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Diversification of the *Perognathus flavus* species group in emerging arid grasslands of western North America

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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 interphotoreceptor retinoid-binding protein (*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. DOI: 10.1644/09-MAMM-A-102.1.

Key words: biogeography, grasslands, Heteromyidae, mitochondrial DNA, nuclear DNA, *Perognathus*, phylogeography, silky pocket mice

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.

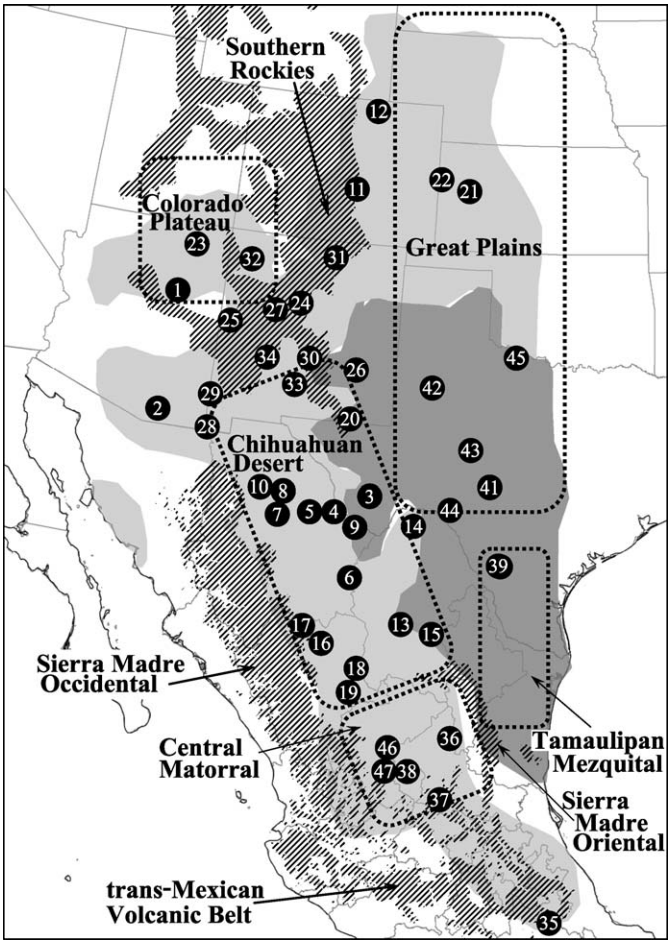

Fig. 1.—Geographic ranges of the 2 species in the *Perognathus flavus* species group redrawn from Hall (1981), with species boundaries estimated from Lee and Engstrom (1991) and Brant and Lee (2006). Light shading reflects the geographic range of *P. flavus* and dark shading the range of *P. merriami*. Locality numbers as per Appendix I. Hatched areas indicate major mountain ranges, and dashed polygons show approximate location of ecoregions from *Wild World Terrestrial Ecoregions of the World* (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.

The foci of our study are 2 nominal species of silky pocket mice that comprise the *P. flavus* species group, *P. flavus* and *P. merriami* (Fig. 1). *P. 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 before 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. 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. 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, examination of allozyme data indicates 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. 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 *Chaetodipus 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 mitochondrial DNA (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 I). Those that were field-collected for this study were handled in accordance with 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 collections. Other specimens included preserved tissues obtained from museum collections or ear clips obtained from private collections (Appendix I).

We sequenced 644 base pairs (bp) of *COIII* and 440 bp of *CR* from up to 10 individuals per locality for a total of 132 specimens (Appendix I; Fig. 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).

The DNA was extracted from preserved tissues using DNeasy kits (Qiagen Inc., Germantown, Maryland). Polymerase chain reaction 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 polymerase chain reaction primers: *COIII*, H8618 and L9232 (Riddle 1995); *CR*, 16007 and 16498 (Kocher et al. 1989; Meyer et al. 1990); and *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 MEGA4 (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 analyses performed separately on each mtDNA sequence, and on a *COIII + CR* combined data set. The *IRBP* sequence was analyzed separately, 1st, 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 2nd, because by combining the 2 we could lose information about the independent history of 1 of the genomes. *P. 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* 4.0b10 (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 analyses were performed using the program TREEFINDER (Jobb 2008) and the same models of evolution used in the Bayesian analyses. Node support for the maximum-likelihood 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 maximum-likelihood 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*, *Chaetodipus formosus*, *P. longimembris*, *P. amplus*, *P. parvus*, *P. fasciatus*, and *P. flavescens* (Appendix I). *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 and 8 outgroup; Appendix I) 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 clocklike evolution using a likelihood ratio test. Maximum-likelihood scores were compared in PAUP* 4.0b10 (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 *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 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 to 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,

Table 1.—Uncorrected pairwise divergence values between major mitochondrial DNA 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

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 (5.6–19.6% divergence; Table 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). For clarity, and not necessarily in accord with current species-level taxonomy, we refer to each major clade using the following naming system: *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; *flavus* clade, a 2nd deep clade that contains most of the individuals currently recognized as *P. flavus*; Meseta Central Matorral clade, a newly recognized lineage with known distribution restricted to the Meseta Central Matorral ecoregion; and 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).

Two of the major clades contain multiple, well-supported phylogroups. A basal near-simultaneous diversification within the *merriami* clade (Fig. 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. 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) were supported by the *IRBP* analysis (Fig. 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) is replaced by a sister relationship between the Meseta Central Matorral and Southern Chihuahua Desert clades in the *IRBP* tree (Fig. 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. 4) might have begun diverging as early as the late Miocene, although some discrepancy exists between the mitochondrial and nuclear genes (Table 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).

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

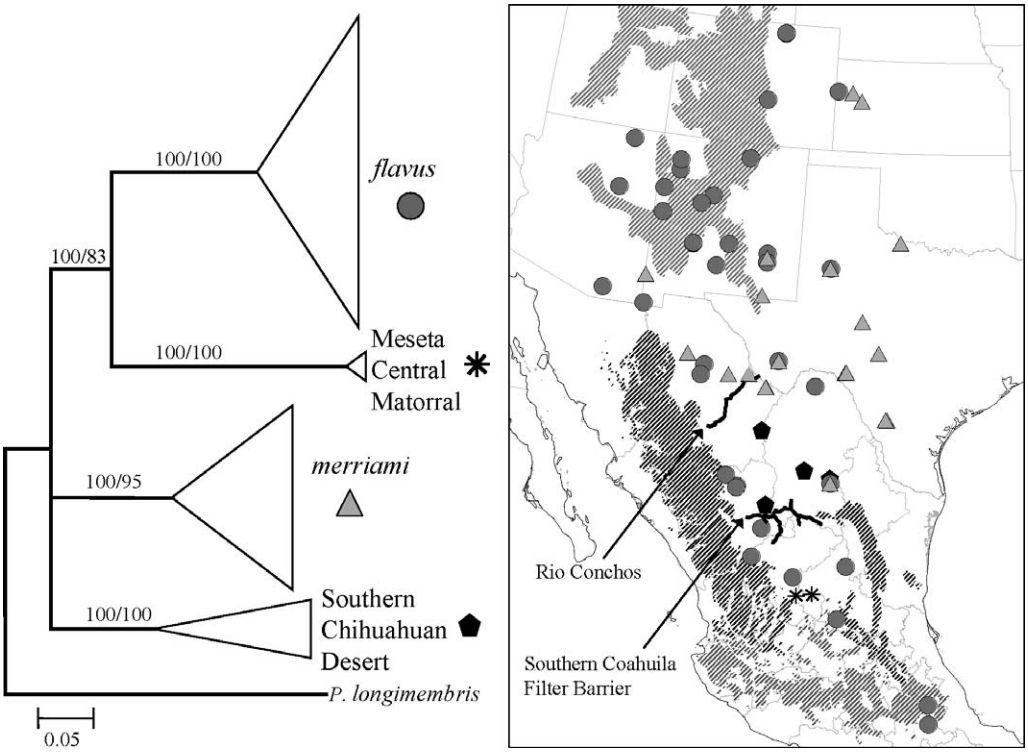

Fig. 2.—Majority rule consensus tree (50%) generated from Bayesian analysis of concatenated mitochondrial DNA sequences from representatives of the *Perognathus flavus* species group, and map showing the distribution of each clade. Hatched areas indicate major mountain ranges. Posterior probability values followed by maximum-likelihood bootstrap values are shown for supported nodes. Scale represents expected changes per site from Bayesian analysis.

Southern Chihuahua Desert clades appear to be narrowly distributed, whereas the *merriami* and *flavus* lineages are more broadly distributed and genetically diverse (Table 1; Fig. 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 (5.6–19.6%; Table 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), and phylogroup divergences within the *merriami* and *flavus* lineages (Table 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 southeast. 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) clearly are not consistent

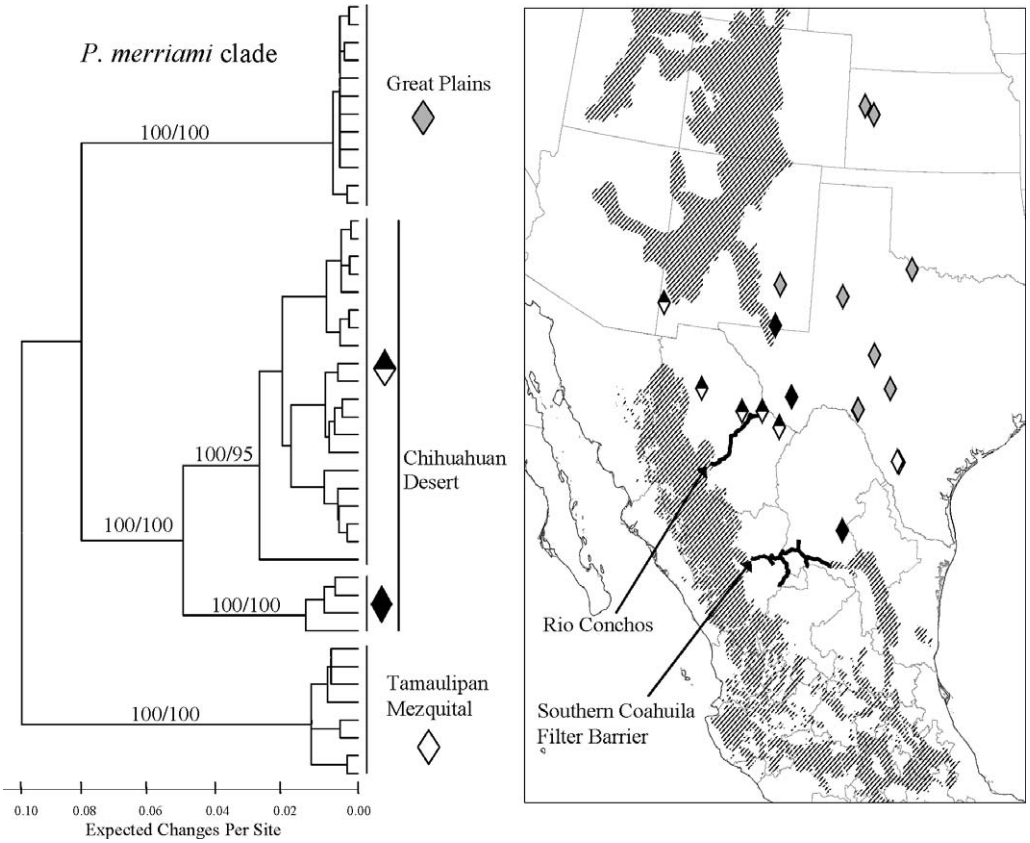

Fig. 3.—Linearized phylogram of 50% majority rule consensus tree generated from Bayesian analysis of concatenated mitochondrial DNA sequences from representatives of the *Perognathus merriami* lineage, and map showing distribution of phylogroups. Hatched areas indicate major mountain ranges. Posterior probability values followed by maximum-likelihood bootstrap values are shown for supported nodes.

with the currently accepted distribution of *P. flavus* and *P. merriami* (Brant and Lee 2006; Lee and Engstrom 1991; Fig. 1). Although examination of our data suggests a general east–west pattern of 2 deeply divergent lineages (the *merriami* and *flavus* clades), the *merriami* clade extends much farther 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 before 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), 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

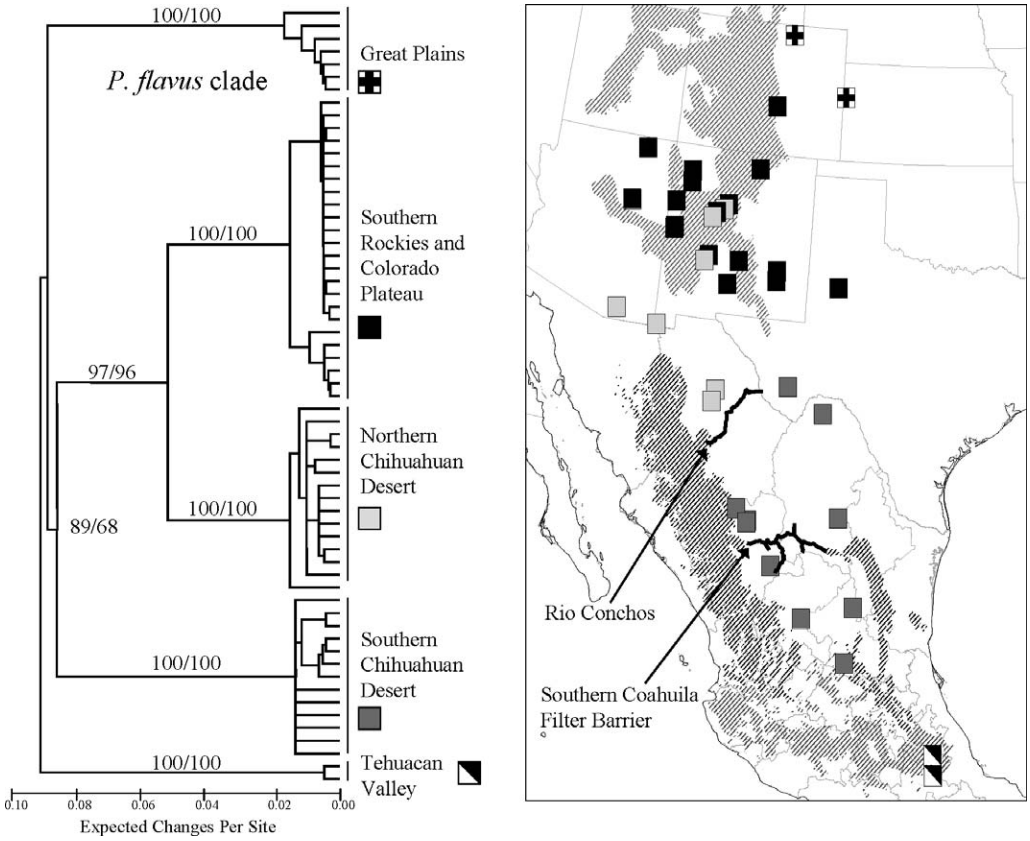

Fig. 4.—Linearized phylogram of 50% majority rule consensus tree generated from Bayesian analysis of concatenated mitochondrial DNA sequences from representatives of the *Perognathus flavus* clade, and map showing distribution of phylogroups. Hatched areas indicate major mountain ranges. Posterior probability values followed by maximum-likelihood bootstrap values are shown for supported nodes.

consistent with the basal trichotomy involving the *flavus*, *merriami*, and Southern Chihuahuan Desert lineages (Fig. 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).

Divergence within the merriami clade.—The 3 phylogroups recovered within the *merriami* lineage (Fig. 3) appear to have diverged coincidentally during the Pliocene (Table 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. 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

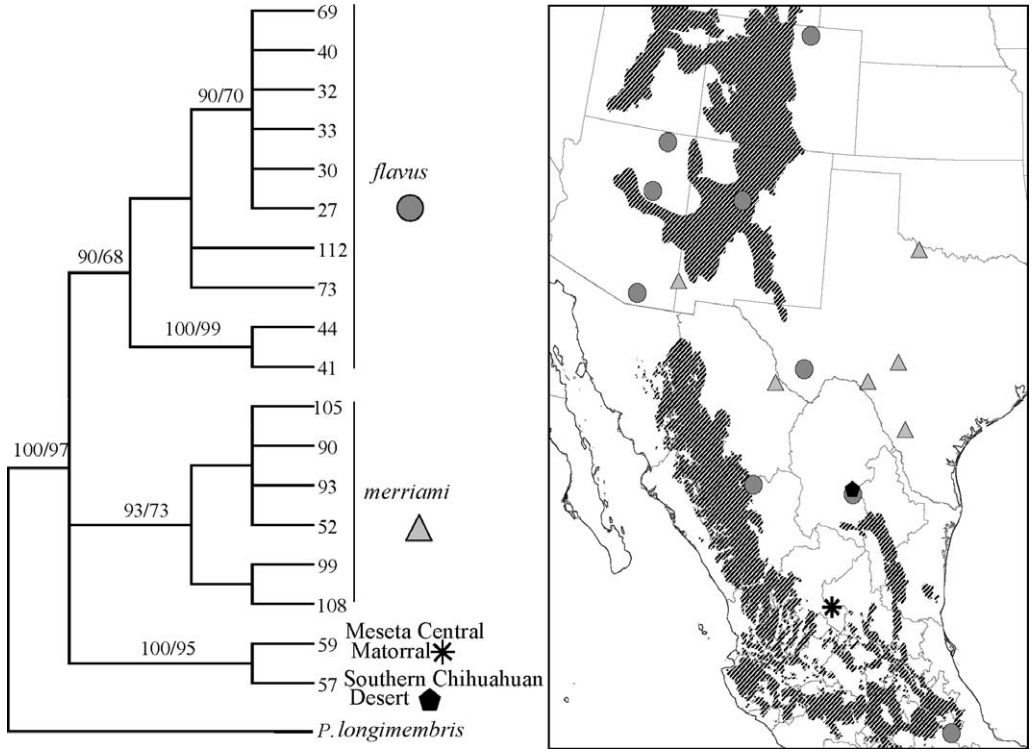

Fig. 5.—Cladogram generated from 50% majority rule consensus from Bayesian analysis of nuclear interphotoreceptor retinoid-binding protein (*IRBP*) gene sequences from representatives of the *Perognathus flavus* species group. Posterior probability values followed by maximum-likelihood bootstrap values are shown for supported nodes. Numbers at the end of branches are mitochondrial DNA haplotype numbers (Appendix I).

Escarpment along the Edwards Plateau, which may be a barrier to northward movement (Fig. 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 *D. 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

Table 2.—Time to most recent common ancestor for select clades in the *Perognathus flavus* species group for the nuclear interphotoreceptor retinoid-binding protein (*IRBP*) and mitochondrial protein-coding gene cytochrome oxidase III (*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)

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 $\leq$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. 3), presence of the eastern group south into Coahuila (Fig. 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) 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. 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. 4; Table 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. 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.

Resumen

Estudiamos la historia evolutiva de un grupo de raton de bolsa sedoso (Heteromyidae: Perognathinae: grupo de especies *Perognathus flavus*) compuesto de las especies *P. flavus* y *P. merriami* para determinar los patrones y causas propuestas de diversificación geográfica a través de pastizales secos y cuencas intermontanas del oeste de Norte America. La región representa un paisaje complejo topográfico con historia neogénica de dramáticas transformaciones geological y climáticas. Análisis de datación y filogenético de ADN mitocondrial favorece una división inicial entre 4 linajes importantes durante el Mioceno tardio y esta hipótesis también es sustentada por el análisis de una porción del gen *IRBP* nuclear. Dos de estos linajes tienen una distribución geográfica restringida al desierto de Chihuahua y dos tienen distribuciones a lo largo de porciones del desierto de Chihuahua, el plateau del Colorado, las Grandes Praderas y el plano de Tamaulipas. Dentro de los dos linajes de amplia distribución, probablemente hubo mayor diversificación geográfica concentrada en el Plioceno, lo cual coincide con el origen hipotético de varias barreras geográficas. Estos resultados son consistentes con modelos de divergencia alopátrica, producto de eventos climáticos y geológicos pre-Pleistoceno, en especial la expansión de pastizales del Mioceno tardío y la evolución Mioceno-Plioceno de la geomorfología de cuencas y cadenas montañosas. Por lo tanto, la estructura biogeografica que muestra el grupo de especies *P. flavus* se puede predecir para más taxa simpátrica.

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APPENDIX I

Specimens examined.—This list includes museum voucher numbers and tissue catalogue information for specimens used in this analysis. The 1st column of numbers refers to localities displayed in Fig. 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. Haplotype refers to the 114 unique haplotypes identified from the combined protein-coding gene cytochrome oxidase III (*COIII*) and a noncoding portion of the control region (*CR*) mitochondrial DNA data set. Phylogroup column identifies the phylogroup to which each individual belongs using the following abbreviations: fGRP, *flavus* Great Plains; fCPSR, *flavus* Colorado Plateau Southern Rockies; fNCHI, *flavus* northern Chihuahuan Desert; fSCHI, *flavus* southern Chihuahuan Desert; fTV, *flavus* Tehuacan Valley; mGRP, *merriami* Great Plains, mCHI, *merriami* Chihuahuan Desert; mTM, *merriami* Tamaulipan Mezquital; MCM, Meseta Central Matorral; and CHI, Southern Chihuahuan Desert. Samples marked B in the Clock column represent individuals used from the *COIII* and interphotoreceptor retinoid-binding protein (*IRBP*) data sets for divergence estimates in BEAST.

Perognathus flavus species group

	Locality	Museum number	Other number	Haplotype	Phylogroup	Clock
1	Arizona, Navajo County, 2 miles E, 3 miles N Winslow	NMMNH 5722	LVT 9261	24	fCPSR	
		NMMNH 5733	LVT 9275	25	fCPSR	
		NMMNH 5734	LVT 9276	26	fCPSR	
		NMMNH 5735	LVT 9277	27	fCPSR	B
2	Arizona, Pima County, Sonoita	NMMNH 5805	LVT 9339	73	fNCHI	B
		NMMNH 5807	LVT 9346	74	fNCHI	
3	Texas, Brewster County, Elephant Mountain	NMMNH 4619	LVT 6545	76	mCHI	
		NMMNH 4626	LVT 6559	33	fSCHI	B
		NMMNH 4631	LVT 6569	77	mCHI	
4	Chihuahua, Mexico, 1 mile E El Mesquite	NMMNH 4554	LVT 6422	93	mCHI	B
		NMMNH 4470	LVT 6423	75	mCHI	
		NMMNH 4555	LVT 6424	97	mCHI	
		NMMNH 4471	LVT 6425	92	mCHI	

Appendix 1.—Continued.

Perognathus flavus species group

	Locality	Museum number	Other number	Haplotype	Phylogroup	Clock
5	Chihuahua, Mexico, 30 km W Coyame	NMMNH 5256	LVT 7584	79	mCHI	
		NMMNH 5258	LVT 7585	80	mCHI	
		NMMNH 5257	LVT 7586	86	mCHI	
		NMMNH 5254	LVT 7587	81	mCHI	
		NMMNH 5255	LVT 7588	79	mCHI	
6	Chihuahua, Mexico, 35 km SW Hercules	NMMNH 5277	LVT 7627	58	CHI	
7	Chihuahua, Mexico, 4 km SW Parrita	NMMNH 2442	LVT 1050	62	fNCHI	
		NMMNH 2443	LVT 1051	63	fNCHI	
		NMMNH 2521	LVT 1052	64	fNCHI	
		NMMNH 2444	LVT 1053	65	fNCHI	
		NMMNH 2525	LVT 1054	65	fNCHI	
		NMMNH 2445	LVT 1055	66	fNCHI	
		NMMNH 2522	LVT 1056	63	fNCHI	
		NMMNH 2446	LVT 1057	67	fNCHI	
		NMMNH 2523	LVT 1058	63	fNCHI	
		NMMNH 2447	LVT 1059	63	fNCHI	
		NMMNH 2524	LVT 1060	68	fNCHI	
8	Chihuahua, Mexico, 6 km E El Sueco	NMMNH 5181	LVT 8726	72	fNCHI	
9	Chihuahua, Mexico, 6 km NW Manuel Benavides	NMMNH 5211	LVT 7596	85	mCHI	
		NMMNH 5263	LVT 7597	98	mCHI	
		NMMNH 5212	LVT 7598	84	mCHI	
		NMMNH 5264	LVT 7599	83	mCHI	
		NMMNH 5213	LVT 7600	82	mCHI	
10	Chihuahua, Mexico, 6 miles NW Ricardo Flores Magon	NMMNH 4459	LVT 6401	96	mCHI	
		NMMNH 4460	LVT 6403	94	mCHI	
11	Colorado, Pueblo County, Fort Carson, Niobrabra	ZM.11527	DTZM 118	39	fCPSR	
12	Colorado, Ward County, Pawnee National Grass Land		PF 0061	44	fGRP	B
			PF 1162	41	fGRP	B
			PF 0028	43	fGRP	
			PF 1218	113	fGRP	
			PF 1331	42	fGRP	
			PF 1358	46	fGRP	
			PF 1585	42	fGRP	
13	Coahuila, Mexico, 1 mile SE Hundido	NMMNH 2586	LVT 1171	110	CHI	
14	Coahuila, Mexico, 2 miles E Agua Nueva	NMMNH 4668	LVT 6634	31	fSCHI	
15	Coahuila, Mexico, 2 km S Santa Teresa	NMMNH 4684	LVT 6676	56	CHI	
		NMMNH 4685	LVT 6677	57	CHI	B
15	Coahuila, Mexico, Plan de Guadalupe	NMMNH 4674	LVT 6648	30	fSCHI	B
		NMMNH 4728	LVT 6649	29	fSCHI	
		NMMNH 4675	LVT 6650	28	fSCHI	
		NMMNH 4676	LVT 6651	78	mCHI	
16	Durango, Mexico, 4 km SSE La Zarca	CNMA 41886		4	fSCHI	
16	Durango, Mexico, 7 miles NNW La Zarca	NMMNH 2473	LVT 1109	35	fSCHI	
		NMMNH 2550	LVT 1110	3	fSCHI	
17	Durango, Mexico, 20 km S, 10 km E Torreon de Canas	NMMNH 3611	LVT 4834	38	fSCHI	
		NMMNH 3612	LVT 4835	32	fSCHI	B
18	Durango, Mexico, 5 km NW La Union	NMMNH 5412	LVT 8864	55	CHI	
19	Durango, Mexico, Hidalgo Atotonilco	NMMNH 4580	LVT 6467	34	fSCHI	
20	New Mexico, Eddy County, 4 miles S, 2 mi W Whites City	NMMNH 4245	LVT 5882	87	mCHI	
21	Kansas, Logan County, 2.5 miles S, 7.5 mi W Russell Springs	MHP 37500		109	mGRP	
21	Kansas, Scott County, 12 miles N, 1 mi W Scott City	MHP 37501		109	mGRP	
22	Kansas, Wallace County, 2.5 miles N, 9.75 mi W Russell Springs	MHP 37502		45	fGRP	
23	Arizona, Navajo County, 3 miles S Kayenta	NMMNH 3226	LVT 702	40	fCPSR	B
24	New Mexico, Bernalillo County, 3 miles N, 5.5 miles W Albuquerque	NMMNH 1907		10	fCPSR	
24	New Mexico, Bernalillo County, 5.5 miles N, 4.5 miles W Albuquerque	NMMNH 1869		69	fNCHI	B
25	New Mexico, Catron County, Quemado, Zuni Salt Lake	MSB 88073	NK 78038	17	fCPSR	
		MSB 88074	NK 78039	18	fCPSR	
		MSB 88075	NK 78040	19	fCPSR	
		MSB 87776	NK 78043	20	fCPSR	
		MSB 88099	NK 78063	21	fCPSR	
26	New Mexico, Chaves County, Bottomless Lakes State Park	MSB 74134	NK 65570	107	mGRP	
26	New Mexico, Chaves County, Bitter Lake NWR, T10S R25E Sec 21	NMMNH 2366		10	fCPSR	
26	New Mexico, Chaves County, Dexter Fish Hatchery, T13S R26E Sec 16	NMMNH 2368		11	fCPSR	

Appendix 1.—Continued.

Perognathus flavus species group

	Locality	Museum number	Other number	Haplotype	Phylogroup	Clock
27	New Mexico, Cibola County, 4 miles S, 1.5 miles W Correo	NMMNH 3943		12	fCPSR	
		NMMNH 3944		70	fNCHI	
		NMMNH 3945		71	fNCHI	
28	New Mexico, Hidalgo County, 2 miles NW Cloverdale	NMMNH 5810	LVT 9869	2	fNCHI	
29	New Mexico, Hidalgo County, Doubtful Canyon, 8 miles N, 0.5 miles W Steins	NMMNH 4417	LVT 6162	88	mCHI	
		NMMNH 4418	LVT 6163	89	mCHI	
		NMMNH 4423	LVT 6168	90	mCHI	B
		NMMNH 4424	LVT 6169	91	mCHI	
		NMMNH 4425	LVT 6170	90	mCHI	
		NMMNH 4428	LVT 6173	95	mCHI	
30	New Mexico, Lincoln County, Valley of Fires Camp Ground	MSB 146017	NK 133625	5	fCPSR	
		MSB 146182	NK 133710	6	fCPSR	
		MSB 146184	NK 133712	7	fCPSR	
31	New Mexico, Sandoval County, Placitas Web	MSB 90727	NK 86776	5	fCPSR	
31	New Mexico, Sandoval County, Star Lake	NMMNH 4161		13	fCPSR	
32	New Mexico, McKinley County, 15 miles N Crownpoint of Hwy 371	MSB 88136	NK 78017	15	fCPSR	
		MSB 88137	NK 78018	16	fCPSR	
32	New Mexico, San Juan County, 57 km S of Farmington	MSB 90210	NK 86410	22	fCPSR	
		MSB 90214	NK 86414	13	fCPSR	
		MSB 90215	NK 86415	10	fCPSR	
		MSB 90218	NK 86418	23	fCPSR	
		MSB 90221	NK 86421	22	fCPSR	
33	New Mexico, Otero County, White Sands Missle Range	MSB 85857	NK 40934	5	fCPSR	
34	New Mexico, Soccoro Co, 10 miles S, 20 miles W San Marcial	NMMNH 3953		10	fCPSR	
		NMMNH 3954		111	fNCHI	
		NMMNH 3955		5	fCPSR	
35	Puebla, Mexico, 1.4 km E San Miguel Zozutla	UAMI 16220		114	fTV	
35	Puebla, Mexico, 3 km S Ciudad Serdan	LSUMZ 36684	M 8609	112	fTV	B
36	San Luis Potosi, Mexico, 3 miles S Matehuala	NMMNH 2514	LVT 1198	36	fSCHI	
37	San Luis Potosi, Mexico, 13 km NE Villa de Reyes	CNMA 43018		3	fSCHI	
38	San Luis Potosi, Mexico, 10 miles S Villa de Ramos	NMMNH 3705	LVT 4912	61	MCM	
38	San Luis Potosi, Mexico, 10 miles W Salinas	NMMNH 3669	LVT 4889	59	MCM	B
39	Texas, Dimmit County, Chaparral Wildlife Management Area	TTU 98021	TK 98098	50	mTM	
		TTU 98132	TK 98101	51	mTM	
		TTU 98137	TK 98154	52	mTM	B
		TTU 98172	TK 98174	53	mTM	
40	Texas, La Salle County, Chaparral Wildlife Management Area	TTU 80785	TK 84610	47	mTM	
		TTU 80898	TK 84686	48	mTM	
		TTU 98474	TK 98046	49	mTM	
		TTU 98501	TK 98235	54	mTM	
41	Texas, Kerr County, Kerr Wildlife Management Area	TTU 98346	TK 111603	101	mGRP	
		TTU 102269	TK 115305	102	mGRP	
41	Texas, Kimble County, Texas Tech University Center at Junction	TTU 77847	TK 78167	108	mGRP	B
42	Texas, Lynn County, 2 miles S, 5 miles E Tahoka	TTU 77563	TK 51911	106	mGRP	
		TTU 77565	TK 51914	14	fCPSR	
43	Texas, Tom Green County, San Angelo State Park	ASNHC 11852	ASK 4480	103	mGRP	
		ASNHC 11858	ASK 5720	104	mGRP	
44	Texas, Val Verde County, 12.8 miles W Del Rio	ASNHC 3901	ASK 1074	8	mGRP	
44	Texas, Val Verde County, Devil's River SNA	ASNHC 11004	ASK 4973	99	mGRP	B
		ASNHC 11006	ASK 4979	100	mGRP	
		ASNHC 11008	ASK 4982	100	mGRP	
45	Texas, Wilbarger County, 2 miles W Harrold	TLB 10568	LVT 2063	105	mGRP	B
46	Zacatecas, Mexico, 1 miles SE Banon	NMMNH 4601	LVT 6509	37	fSCHI	
47	Zacatecas, Mexico, 2 miles E San Jeronimo	NMMNH 4497	LVT 6479	60	MCM	

Outgroup 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 nelsoni	Durango, Mexico, 7 miles NNW La Zarca	NMMNH 2472	LVT 1107	AY926431
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 Seminoe	NMMNH 3240	LVT 2525	AY926421
P. flavescens	Nebraska, Sheridan County, 27 miles N Lakeside	NMMNH 3242	LVT 2527	AY926422
P. longimembris	Baja California, Mexico, 27 km S Punta Prieta	NMMNH 2978	LVT 2191	AY926420
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