 98474	TK 98046	
		TTU 98501	TK 98235	
41	Texas, Kerr County, Kerr Wildlife Management Area	TTU 98346	TK 111603	
		TTU 102269	TK 115305	
41	Texas, Kimble County, Texas Tech University Center at Junction	TTU 77847	TK 78167	B
42	Texas, Lynn County, 2 mi S, 5 mi E Tahoka	TTU 77563	TK 51911	
		TTU 77565	TK 51914	
43	Texas, Tom Green County, San Angelo State Park	ASNHC 11852	ASK 4480	
		ASNHC 11858	ASK 5720	
44	Texas, Val Verde County, 12.8 mi W Del Rio	ASNHC 3901	ASK 1074	
44	Texas, Val Verde County, Devil's River SNA	ASNHC 11004	ASK 4973	B

		ASNHC 11006	ASK 4979	
		ASNHC 11008	ASK 4982	
45	Texas, Wilbarger County, 2 mi W Harrold	TLB 10568	LVT 2063	B
46	Zacatecas, Mexico, 1 mi SE Banon	NMMNH 4601	LVT 6509	
47	Zacatecas, Mexico, 2 mi E San Jeronimo	NMMNH 4497	LVT 6479	

APPENDIX 2

SPECIMENS EXAMINED - *PEROGNATHUS FASCIATUS* SPECIES GROUP

The first column corresponds to numbered localities in Fig 3.1 (some numbers refer to more than one specific locality). Abbreviations are as follows: ASNHC and ASK – Angelo State Natural History Collection, LVT – University of Nevada, Las Vegas tissue collection, MHP – Sternberg Museum, MSB and NK – Museum of Southwestern Biology, NMMNH – New Mexico Museum of Natural History, PF – personal collection of Paul Stapp, TTU and TK – The Museum Texas Tech, RAM – Royal Alberta Museum, ZM – Denver Museum of Nature and Science. Samples used in the BEAST analysis are labeled with B under Clock column.

	Locality	Museum number	other number	Clock
1	Canada, Alberta, 7.5 miles S, 5 miles E of Cavendish	RAM01.16.8		
1	Canada, Alberta, 7 miles S, 2 miles E of Cavendish	RAM01.16.9		
1	Canada, Alberta, 1mile S, 2 miles E of Cavendish	RAM01.16.10		
1	Canada, Alberta, 0.75 miles S, 2 miles E of Cavendish	RAM01.16.11		
1	Canada, Alberta, Canadian Forces Base Suffield, near Medicine Hat	RAM95.30.3		
		RAM95.30.4		
		RAM95.30.5		
2	Arizona, Coconino County, 7 miles N Cameron	NMMNH3221	LVT694	
3	Arizona, Navajo County, Petrified Forerst National Park, 1.0 miles S, 0.4 miles E Rainbow Forest Museum	MSB123108	NK39511	
4	Colorado, Custer County, 9 miles NE Silver Cliff	ZM.12004	LVT9897	
		ZM.12005	LVT9898	B
5	Colorado, Moffat County	MSB76580	NK56312	
		MSB76579	NK56327	
6	Colorado, Ward County, Pawnee NGL	tissue only	PF1357	

7	Colorado, Weld County, 4 miles S Roggen	NMMNH3249	LVT2534	
8	Kansas, Dickinson County, 2 miles N, 3 miles W Abilene	MHP37499		
9	Kansas, Finney County, 4 miles S Holcomb	NMMNH3251	LVT2536	
		NMMNH3253	LVT2538	
		NMMNH3254	LVT2539	
10	Montana, Roosevelt County 9 miles SE Bainville	NMMNH3264	LVT2549	
		NMMNH3265	LVT2550	
11	North Dakota, Ransom County, Sheyenne NGL	tissue only	LVT9940	
		tissue only	LVT9941	
		tissue only	LVT9942	B
		tissue only	LVT9943	
		tissue only	LVT9944	
		tissue only	LVT9945	
12	Nebraska, Sheridan County, 27 miles N Lakeside	NMMNH3241	LVT2526	
		NMMNH3242	LVT2527	
13	New Mexico, Chaves County, 5 miles W Kenna	NMMNH3259	LVT2544	
		NMMNH3260	LVT2545	
14	New Mexico, De Baca County, 16 miles S, 3 miles E. Taiban	ASNHC3658	ASK1091	
15	New Mexico, Lea County, 20 miles W Hobbs	NMMNH3258	LVT2543	
16	New Mexico, McKinley Co, 1 miles N NM HWY 53 on Zuni	MSB86409	NK76125	
		MSB88493	NK83440	B
17	New Mexico, Mora County, 6 miles N Logan	NMMNH3255	LVT2540	
18	New Mexico, San Juan County, 38 miles S Farmington	NMMNH3267	LVT2307	
		NMMNH3268	LVT2308	
19	New Mexico, Socorro County, Sevilleta National Wildlife Refuge, Rio Salado GL	MSB67861	NK19796	
		MSB140993	NK45148	B
		MSB140978	NK47056	
		MSB67865	NK24305	
20	Texas, Ward County, Monahans Sandhills State Park, 1 mile N headquarters building	TTU100239	TK69523	

		TTU100241	TK69570	
		TTU100240	TK69602	B
21	Utah, San Juan County, 16 miles N Monticello	tissue only	LVT9905	B
		tissue only	LVT9906	
		tissue only	LVT9907	
		tissue only	LVT9908	
		tissue only	LVT9909	
		tissue only	LVT9910	
22	Utah, Uintah County, 6 miles S Bonanza	tissue only	LVT9920	
		tissue only	LVT9921	
		tissue only	LVT9922	
23	Utah, Emery County, 7 miles S Green River	tissue only	LVT9913	
		tissue only	LVT9914	
		tissue only	LVT9915	
		tissue only	LVT9916	
		tissue only	LVT9917	
		tissue only	LVT9918	B
24	Utah, Grand County, 10 miles N Moab	NMMNH3222	LVT698	
		NMMNH3223	LVT699	
		NMMNH3224	LVT700	
		NMMNH3225	LVT701	
25	Utah, San Juan County, Mexican Hat	MSB76896	NK55146	
		MSB76894	NK55134	
		MSB76895	NK55142	
26	Wyoming, Carbon Co, 10 miles S Seminoe Dam	NMMNH3240	LVT2525	
27	Wyoming, Natrona County, 25 miles NW Independence Rock	NMMNH5792	LVT9314	B
		NMMNH5793	LVT9315	
		NMMNH5794	LVT9316	
28	Wyoming, Sweetwater Co, 10 miles SE Eden	NMMNH3266	LVT2551	
28	Wyoming, Sweetwater County, 28 miles N Green River on CR5	NMMNH5801	LVT9331	

		NMMNH5802	LVT9332	B
		NMMNH5803	LVT9333	
29	Wyoming, Sweetwater Co, 18.1 miles S Bitter Creek	NMMNH5774	LVT9287	
29	Wyoming, Sweetwater Co, 25 miles S Bitter Creek	NMMNH5769	LVT9282	
		NMMNH5770	LVT9283	
		NMMNH5771	LVT9284	

APPENDIX 3

SPECIMENS EXAMINED - *DIPODOMYS ORDII* AND *D. COMPACTUS*

The first column corresponds to numbered localities in Figure 4.1 (some numbers refer to more than one specific locality). Abbreviations are as follows: ASNHC and ASK – Angelo State Natural History Collection, LVT – University of Nevada, Las Vegas tissue collection, MHP – Sternberg Museum, NMMNH – New Mexico Museum of Natural History, RAM – Royal Alberta Museum. Samples used in the BEAST analysis are labeled with B under Clock column.

	Locality	Museum number	Tissue number	Clock
1	Canada, Alberta, 13.5 miles north, 4 miles west of Burstall	RAM 04.7.1		
	Canada, Alberta, 1 mile north, .5 miles east of Social Plains	RAM 04.3.1		B
2	Mexico, Chihuahua,27 km S, 12 km E El Sueco		LVT 8730	
	Mexico, Chihuahua,27 km S, 12 km E El Sueco		LVT 8731	
	Mexico, Chihuahua,27 km S, 12 km E El Sueco		LVT 8732	
	Mexico, Chihuahua,27 km S, 12 km E El Sueco		LVT 8733	
	Mexico, Chihuahua,27 km S, 12 km E El Sueco		LVT 8734	
3	Mexico, Coahuila, 2 mi E Agua Nueva		LVT 6619	
	Mexico, Coahuila, 2 mi E Agua Nueva		LVT 6620	
	Mexico, Coahuila, 2 mi E Agua Nueva		LVT 6621	
	Mexico, Coahuila, 2 mi E Agua Nueva		LVT 6622	
	Mexico, Coahuila, 2 mi E Agua Nueva		LVT 6623	
4	Mexico, Coahuila, 5 km E San Francisco		LVT 7641	
	Mexico, Coahuila, 5 km E San Francisco		LVT 7643	
5	Mexico, Durango, 7 mi NNW La Zarca		LVT 1108	
6	Mexico, Durango, 20 km S, 10 km E Torreon de Cana		LVT 4827	
	Mexico, Durango, 20 km S, 10 km E Torreon de Cana		LVT 4831	
	Mexico, Durango, 20 km S, 10 km E Torreon de Cana		LVT 4832	

	Mexico, Durango, 20 km S, 10 km E Torreon de Cana	LVT 4833	
	Mexico, Durango, 20 km S, 10 km E Torreon de Cana	LVT 4837	
7	Mexico,Durango, Hidalgo Atotonilco	LVT 6463	B
8	Mexico, Zacatecas, 2 mi E San Jeronimo	LVT 6473	B
9	Mexico, Zacatecas, 1 mi SE Banon	LVT 6510	
	Mexico, Zacatecas, 1 mi SE Banon	LVT 6511	
	Mexico, Zacatecas, 1 mi SE Banon	LVT 6512	
	Mexico, Zacatecas, 1 mi SE Banon	LVT 6514	
	Mexico, Zacatecas, 2 mi SE Banon	LVT 6515	
	USA, Arizona, Cochise County, 6 mi SW Apache, jct. Price		B
10	Canyo	LVT 5860	
11	USA, Arizona, Coconino County, House Rock Valley	LVT 9362	B
12	USA, Arizona, Pima County,10 miles NE Sonoita	LVT 9864	
13	USA, California, ModocCounty, 5 mi E Eagleville	LVT 8907	
14	USA, Colorado, Baca County, 5 miles E. Campo	LVT 9272	
	USA, Colorado, Baca County, 5 miles E. Campo	LVT 9273	
15	USA, Colorado, Moffat County, 40 mi NW Craig	LVT 9892	
	USA, Colorado, Moffat County,19 mi NW Craig	LVT 9893	
16	USA, Colorado, Saquache County,1mile S, 2 miles W. Moffat	LVT 9269	
	USA, Colorado, Saquache County,1mile S, 2 miles W. Moffat	LVT 9270	
17	USA, Idaho, Owyhee County, 5 mi W Murphy	LVT 8984	
18	USA, Kansas, Gove County,	KK 1981	
19	USA, Kansas, Meade County,	KK 1638	
	USA, Kansas, Seward County,	KK 1219	
	USA, Kansas, Seward County,	KK 1260	
	USA, Kansas, Seward County,	KK 1265	
	USA, Kansas, Seward County,	KK 1310	
20	USA, Kansas, Morton County,	KK 863	
21	USA, Kansas, Osborne County,	KK 1581	
22	USA, Kansas, Rawlins County,	KK 1030	
	USA, Kansas, Rawlins County,	KK 1909	

	USA, Kansas, Rawlins County,		KK 1910	
	USA, Kansas, Rawlins County,		KK 1911	
23	USA, Nevada, Churchill County, 10 mi W Fallon		LVT 9577	
	USA, Nevada, Churchill County, 10 mi W Fallon		LVT 9578	
24	USA, Nevada, Elko County, Tecoma		LVT 9654	
	USA, Nevada, Elko County, Montello		LVT 9268	B
	USA, Nevada, Elko County, Montello		LVT 9663	
25	USA, Nevada, Eureka County, 5 mi SSW Beowawe		LVT 9637	
	USA, Nevada, Eureka County, 5 mi SSW Beowawe		LVT 9643	
	USA, Nevada, Eureka County, 3 mi SSW Beowawe		LVT 9647	
26	USA, Nevada, Lincoln County, Lake Valley 3		LVT 7820	
	USA, Nevada, Lincoln County, Lake Valley 3		LVT 7828	
	USA, Nevada, White Pine County, Spring Valley 4		LVT 7897	
27	USA, New Mexico, Bernalillo County,3 mi N, 4.5 mi W Albuquerque	NMMNH 2276		
28	USA, New Mexico, Dona Ana County, Abondoned Ranch, 25 mi. W. El Paso		LVT 0381	
29	USA, New Mexico, Grant County, 2.6 mi N, 1.8 mi E Redrock		LVT 6121	
	USA, New Mexico, Grant County, 2.6 mi N, 1.8 mi E Redrock		LVT 6122	
30	USA, New Mexico, Lea County, 4 mi. S, 3 mi. W Maljamar	NMMNH 3858		B
31	USA, New Mexico, Taos County, Urraca State Wildlife Area, 14 mi.	NMMNH 1864		
	USA, New Mexico, Taos County, Urraca State Wildlife Area, 14 mi.	NMMNH 2311		
32	USA, Oregon, Harney County, Fields		LVT 8964	
33	USA, Oregon, Lake County, Alkali Lake		LVT 8935	B
34	USA, Texas, Brewster County, Elephant Mountain WMA		LVT 6549	B
	USA, Texas, Brewster County, Elephant Mountain WMA		LVT 6553	
	USA, Texas, Brewster County, Elephant Mountain WMA		LVT 6554	
	USA, Texas, Brewster County, Elephant Mountain WMA		LVT 6556	
35	USA, Texas, Dimmit County,		ASK 5013	B

	USA, Texas, Dimmit County,	ASK 5015	
	USA, Texas, Dimmit County,	ASK 5016	
36	USA, Utah, Millard County, 16 mi S Delta	LVT 8595	B
	USA, Utah, Millard County, 16 mi S Delta	LVT 8604	
37	USA, Utah, Tooele County, Rush Valley	LVT 8605	
	USA, Utah, Tooele County, Rush Valley	LVT 8607	
38	USA, Utah, San Juan County, 1 mile N Mexican Hat	LVT 9900	
	USA, Utah, San Juan County, 1 mile N Mexican Hat	LVT 9901	
39	USA, Utah, San Juan County, 16 miles N Monicello	LVT 9904	
	USA, Utah, San Juan County, 16 miles N Monicello	LVT 9912	
40	USA, Utah, Emery County, 7 miles S Green River	LVT 9919	B
41	USA, Wyoming, Carbon County, 23 mi N Sinclair	LVT 9290	
	USA, Wyoming, Carbon County, 23 mi N Sinclair	LVT 9291	
41	USA, Wyoming, Natrona County, 25 mi NW Independence Rock	LVT 9324	
42	USA, Wyoming, Sweetwater County, 27 mi N 37 mi E Rock Springs	LVT 9293	
43	USA, Wyoming, Sweetwater County, Seedskadee National Wildlife Refuge	LVT 9294	
	USA, Wyoming, Sweetwater County, Seedskadee National Wildlife Refuge	LVT 9295	
43	USA, Wyoming, Sweetwater County, 28 mi N Green River on CR5	LVT 9334	
44	USA, Wyoming, Sweetwater County, 51 mi S Rock Springs	LVT 9889	
45	USA, Wyoming, Weston County, 24 mi SW Newcastle	LVT 9301	
45	USA, Wyoming, Weston County, 19 mi SW Newcastle	LVT 9302	
46	USA, Texas, Klegberg County, Padre Island National Seashore	LVT 2060	B

APPENDIX 4

SPECIMENS EXAMINED - OUTGROUP TAXA

Outgroup taxa used in molecular clock analyses. Abbreviations are as follows: NMMNH – New Mexico Museum of Natural History, LVT – University of Nevada, Las Vegas tissue collection, TLB – sample from Troy L. Best.

Species	Locality	Museum number	Other number	*COIII* GenBank number
Chaetodipus formosus	California, Riverside County, 9 miles W, 1 miles S Quien Sabe	NMMNH 2395	LVT 987	AY926424
Dipodomys californicus	California, Tehama County, 6 miles NE Dales		LVT 2037/ TLB 10357	AY926435
D. deserti	Nevada, Clark County, St. Thomas Gap		LVT 2083	AY926448
D. nelsoni	Mexico, Durango, 7 miles NNW La Zarca	NMMNH 2472	LVT 1107	AY926431
D. spectabilis	New Mexico, Socorro County, San Mateo Mountains, Nogal Canyon		LVT 2470	AY926449
Microdipodops megacephalus	Nevada, Lincoln County, 6 miles N, 31 miles W Hiko		LVT 5155	AY926429
M. pallidus	Nevada, Lincoln County, 7 miles N, 6.45 miles Tempiute		LVT 1573	AY926428
P. amplus	Arizona, Pima County, 0.5 miles N Organ Pipe Cactus National Monument	NMMNH 3297	LVT 403	AY926414
P. fasciatus	Wyoming, Carbon County, 10 miles S Semilesnoe	NMMNH 3240	LVT 2525	AY926421
P. flavescens	Nebraska, Sheridan County, 27 miles N Lakeside	NMMNH 3242	LVT 2527	AY926422
P. flavus	Chihuahua, Mexico, 4 km SW Parrita	NMMNH 2442	LVT 1050	GQ470299

P. longimembris	Mexico, Baja California, 27 km S Punta Prieta	NMMNH 2978	LVT 2191	AY926420
P. merriami	Texas, Val Verde County, Devil's River SNA	ASNHC 11006	ASK 4979	GQ470343
P. parvus	Utah, Wayne County, 9 miles S, 2 miles W Hanksville	NMMNH 3186	LVT 1816	AY926418
P. parvus	Washington, Adams County, 4 miles S, 6 miles E Ritzville	NMMNH 3198	LVT 1920	AY926419

APPENDIX 5

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VITA

Graduate College
University of Nevada, Las Vegas

Sean A. Neiswenter

Degrees:

Bachelor of Science, Biology 2002
Angelo State University

Master of Science, Biology 2004
Angelo State University

Special Honors and Awards:

Carr Research Fellowship, Angelo State University, 2004
Elmer C. Birney Award, American Society of Mammalogists, 2009
BIOS Publication Award, University of Nevada, Las Vegas, 2010

Publications:

Neiswenter, S. A. and B. R. Riddle. 2010. Evolution of silky pocket mice in the *Perognathus flavus* species group: diversification associated with emerging arid grasslands in western North America. Journal of Mammalogy 98:348-362.

Matthews, A. K., **S. A. Neiswenter**, L. K. Ammerman. 2010. Trophic ecology of free-tailed bats *Nyctinomops femorosaccus* and *Tadarida brasiliensis* (Chiroptera: Molossidae) from Big Bend National Park, Texas. Southwestern Naturalist 55:340-346.

Neiswenter, S. A., R. C. Dowler, and J. H. Young. 2010. Activity patterns and movements of two skunk species in Texas. Southwestern Naturalist 55:16-21.

Neiswenter, S. A. and R. C. Dowler. 2007. Habitat use of skunks in west central Texas. Journal of Wildlife Management 71 (2): 583-588.

Neiswenter, S. A., D. B. Pence, and R. C. Dowler. 2006. Helminths of sympatric striped, hog-nosed, and spotted skunks in west central Texas. Journal of Wildlife Diseases 42(3): 511-517.

Dissertation Title: Phylogeography of Three Heteromyid Taxa: Insight on the Evolution of a North American Arid Grassland Rodent Guild

Dissertation Examination Committee:

Chairperson, Brett R. Riddle, Ph. D.
Committee Member, Dave J. Hafner, Ph. D.
Committee Member, John Klicka, Ph. D.
Committee Member, Dan B. Thompson, Ph. D.
Graduate Faculty Representative, Chad L. Cross, Ph. D.