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DNA Data Support a Rapid Radiation of Pocket Gopher Genera (Rodentia: Geomyidae)

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In this study, we address the question of phylogenetic relationships in the Geomyidae, focusing primarily on intergeneric relationships within the tribe Geomyini. Our study makes use of DNA sequences from two mitochondrial and two nuclear genes, and we use model-based methods of phylogenetic analysis to infer relationships and determine the level of support for each proposed relationship. Relationships among geomyine pocket gopher genera remain only partially resolved despite a number of earlier attempts to reconstruct their phylogenetic history and despite the newly generated sequence data analyzed in this study. This lack of resolution does not appear to result from insufficient or inappropriate DNA data, nor is it caused by inadequate sampling of taxa. Rather, molecular data and fossil data together lead to the conclusion that diversification within the Geomyini likely occurred during a geologically brief period in the Blancan. Rapid climate change during the Blancan, the origin of patchily distributed grasslands, and the evolution of hypsodonty may have triggered the rapid diversification that eventually produced the five extant genera of the Geomyini.

KEY WORDS: Blancan, Geomyidae, Geomyini, Pliocene, pocket gophers, rapid radiation.

INTRODUCTION

Pocket gophers (Rodentia: Geomyidae) are a frequently studied group of rodents from many aspects, including ecology, behavior, physiology, comparative anatomy, functional morphology, and host–parasite coevolution. For example, the 1990–1999 index of the *Journal of Mammalogy* lists a total of 25 publications on pocket gophers in this single journal, with each of the six extant genera receiving multiple listings (American Society of Mammalogists, 2001). Despite this widespread interest in pocket gophers, some of the most fundamental events in the evolutionary history of the family have yet to be elucidated. Unfortunately, the geomyid fossil record is still too limited to resolve intergeneric relationships among modern genera of the family (Kurtén and Anderson, 1980; Savage and Russell,

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1983). The few neontological studies that have addressed intergeneric relationships in the Geomyidae (Russell, 1968a; Hafner, 1982; Honeycutt and Williams, 1982) have produced vastly different phylogenetic hypotheses. These disagreements may be an artifact of the low-resolution techniques used in these studies (e.g., comparative morphology, allozyme analysis, comparative immunology) compounded by the absence of model-based methods for phylogenetic analysis. Alternatively, the lack of agreement among these early attempts to resolve geomyid relationships may reflect a real evolutionary radiation among pocket gopher genera that may have occurred too rapidly and too far in the past to resolve today, even with the aid of high-resolution techniques and modern methods of phylogenetic analysis.

In this study, we apply DNA sequence data and a growing body of information about mammalian genome evolution to the question of relationships in the Geomyidae, focusing primarily on intergeneric relationships within the tribe Geomyini. Our study makes use of DNA sequences from two mitochondrial and two nuclear genes. The mitochondrial genes cytochrome *b* (cyt *b*) and cytochrome *c* oxidase subunit I (COI) have been useful in a number of other phylogenetic studies of mammals, including pocket gophers (Hafner *et al*., 1994; Demastes *et al*., 2002). The nuclear genes, β-fibrinogen and recombination activating gene 1 (Rag1) have seen less use, but appear to be of phylogenetic utility in other mammals (Seddon *et al*., 2001; Steppan *et al*., 2004). Because the importance of model choice in phylogenetic analysis has been demonstrated clearly (Sullivan and Swofford, 1997), we use model-based methods of phylogenetic analysis to infer intergeneric relationships and determine the level of support for each proposed relationship.

Taxonomic History of the Geomyidae

Much of current taxonomy in the Geomyidae dates to Russell's (1968a) treatment of the family. Russell recognized two extant tribes: the Thomomyini, which includes only the genus *Thomomys*, and the Geomyini, which includes all other extant genera of pocket gophers (*Cratogeomys*, *Geomys*, *Orthogeomys*, *Pappogeomys*, and *Zygogeomys*; Fig. 1). Russell's (1968a,b) analysis of the group attempted to address phylogenetic relationships among pocket gophers, including both extinct and extant taxa, based exclusively on comparative morphology. Russell assumed a close relationship among *Pappogeomys* and *Cratogeomys* species, as did other authors before him (e.g., Merriam, 1895; Wood, 1955). However, Russell (1968b) treated these taxa as subgenera of a single genus (*Pappogeomys*), thereby departing from earlier taxonomy in which both taxa were recognized at the generic level (e.g., Simpson, 1945). Russell also recognized *Heterogeomys* Merriam 1895 and *Macrogeomys* Merriam 1895 as subgenera of *Orthogeomys*, thereby reducing the total number of geomyid genera from eight (Wood, 1955) to five (Russell, 1968a).

In attempting to describe relationships among the genera of pocket gophers, Russell (1968a, p. 568) suggested that "*Orthogeomys* has closer affinities with *Zygogeomys* than with any of the other genera." Beyond that, Russell made no comment about intergeneric relationships in the Geomyidae, except to suggest that there was an evolutionary radiation near the end of the Hemphillian or the beginning of the Blancan North American Land Mammal Age (ca. 5 mya) that produced at least four lineages "at essentially the same time . . . presumably from the same ancestral stock" (Russell, 1968a, p. 558). Each of these lineages, he suggested, led to one of four modern genera (*Geomys*, *Orthogeomys*, *Pappogeomys* [including *Cratogeomys*], and *Zygogeomys*).

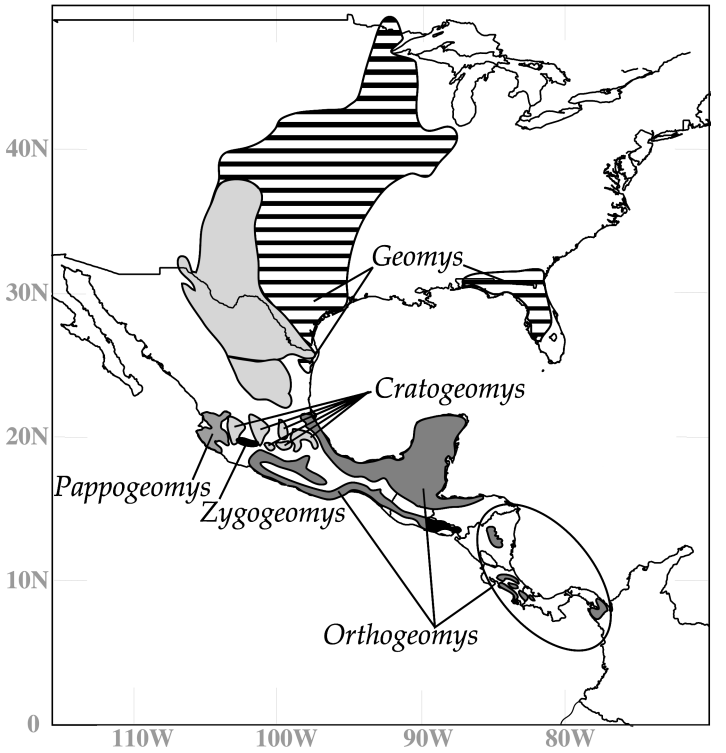

Fig. 1. Approximate distribution of genera in the pocket-gopher tribe Geomyini throughout North and Central America. *Thomomys* species (tribe Thomomyini; not shown) range primarily through the western United States and the northern half of Mexico. Distributions are redrawn from Demastes *et al*. (1996, 2002), Hafner (1991), and Hall (1981).

Hafner's (1982) analysis of protein distance data and comparative immunology provided strong support for reciprocal monophyly of the two tribes, Geomyini and Thomomyini, in the family Geomyidae. This analysis also suggested that Russell was correct to recognize *Orthogeomys* more broadly to include the subgenera *Orthogeomys*, *Heterogeomys*, and *Macrogeomys*. Hafner's (1982) protein data suggested a sister-genus relationship between *Orthogeomys* taxa and *Zygogeomys* and between *Pappogeomys* and *Geomys*. In both cases, however, internodes were short (protein distance = 0.05) relative to the branches that they supported (protein distance = 0.25–0.31), potentially corroborating Russell's (1968a) idea of a rapid evolutionary radiation leading to modern genera in the tribe Geomyini.

Honeycutt and Williams (1982) examined protein data in several species of *Cratogeomys* and one species each of *Geomys*, *Orthogeomys*, *Pappogeomys*, and *Zygogeomys*. Given the levels of genetic divergence between *Cratogeomys* and *Pappogeomys*, these authors resurrected the genus name *Cratogeomys*, bringing the recognized number of extant pocket gopher genera up to six. A locus-by-locus analysis of the protein data indicated sister-genus relationships between *Pappogeomys* and *Cratogeomys* (supported by two loci)

and between *Orthogeomys* and *Zygogeomys* (supported by one locus). *Geomys* appeared as the earliest offshoot in the geomyine lineage. Distance analysis of the same data yielded a radically different arrangement, perhaps, as the authors suggested, resulting from convergence in electrophoretic mobility at a few loci. Our reanalysis of Honeycutt and Williams' (1982) data (their table 1) using the parsimony approach implemented in PAUP* (Swofford, 2002) yields a third arrangement of genera (not shown). Therefore, presumed relationships among the genera of the Geomyini based on Honeycutt and Williams' (1982) data set are highly dependent on the method of analysis chosen. This outcome would be expected if Russell (1968a) was correct in his description of a rapid evolutionary radiation within the tribe.

MATERIALS AND METHODS

Data Collection and Alignment

DNA sequences were obtained for mitochondrial and nuclear genes from 23 pocket gopher individuals and one outgroup specimen (*Chaetodipus penicillatus*) from the closely related rodent family, Heteromyidae (Wahlert, 1985). Sequences were submitted to GenBank and specimen data, including collector information, locality data, and museum accession numbers, are included with each GenBank entry (Appendix).

Cyt *b* sequences new to this study were generated using polymerase chain reaction (PCR) products generated using the primers L14724 and H15154 as described by Spradling *et al*. (2001). The COI gene was amplified using the primers CO1-5285F (5′-CCY CTG TNY TTA GAT TTA CAG TCT A-3′) and CO1-6929R (5′-ACA ARG TTA TGT AAT DDT TTT ACT A-3′). Each 50-μL reaction included 0.2 units of Taq polymerase (Applied Biosystems, Foster City, California), 1× reaction buffer (Applied Biosystems), 4 mM $MgCl_2$, 0.5 mM each dNTP, and 0.4 μM each primer. Thermal cycles included an initial denaturing step at 94°C (1 min), followed by 30 cycles of 94°C (45 s), 55°C (50 s), and 72°C (60 s), and a final extension at 72°C (5 min). PCR products were purified using the QIAquick PCR Purification Kit (QIAGEN, Inc., Valencia, California). Approximately 55 ng of the purified PCR product was used for sequencing. Forward and reverse strand sequences were determined for consistency. Sequencing primers included CO1-5285F, CO1-6929R, Gco1F1 (5′-CCY CGN ATA AAT AAY ATA AG-3′), Gco1R1 (5′-GTR AAA TGR ATT TTT GCT CA-3′), and CO1-570F (5′-MTG ATC AGT YHT AAT YAC TG-3′).

The seventh intron of the nuclear β-fibrinogen gene, along with portions of the surrounding exons, was amplified using primers FIB-B17U and FIB-B17L (Prychitko and Moore, 1997). The resulting PCR fragment was cloned and sequenced for three pocket gophers (data not included here). Near the beginning and middle of the intron, there are several long repeat regions that are difficult to sequence. Therefore, phylogenetic analysis was restricted to a 492-bp region at the end of the intron plus the adjoining 61-bp region of exon 8. Each initial 20-μL PCR included 1 × PCR Master Mix (Promega Corporation, Madison, Wisconsin) and primers FIB-B17U and FIB-B17L (5 μM each). Cycling conditions involved an initial denaturation (95°C, 1 min), followed by 40 cycles of 95°C (1 min), 42°C (45 s), and 72°C (1 min), and a final extension at 72°C (10 min). PCR products were cleaned using the QIAquick PCR Purification Kit (QIAGEN, Inc., Valencia, California) and reamplified (1 μL of the cleaned product in a 50-μL reaction with FIB-B17L and B3R [5′-CAC ACT CCA GAC TTC TTT C-3′]). Amplification conditions differed from the

Table I. Models Used in Bayesian and Maximum-Likelihood Analyses with *Chaetodipus* as Outgroup

Analysis	Partition	Model[a]	Base Frequency A, C, G, T[b]	TS:TV[c]	Alpha[d]	I[e]	Rate Matrix[f]
Maximum-likelihood analysis of 4 genes		GTR + I + G	0.30, 0.23, 0.16, 0.31	NA	0.6	0.5	2.8, 10.4, 2.5, 0.6, 30.9, 1.0
Bayesian analysis							
	COI	GTR + I + G	NA	NA	1.2	0.6	1.0, 20.5, 1.0, 0.5, 18.0, 1.0
	cyt b	TrN + I + G	NA	NA	1.0	0.5	1.0, 6.4, 1.0, 1.0, 15.0, 1.0
	β-fibrinogen	HKY + G	NA	NA	0.5	0	1.0, 4.0, 0.3, 0.3, 4.0, 1.0
	Rag1	HKY	NA	1.3	NA	0	NA

[a]GTR = Rodríguez *et al.* (1990); TrN = Tamura and Nei (1993); HKY = Hasegawa *et al.* (1985); I = invariant sites; G = gamma distribution.
[b]NA = not applicable.
[c]Transition to transversion ratio; NA = not applicable.
[d]Shape parameter of the gamma distribution.
[e]Proportion of invariant sites.
[f]Rates for AC, AG, AT, CG, CT, and GT substitutions; NA = not applicable.

initial reactions only in the number of cycles (35) and the annealing temperature (52°C). FIB-B17L and R2 (5′-GTG GTA GTG CAG TCA AAC TCA GGC C-3′) were used to sequence both strands of DNA.

Amplification of a portion of Rag1 was performed using primers Rag1-S70 and Rag1-S115 (5 μM each; Steppan *et al*., 2004) with 1 × PCR Master Mix (Promega Corporation, Madison, Wisconsin) in a 20-μL reaction. Thermal parameters included an initial denaturation at 95°C (1 min), followed by 40 cycles of 95°C (1 min), 42°C (45 s), and 72°C (1 min), and a final extension at 72°C (10 min). When necessary to improve yield, a second amplification was performed on cleaned PCR products using internal primers Rag1-F (5′-GCT GGA GTT CAG AAG CCA GTC C-3′) and Rag1-Rb (5′-GGT ACT GAG ATG GAT CTT ACT GC-3′). Conditions for this reaction differed from the initial amplification only in the number of cycles (35) and the annealing temperature (52°C). Rag1-F and Rag1-Rb were used to sequence both strands of DNA.

Cyt *b*, COI, β-fibrinogen, and Rag-1 PCR products were prepared for sequencing using the QIAquick PCR Purification Kit (QIAGEN, Inc., Valencia, California). Sequencing reactions were performed using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California) and were assessed using the ABI Prism 377 Genetic Analyzer (Applied Biosystems, Foster City, California).

ClustalX (Thompson *et al*., 1997) was used to help align β-fibrinogen intron sequences. Other sequences were easily aligned by eye. At the end of the COI gene, it appears that there have been multiple insertion/deletion (indel) events in the evolutionary history of pocket gophers. Therefore, the stop codon and up to 10 bp of sequence upstream of the stop codon were eliminated from phylogenetic analysis, thereby terminating each sequence with the same set of conserved amino acids.

Data Analysis

Modeltest (Posada and Crandall, 1998) was used in conjunction with PAUP* (Swofford, 2002) to choose an appropriate model for each gene. The models and parameter values indicated by the hierarchical likelihood-ratio test option of Modeltest (Tables I and II) were incorporated into commands used by MrBayes v3.0B4 (Huelsenbeck and Ronquist, 2001) to perform Bayesian estimations of phylogeny for each gene. The sequence data for the four loci combined also were analyzed using a Bayesian approach, with each gene forming a different partition in the data set, and with each partition analyzed using its own model and parameter values determined using Modeltest (Tables I and II). In all Bayesian analyses, runs were initiated with random starting trees. Ten million generations (burnin = 2000) were run with four incrementally heated chains sampled at intervals of 100 generations. The first 2000 trees (burnin) were discarded; this number of trees was well past the point of convergence on a stable likelihood value for each of the analyses (Hall, 2001). The retained trees were used to generate 50% majority-rule consensus trees, average branch lengths, and posterior probabilities.

Maximum likelihood and parsimony analyses were performed using PAUP* (Swofford, 2002). Maximum-likelihood models were determined for the combined data (four genes) using Modeltest (Posada and Crandall, 1998; Tables I and II). Optimal maximum-likelihood trees were determined from at least 100 heuristic searches, each with random taxon-input order. Maximum-likelihood bootstrap values were determined using

Table II. Models Used in Bayesian and Maximum-Likelihood Analyses with *Thomomys* as Outgroup

Analysis	Partition	Model[a]	Base Frequency A, C, G, T[b]	TS:TV[c]	Alpha[d]	I[e]	Rate Matrix[f]	Other Information
Bayesian analysis, Fig. 4A								
	COI	GTR + I + G	NA	NA	1.3	0.6	1.1, 21.0, 1.1, 0.5, 18.3, 1.0	also Fig. 2A
	cyt b	TrN + I + G	NA	NA	1.2	0.5	1.0, 6.6, 1.0, 1.0, 14.9, 1.0	also Fig. 2A
	β−fibrinogen	HKY + G	NA	1.8	0.6	0	NA	also Fig. 2C
	Rag1	HKY + G	NA	4.0	0.2	0	NA	also Fig. 2D
Bayesian analysis, Fig. 4B								
	mtDNA 3rd positions RY	JC + G	NA	NA	0.4	0	NA	also Fig. 2B
	mtDNA 1st and 2nd positions	TrN + I + G	NA	NA	1.5	0.9	1.0, 5.2, 1.0, 1.0, 26.1, 1.0	also Fig. 2B
	β−fibrinogen	HKY + G	NA	1.8	0.6	0	NA	also Fig. 2C
	Rag1	HKY + G	NA	4.0	0.2	0	NA	also Fig. 2D
Maximum-likelihood tree not shown (bootstraps in Fig. 4A)	all genes	TrN + I + G	0.31, 0.25, 0.14, 0.29	NA	1.2	0.7	1.0, 7.4, 1.0, 1.0, 14.5, 1.0	1000 bootstrap replicates
Maximum-likelihood tree not shown (bootstraps in Fig. 4B)	all genes	TrN + I + G	0.26, 0.25, 0.21, 0.29	NA	0.3	0.6	1.0, 2.4, 1.0, 1.0, 5.2, 1.0	250 bootstrap replicates

[a] GTR = Rodríguez *et al.* (1990); HKY = Hasegawa *et al.* (1985); JC = Jukes and Cantor (1969); TrN = Tamura and Nei (1993); I = invariant sites; G = gamma distribution.
[b] NA = not applicable.
[c] Transition to transversion ratio; NA = not applicable.
[d] Shape parameter of the gamma distribution.
[e] Proportion of invariant sites.
[f] Rates for AC, AG, AT, CG, CT, and GT substitutions; NA = not applicable.

heuristic searches, each with random taxon-input order (250–1000 replicates; Tables I and II). All parsimony trees were determined using 1000–5000 heuristic searches, each with random taxon-input order and tree-bisection and reconnection (TBR) branch swapping. The partition-homogeneity test was implemented using PAUP* (Swofford, 2002), with each gene forming a data partition. For this test, 2500–10,000 parsimony analyses were performed, each with a heuristic search employing TBR branch swapping and random taxon-input order. Executable data files for Bayesian and maximum-likelihood analyses are available from TreeBASE (http://www.treebase.org).

Tests for violation of the molecular clock assumption among species of the Geomyini were performed using Naoko Takezaki's program Lintre, available at http://iubio.bio.indiana.edu/soft/molbio/evolve/lintr. Neighbor-joining trees of relationships in the Geomyini were constructed using the Tamura and Nei (1993) model of the program with a gamma distribution. *Thomomys bottae* was included as an outgroup. Mitochondrial and nuclear genes were analyzed together, both including third position transitions for mitochondrial genes and excluding them (alpha shape parameter for the gamma distribution = 1.2 and 0.3, respectively). Subsets of the data also were analyzed for rate constancy (mitochondrial genes alone, alpha = 1.3; mitochondrial genes alone, excluding third position transitions, alpha = 1.5; β-fibrinogen, alpha = 0.6; Rag1, alpha = 0.2). The two-cluster test (Takezaki *et al*., 1995) was used to evaluate rate heterogeneity among taxa. Relative-rate tests were performed on some pairs of taxa in the Geomyini using *G. texensis* as the outgroup for estimating the raw number of substitutions in each pocket gopher lineage since the time that they shared a common ancestor, as described by Li and Graur (1991). These calculations were based on absolute number of sequence differences between pairs of taxa. A binomial test (Mindell and Honeycutt, 1990) was used to determine if differences were statistically significant based on a two-tailed test (Allard and Honeycutt, 1992; DeWalt *et al*., 1993). Divergence times were estimated on Bayesian trees from the full data set using the NPRS method and Powell algorithm of Sanderson (1997) via M. J. Sanderson's r8s program, available at http://ginger.ucdavis.edu/r8s/.

RESULTS AND DISCUSSION

Comparisons of Genes

A total of 3017 bp of DNA was sequenced for each taxon. Among pocket gophers, base frequencies were similar among taxa for nuclear genes analyzed both separately and together ($\chi^2 = 1.78$–7.329818, df = 66, $p > 0.999$). Likewise, mitochondrial first and second positions appeared to have similar base composition among taxa ($\chi^2 = 0.90$–7.61, df = 66, $p > 0.999$). However, mitochondrial third positions did show evidence of heterogeneity in base composition when taxa of both the Geomyini and Thomomyini were included ($\chi^2 = 156.29$, df = 66, $p < 0.001$). *Thomomys* species showed slightly higher mean proportions of guanine and thymine than did geomyine species (G = 0.06 vs. 0.04 and T = 0.36 vs. 0.30, respectively) and slightly lower mean proportions of adenine and cytosine (A = 0.39 vs. 0.41 and C = 0.20 vs. 0.24, respectively). Within the ingroup, however, there was no significant heterogeneity in base composition of mitochondrial third positions ($\chi^2 = 49.03$, df = 48, $p = 0.43$).

It has been suggested that nuclear-gene introns evolve at a rate that is 5–10 × slower than the average rate of evolution of mtDNA genes (Hewitt, 2001). Thus, it was not

surprising that the mitochondrial genes studied here contained many more variable sites and larger levels of sequence divergence than did the nuclear genes, although all four loci yielded a large number of variable and potentially phylogenetically informative characters (Table III). Of the two nuclear loci examined, the Rag-1 gene appeared to have a slightly slower rate of evolution than did the β-fibrinogen intron (Table III), possibly indicating a lower level of natural selection on the intron.

Outgroup Choice

A partition-homogeneity test (as implemented in PAUP*, Swofford, 2002) found no significant heterogeneity among data sets for the individual genes either with the heteromyid outgroup, *C. penicillatus*, included or with it excluded, (2500 replicates, $p = 0.89$ and 10,000 replicates, $p = 0.67$, respectively). Because the utility of the partition-homogeneity test has been questioned recently (Barker and Lutzoni, 2002), trees resulting from Bayesian and parsimony analyses of each gene were compared to determine if the different genes reflect different individual histories (Huelsenbeck *et al*., 1996). Although the topology of the four trees differed somewhat, conflicting nodes were not strongly supported as measured by posterior probability and bootstrapping. Thus, there is no evidence that the data from the four genes show conflicting phylogenetic histories.

Partitioned Bayesian analysis (Table I) of the four genes shows strong support (100% posterior probability) for monophyly of the tribe Geomyini. Posterior probability values may be artificially high (Cummings *et al*., 2003), but a monophyletic Geomyini also appears in 100% of parsimony bootstrap replicates based on the four genes with all characters weighted equally (5000 heuristic searches) and 94% of maximum-likelihood bootstrap trees (Table I). Therefore, the Geomyini appears to be monophyletic with respect to *Thomomys*, and *Thomomys* may serve as an appropriate outgroup.

Within *Thomomys*, Thaeler (1980) recognized two subgenera, *Megascapheus* and *Thomomys*, based on differences in chromosome number and several morphological features. The nuclear and mitochondrial sequences examined in this study support the distinctness of these two groups. Species in the subgenus *Megascapheus* and species in the subgenus *Thomomys* differ from each other only slightly less than each differs from genera in the Geomyini (Table IV), suggesting that divergence between *Megascapheus* and *Thomomys* followed soon after the divergence of the Geomyini and the Thomomyini.

Although monophyly of the genus *Thomomys* has been tentatively supported by Smith (1998), the DNA data analyzed in this study provide only weak support for *Thomomys* monophyly. A variety of parsimony, maximum-likelihood, and Bayesian (Table I) analyses were explored. These analyses were performed both including mitochondrial third positions and excluding them due to apparent substitutional saturation (plots of mitochondrial third-position transversions vs. all other substitutions indicated strong evidence of saturation). For example, using all four genes (with third positions removed from the mitochondrial data for reasons explained above) and using *Chaetodipus penicillatus* as the outgroup, parsimony analysis supports *Thomomys* monophyly, but parsimony bootstrap support is only 40%. Bayesian analysis of the same data yields a trichotomy involving the subgenus *Megascapheus*, the subgenus *Thomomys*, and the branch leading to the Geomyini. Including third positions in parsimony analysis produces a tree in which *Thomomys* is not monophyletic. Expanding the outgroup to include more heteromyid taxa does nothing to

Table III. Numbers of Characters and Levels of Sequence Divergence by Gene for Pocket Gophers[a]

	COI	Cytochrome *b*	β-Fibrinogen	Rag-1	All loci combined
# of characters	1538	400	527	552	3017
# of variable characters (%)	567 (37%)	173 (43%)	75 (14%)	60 (11%)	875 (29%)
# of potentially parsimony-informative characters	523 (74–1st position, 6–2nd position, 443–3rd position)	145 (30–1st position, 4–2nd position, 111–3rd position)	37 (noncoding intron sequence)	34 (10–1st position, 4–2nd position, 20–3rd position)	739
Minimum and maximum uncorrected sequence divergence between taxa in Geomyini	2.5–17.9%	2.2–18.8%	0.2–4.6%	0–2.4%	1.8–12.8%

[a]The outgroup *Chaetodipus* is not included in calculations shown here.

Table IV. Average Sequence Divergence (and Range) Between Selected Taxa by Gene[a]

Taxonomic Comparison	All Loci Combined (3rd Positions of Mitochondrial Genes RY-Coded)	COI and Cyt *b* (3rd Positions RY-Coded)	β-Fibrinogen	Rag-1
Pappogeomys vs. *Cratogeomys*	4.0% (3.6–4.2%)	5.7% (5.3–6.0%)	0.5% (0.2–0.8%)	1.3% (0.9–1.6%)
Pappogeomys vs. *Orthogeomys*	4.7% (4.4–5.2%)	6.6% (6.2–7.4%)	1.8% (1.2–2.4%)	1.3% (0.5–2.0%)
Pappogeomys vs. *Zygogeomys*	4.8%	6.3%	2.2%	3.5%
O. grandis vs. *Pappogeomys*, *Cratogeomys*, and *Zygogeomys*	4.8% (4.3–5.2%)	6.7% (6.2–7.1%)	1.6% (1.0–3.0%)	1.4% (0.7–2.4%)
Within *Orthogeomys*: subgenus *Orthogeomys* vs. subgenera *Heterogeomys* and *Macrogeomys*	5.1% (4.9–5.4%)	6.8% (6.4–7.0%)	3.2% 2.4–3.6%	1.6% (1.3–1.8%)
O. grandis (subgenus *Orthogeomys*) vs. *Zygogeomys*	5.2% (5.2–5.2%)	6.8% (6.7–6.9%)	2.8% (2.6–3.0%)	2.3% (1.6–4.0%)
Zygogeomys vs. *Cratogeomys*	5.4% (5.2–5.7%)	7.3% (7.0–7.6%)	2.3% (2.0–2.6%)	1.8% (1.5–2.2%)
Geomys vs. other Geomyini	6.0% (5.3–6.9%)	7.9% (6.9–9.1%)	3.6% (2.8–4.6%)	1.7% (0.9–2.5%)
Within *Thomomys*: subgenus *Megascapheus* vs. subgenus *Thomomys*	7.0% (6.4–7.9%)	9.4% (8.8–10.6%)	3.3% (2.6–4.0%)	2.4% (1.6–3.3%)
Geomyini vs. Thomomyini	7.7% (6.8–8.4%)	10.4% (9.2–11.7%)	3.6% (2.0–5.6%)	2.7% (1.6–4.0%)

[a]Taxonomic comparisons are arranged by increasing sequence divergence.

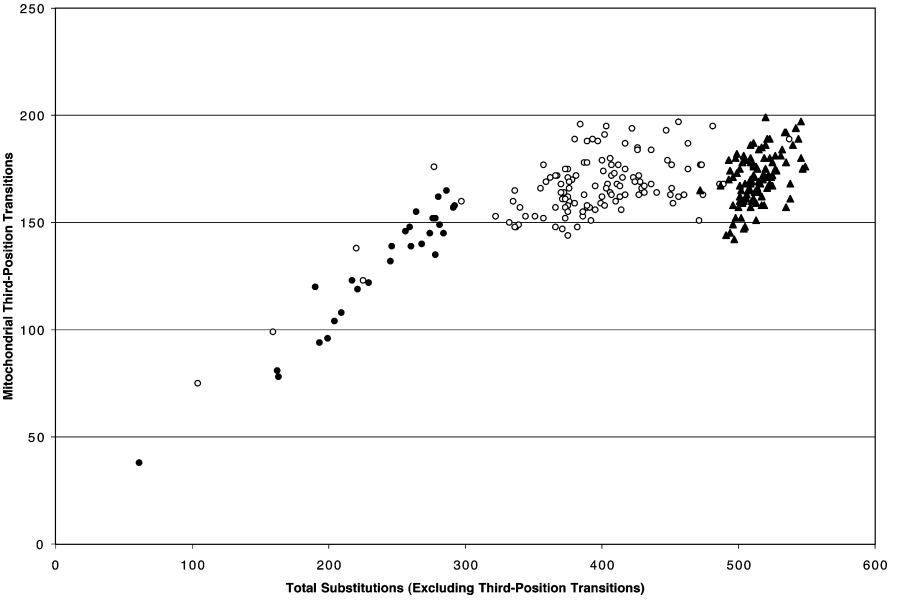

Fig. 2. Pairwise comparisons of number of mitochondrial third-position transitions versus all other mitochondrial substitutions. Comparisons within *Cratogeomys* (filled circles) show no evidence of substitutional saturation in these rapidly evolving characters. Comparisons between other taxa within the Geomyini (open circles) show evidence of substitutional saturation among some of the more genetically divergent taxa. Comparisons between taxa in different tribes (Geomyini vs. Thomomyini, triangles) show clear evidence of substitutional saturation in third position transitions.

resolve relationships among these three lineages based on COI sequences in a variety of analyses (S. V. Brant, unpublished data). Among the data from these four genes, there are few synapomorphies in the sequence data uniting all *Thomomys* taxa, which might be expected if the *Thomomys* subgenera diverged shortly after the genus split from basal geomyid stock. Hence, while there is little corroboration of a monophyletic *Thomomys* based on these data, there is strong support for monophyly of the Geomyini with respect to *Thomomys*. Therefore, to reduce the effect of substitutional saturation on analyses of geomyine relationships, *C. penicillatus* was pruned from subsequent analyses and the six *Thomomys* species were used to root the Geomyini.

Relationships within the Geomyini

A plot comparing number of mitochondrial third-position transitions to total number of other mitochondrial substitutions in pairwise comparisons of pocket gophers (Fig. 2) indicates substitutional saturation in comparisons between *Thomomys* and genera of the Geomyini. There may also be substitutional saturation in the more divergent comparisons between taxa within the Geomyini (Fig. 2). Third position transversions did not appear to be saturated in a similar plot of the mitochondrial data (not shown). Therefore, in some analyses, basal relationships among genera in the Geomyini were explored with mitochondrial third positions recoded as R and Y to indicate purines and pyrimidines, effectively eliminating third-position mitochondrial transitions from these analyses (referred to hereafter as the "RY-coded" data; Phillips *et al*., 2001).

Bayesian analysis of mitochondrial genes yields a different set of relationships depending on whether third position transitions are included in the analysis or not (Fig. 3A and B). As expected, mitochondrial genes appear to provide better resolution of relationships within genera than do nuclear genes (Fig. 3). Surprisingly, although nuclear genes analyzed separately appear to resolve relationships among species of the Thomomyini, they provide little resolution of relationships of the genera within the Geomyini (Fig. 3C and D). Although Rag1 data conflict with mitochondrial data in placing *Pappogeomys* with three of the *Orthogeomys* species (Fig. 3D), the high posterior probability value associated with this node corresponds with relatively weak bootstrap support (78% of 1000 maximum-likelihood bootstrap replicates and 62% of 1000 parsimony bootstrap replicates).

Partitioned Bayesian analysis of the four genes together (Table II) produced trees that are well supported by both posterior probability and bootstrap values at many nodes (Fig. 4). All methods of phylogenetic analysis (Bayesian, parsimony, and maximum likelihood with RY and non-RY coded data) indicate that *Geomys* is an early offshoot within the Geomyini. Although support is generally weaker, it also appears that *Zygogeomys* may have diverged from remaining members of the Geomyini shortly thereafter (Fig. 4). Maximum-likelihood analysis of the four genes without RY coding (Table II) produces a tree with the same topology as the Bayesian tree shown in Fig. 4A (–ln = 18101.6). Maximum-likelihood analysis of RY-coded data (Table II) also yields a tree with this topology, except that the relationships within *Cratogeomys* mirror those produced by Bayesian analysis of RY-coded data (Fig. 4B; –ln = 10312.6). Parsimony analysis of the RY-coded data produces the same tree shown in Fig. 4B, but in the parsimony tree the genus *Orthogeomys* is monophyletic. This relationship, however, is seen in less than 50% of parsimony or maximum-likelihood bootstrap replicates (10,000 and 250 heuristic-search replicates, respectively). Although these data do not provide clear support for *Orthogeomys* monophyly, it is apparent that *O. grandis* is genetically the most divergent of the *Orthogeomys* species, with *O. grandis* differing from other *Orthogeomys* species at about the same level that it differs from *Cratogeomys*, *Pappogeomys*, or *Zygogeomys* (Table IV).

As Russell (1968a,b) predicted, *Cratogeomys* and *Pappogeomys* are sister taxa in all trees resulting from RY-coded data, but support for this relationship is weak (Fig. 4B). When mitochondrial third-position transitions are included in analyses, *Pappogeomys* tends to associate with three of the *Orthogeomys* species, but again, support for this possible relationship is weak (Fig. 4A). Although the phylogenetic position of *Pappogeomys* cannot be determined with certainty based on these data, it is clear that *Pappogeomys* and *Cratogeomys* are distinct from one another, supporting Honeycutt and Williams' (1982) decision to recognize both taxa at the generic level. *Pappogeomys* and *Cratogeomys* taxa are only slightly more similar in sequence than are *Pappogeomys* and *Orthogeomys* or *Pappogeomys* and *Zygogeomys* (Table IV). Therefore, these data provide support for recent trends in the literature to follow Honeycutt and Williams (1982) in recognizing *Pappogeomys* and *Cratogeomys* as separate genera.

Not surprisingly, mitochondrial third-position transitions appear to be important in resolving relationships among species within the genus *Cratogeomys*. Relationships based on non-RY coded data (Fig. 4A) correspond well with previous morphological analyses (Russell, 1968b) in placing *C. merriami* with other members of the *castanops* species group. Additionally, nodes associating *Cratogeomys* species generally carry better bootstrap support when mitochondrial third-position transitions are included in the analysis (Fig. 4A)

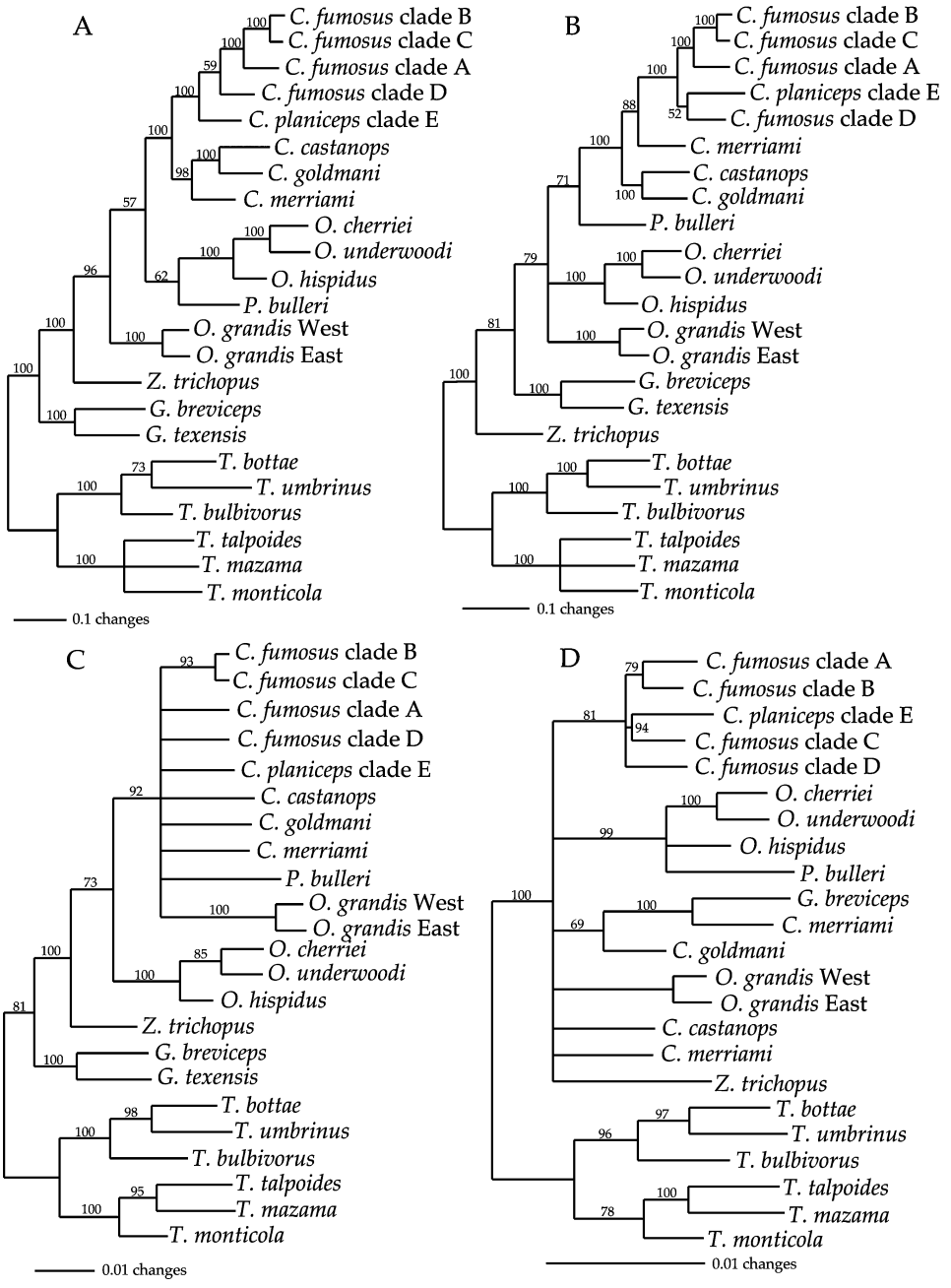

Fig. 3. Bayesian analysis of mitochondrial and nuclear genes, using *Thomomys* as the outgroup. Posterior probability values greater than 50% are shown above each node. (A) Mitochondrial genes, all third position substitutions included; (B) mitochondrial genes, RY coding eliminates third position transitions; (C) β-fibrinogen; (D) Rag1. Model differs for each analysis (Table II).

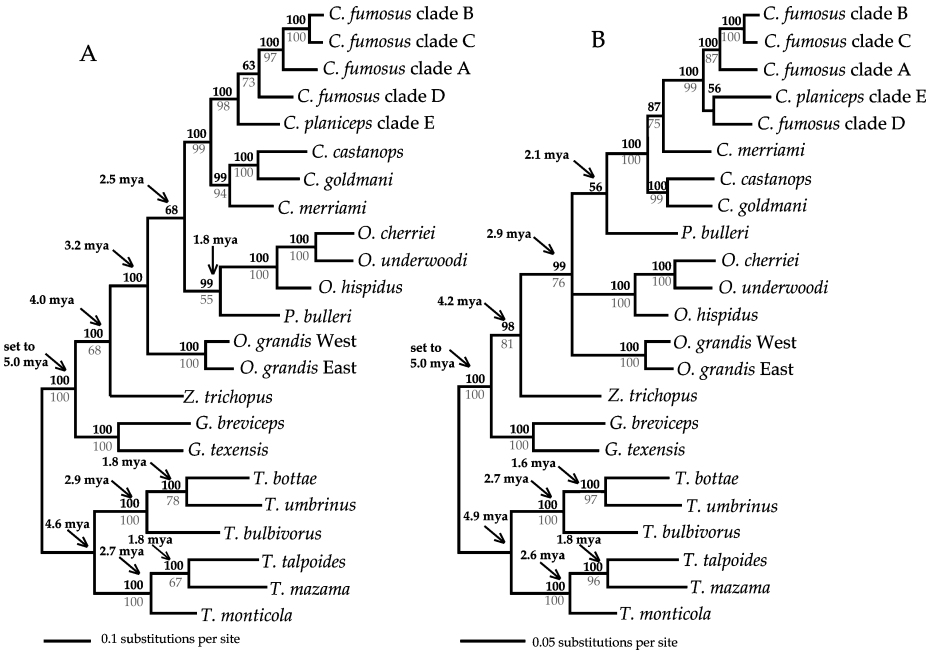

Fig. 4. Bayesian analysis of relationships in the tribe Geomyini, using *Thomomys* as the outgroup, based on data from two mitochondrial and two nuclear genes. Data are partitioned by gene (Table II), using different parameters for each partition. (A) Mitochondrial third-position transitions included in the analysis; (B) mitochondrial third-positions RY coded. Bayesian posterior probability values greater than 50% are shown in dark text above nodes, maximum likelihood bootstrap values (Table II) greater than 50% are shown in gray below nodes. Intergeneric divergence dates correspond to output from NPRS analysis (Sanderson, 1997) using a date of 5.0 mya for the initial divergence within the Geomyini as a calibration point.

than when RY-coding is used (Fig. 4B). On the other hand, for determining relationships among geomyine genera, RY-coded data may have a greater potential for recovering true relationships as these analyses show better bootstrap support for relationships within the Thomomyini (Fig. 4B) than do analyses of non-RY coded data (Fig. 4A). Because *Thomomys* species show levels of sequence divergence similar to those between geomyine genera, and because there is evidence of saturation in mitochondrial third-position transitions for many of these comparisons (Fig. 2), analyses of RY-coded data may be more appropriate for this level of divergence.

Tree-based analyses of evolutionary rate within the Geomyini (Takezaki *et al*., 1995) indicated significant heterogeneity in rate of evolution whether RY coding was used for third positions or not ($Q = 81.1$ and 77.0, respectively, df $= 16$, $p < 0.001$). Rate heterogeneity was evident for both nuclear and mitochondrial genes. β-fibrinogen, Rag-1, combined mitochondrial genes, and RY-coded mitochondrial genes each yielded evidence of significant heterogeneity in rate of evolution (Q-values $= 76.6$–81.9, df $= 16$, $p < 0.001$). Elimination of four taxa, *O. cherriei*, *O. hispidus*, *O. underwoodi*, and *P. bulleri*, was necessary to produce a group of geomyines that did not show any significant heterogeneity in rate of evolution in the full data sets (non-RY coded data: $Q = 9.0$, df $= 9$, $p = 0.44$; RY-coded data: $Q = 9.9$, df $= 9$, $p = 0.36$). Based on Lintre results, *Pappogeomys* contributes to the overall heterogeneity in rate of evolution by exhibiting a relatively slow rate of evolution,

while the *Orthogeomys* species seem to have an elevated rate of evolution. Binomial tests used for comparing relative rates of evolution between pairs of taxa confirm that *P. bulleri* has a significantly slower relative rate of evolution when compared to many, but not all, of the *Cratogeomys* species ($p = 0.02$–0.08). *Orthogeomys cherriei* has a significantly faster rate of evolution than does *O. grandis* (binomial test, $p = 0.02$). *Orthogeomys underwoodi* and *O. hispidus* also show a greater number of nucleotide substitutions than are seen in *O. grandis*, but there is no statistically significant difference in rate of evolution among these taxa (binomial test, $p = 0.24$–0.25). Among-taxon evolutionary rate heterogeneity could explain the tendency of *P. bulleri* (a monotypic genus with a long branch) to associate with the more rapidly evolving *Orthogeomys* species in some analyses of the molecular data (Fig. 4A; Omilian and Taylor, 2001) rather than with its proposed sister genus, *Cratogeomys* (Russell, 1968a). Furthermore, if Russell (1968a) was correct in placing *O. grandis* in the same genus as the other *Orthogeomys* species, then heterogeneity in rate of evolution may play a role in the apparent inability of these molecular data to recover that relationship.

Tree Support

Several lines of evidence suggest that the data reported in this study would be of phylogenetic utility if the true history of these genera involved anything other than a rapid phylogenetic radiation. First, the combined 3017 nucleotides of sequence data yield a large number of potentially phylogenetically informative characters from both rapidly evolving and slowly evolving genes (Table III). Second, the data easily recover relationships both older and younger than the intergeneric polytomy in question. For example, monophyly of the Geomyini is supported by 100% posterior probability values and by 94–100% of maximum-likelihood and parsimony bootstrap replicates when the tree is rooted with *Chaetodipus*. Likewise, relationships that are a product of later diversification (e.g., the *Cratogeomys* radiation) are recovered by these data with robust support (Fig. 4). Third, it appears that the characters used in this analysis are evolving at an appropriate rate for the question at hand. Of the characters included in this analysis, mitochondrial third-position transitions are most susceptible to saturation given their higher rate of substitution (Table III). Within the Geomyini, there is some evidence of saturation in third position transitions among the most genetically divergent taxa (Fig. 2), but no evidence of substitutional saturation in other characters, including third position transversions. Analysis of RY-coded data, which eliminates third position transitions from consideration, still includes 443 potentially parsimony informative characters. Finally, relationships within the Thomomyini are resolved with high posterior probability and bootstrap support (Fig. 4B), and these relationships involve approximately the same levels of genetic divergence that are seen among genera in the Geomyini (Table IV).

Given that taxon sampling in this study is nearly exhaustive, especially in the least resolved areas of the tree (Fig. 3), it is unlikely that additional samples will enhance phylogenetic resolution in the Geomyini (Johnson, 2001). For example, *Zygogeomys* and *Pappogeomys* are monotypic genera (Hafner and Barkley, 1984; Demastes *et al*., 2003), and diversity within the genus *Orthogeomys* is well represented in this study. The only species missing from the subgenus *Orthogeomys* is *O. cuniculus*, a species known from a single locality (Hall, 1981) and for which tissues are not available. Similarly, the only species missing from the subgenus *Heterogeomys* is *O. lanius*, which is another taxon restricted to a single locality and judged by Hall (1981) to most likely be conspecific with *O. hispidus*

(included here). Finally, taxon-sampling within the *Orthogeomys* subgenus *Macrogeomys* and within the *Cratogeomys* lineage is broad and representative of all the major lineages identified in previous molecular studies (Sudman and Hafner, 1992; Demastes *et al*., 2002). Therefore, in the areas of the tree where relationships are most difficult to determine, taxon sampling likely cannot be improved.

Branch Lengths and Timing of Diversification

Modern pocket gopher genera first appear in the fossil record beginning in the Blancan North American Land Mammal Age (Russell, 1968a; Korth, 1994), which began approximately 5 mya (Lindsay *et al*., 2002). *Geomys* first appears in the earliest Blancan deposits (Kurtén and Anderson, 1980; Martin *et al*., 2002). Prior to the Blancan, there are numerous records of the Geomyidae in the fossil record, but all representatives belong to more primitive genera that are quite distinct morphologically from extant pocket gopher genera (Korth, 1994). The first record of *Cratogeomys* (*C. bensoni*) occurs in formations 4.2- to 3.2-my-old (Savage and Russell, 1983). Therefore, it seems reasonable to suggest that *Geomys* diverged from other members of the Geomyini in the early Blancan, probably no earlier than about 5 mya. Dating the divergence between these taxa at 5 mya, other intergeneric divergence points can be viewed in a relative time perspective that takes into account observed among-taxon heterogeneity in rate of evolution (Sanderson, 1997). For example, using branch lengths from the Bayesian analysis of the four genes with mitochondrial RY-coding (Fig. 4B), *Zygogeomys* likely diverged from other genera about 4.2 mya, shortly after the *Geomys* divergence.

The most poorly resolved intergeneric relationships involve the more recent radiation including *Cratogeomys*, *Pappogeomys*, and the two *Orthogeomys* lineages. On the basis of molecular dating analysis, these divergence events occurred between 2.1 and 2.9 mya (Fig. 4B). Therefore, five lineages of the tribe Geomyini (*Cratogeomys*, *Pappogeomys*, two lineages of *Orthogeomys*, and *Zygogeomys*), would have arisen within a period from 4.2 to 2.1 mya, creating a number of small internodal branches, each of which represents a small percentage of the total time since divergence. Thus, a short time of common ancestry between each pair of genera, followed by a relatively long period since common ancestry, is suggested by the molecular data (Fig. 4).

Molecular estimates may suggest a slightly later divergence among many of the geomyine genera than does the fossil record. The next modern genus to appear after *Geomys* is represented by the 4.2- to 3.2-my-old specimen of *C. bensoni*. However, it is not clear, based on available material, whether this specimen is really *Cratogeomys* or *Pappogeomys* (Tomida, 1987), and the possibility exists that this specimen may actually represent an ancestor of both lineages. Regardless, the fossil record suggests an even more rapid radiation among genera than is suggested by this analysis of the molecular data. Such relationships are expected to be difficult, if not impossible, to resolve using stochastically evolving molecules, because slowly evolving characters may not change within the time of common ancestry and rapidly evolving characters will retain little useful information about the brief period of common ancestry that occurred so long ago (Lanyon 1988).

CONCLUSIONS

Relationships among extant pocket gopher genera remain incompletely resolved despite previous studies of this group (Russell, 1968a; Hafner, 1982; Honeycutt and Williams,

1982) and despite the newly generated sequence data analyzed herein. This lack of resolution does not appear to result from insufficient or inappropriate DNA data, nor is it caused by inadequate taxon sampling. Rather, fossil and molecular data both lead to the conclusion that diversification within the tribe Geomyini likely occurred during a geologically brief period of the Blancan. This, compounded by the problem of determining relationships in the presence of among-taxon evolutionary rate heterogeneity, may make such relationships irresolvable using currently available techniques (Lanyon, 1988; Omilian and Taylor, 2001).

The Blancan appears to have been an especially important time in geomyid evolution. The geomyid subfamily Geomyinae, to which all modern pocket gophers belong, reached its highest species diversity (of the Tertiary Period) in the Blancan (Korth, 1994). The Blancan also witnessed a sharp increase in generic diversity among geomyids, with the number of pocket gopher genera more than doubling compared to the previous North American Land Mammal Age (Hemphillian; ca. 10–4.5 mya; Korth, 1994). Molecular evidence from living pocket gopher species is consistent with a rapid Blancan radiation.

The ultimate cause of the Blancan geomyid radiation is unknown. However, near the end of the Hemphillian and beginning of the Blancan, the North American climate became increasingly arid, resulting in a shift from extensive savannas to extensive true grasslands in midcontinental and western North America (Webb and Opdyke, 1995). Webb and Opdyke (1995) point out that, even though true grasslands covered much of this area, a number of regions of North America supported very different habitats, with a notable increase in provincialism during the Blancan. Perhaps habitat patchiness increased geographic subdivision among early members of the Geomyini, leading to isolation of populations and evolution of new taxa. Another possible explanation for the apparently rapid increase in pocket gopher diversity in the Blancan could be the attainment of hypsodonty (high-crowned and evergrowing cheek teeth). Korth (1994) describes a gradual increase in pocket gopher hypsodonty throughout the Pliocene, probably in response to the evolution of abrasive grass species. Cerling *et al*. (1997) postulate that hypsodont lineages of mammals were adaptively superior to nonhypsodont lineages during the Blancan, which may explain why the cricetid rodents, especially the more hypsodont forms, experienced an adaptive radiation in the Blancan (Webb, 1977). Thus, it is possible that the attainment of hypsodonty in pocket gophers during a time of expanding grasslands represents a key innovation in the evolution of modern pocket gophers.

The lack of clear phylogenetic resolution produced when studying rapid evolutionary radiations may be frustrating to systematists, but rapid radiations represent, nonetheless, fascinating periods in evolutionary history. When the fossil record and molecular data both indicate a rapid increase in taxonomic diversity, we are challenged to discover the ultimate cause of the radiation. In the case of the Geomyini, it appears that rapid climate change during the Blancan, the origin of patchily distributed grasslands, and the evolution of hypsodonty may have triggered the diversification that eventually produced five modern genera of the tribe Geomyini within a geologically brief timespan.

APPENDIX

Specimens Examined

GenBank accession numbers for the four genetic loci examined. Accessions marked with an asterisk are from previously published works. *Cratogeomys* taxonomy follows

Hafner *et al*. (in press; clades also correspond to Demastes *et al*., 2002). Voucher specimens are deposited at the Louisiana State University Museum of Natural Science (Baton Rouge), Tarleton State University (Tarleton, Texas), and Centenary College (Shreveport, Louisiana). Locality data, collector information, and museum accession numbers are reported in the GenBank database.

	cyt *b*	COI	Rag-1	β-fibrinogen
Cratogeomys fumosus, clade A	AF302179*	AY506563	AY506566	AY506569
C. fumosus, clade B	AF302174*	AY331073	AY331217	AY331238
C. fumosus, clade C	AF302170*	AY331074	AY331218	AY331239
C. fumosus, clade D	AF302165*	AY331075	AY331219	AY331240
C. planiceps, clade E	AF302183*	AY506564	AY506567	AY506570
C. castanops	AF302171*	AY331076	AY331220	AY331241
C. goldmani	AF302176*	AY331077	AY331221	AY331242
C. merriami	AF302158*	AY331078	AY331222	AY331243
Geomys breviceps	L28736*	AY331085	AY331229	AY331250
G. texensis	AY331214	AY331086	AY331230	AY331251
Orthogeomys cherriei	L38473*	AY331079	AY331223	AY331244
O. grandis (West)	AY331212	AY331083	AY331226	AY331247
O. grandis (East)	AY331213	AY331082	AY331227	AY331248
O. hispidus	L38470*	AY331081	AY331225	AY331246
O. underwoodi	AY331211	AY331080	AY331224	AY331245
Pappogeomys bulleri	AF302177*	AY331084	AY331228	AY331249
Zygogeomys trichopus	L38465*	AY331087	AY331231	AY331252
Thomomys bottae	U65289*	AY331088	AY331232	AY331253
T. bulbivorus	AF155867*	AY331090	AY331234	AY331255
T. mazama	AY331216	AY331092	AY331236	AY331257
T. monticola	U65292*	AY506565	AY506568	AY506571
T. talpoides	AY331215	AY331091	AY331235	AY331256
T. umbrinus	U65289*	AY331089	AY331233	AY331254
Chaetodipus penicillatus	AF155868	AY331093	AY331237	AY509148

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