Journal of Mammalian Evolution, Vol. 6, No. 3, 1999

MtDNA Evidence for Repeated Pulses of Speciation Within Arvicoline and Murid Rodents

Chris J. Conroy[1,2,3] and Joseph A. Cook[1]

We examined temporal aspects of phylogenetic relationships among 5 murid rodent subfamilies and 11 arvicoline genera based on DNA sequences of the cytochrome *b* gene (n = 92) and ND4 gene (n = 17). We found monophyly for Muridae but a polytomy among murid subfamilies. Arvicolinae was monophyletic, but most genera within this subfamily arose from a polytomy. *Microtus* was monophyletic, but within the genus, species arose rapidly. This pattern of nested pulses (polytomies) was recovered across parsimony, distance, and likelihood methods and indicates that accumulation of taxonomic diversity in murids was sporadic, rather than gradual. Arvicolines appeared in the Late Miocene and diversified later, between 3 and 5 million years ago. A relatively high rate of sequence evolution (i.e., 2.3% in third-position transversions per million years) helps reconcile the diversification of fossils and mtDNA lineages.

KEY WORDS: vole; murids; parsimony; likelihood; molecular clock.

INTRODUCTION

The hollow curve distribution of species richness, where a few taxa (e.g., genera) are species rich but most are depauperate within a particular family (Fig. 1), has stimulated considerable discussion among biologists (Anderson, 1974; Stanley, 1979) because it is a common feature of higher taxonomic categories. Studies aimed at examining this uneven distribution have generally avoided an explicit phylogenetic framework (e.g., Huston, 1995), yet phylogenies based on molecular techniques have provided insight into the apparent disparity in diversification rates among lineages (e.g., Sanderson and Donoghue, 1996).

The murid rodent subfamily Arvicolinae has been advanced as a classic example of the hollow curve distribution (Reig, 1989) (Fig. 1). Previous attempts to classify or reconstruct the phylogenetic history of the Arvicolinae have been based on morphology, allozymes, karyotypes (reviewed by Musser and Carleton, 1993), and restriction fragments of nuclear and mitochondrial DNA (DeBry, 1992; Modi, 1996). Because few studies have shared many taxa, comparisons across studies have been difficult. Reconstructing the history of arvicolines has been further complicated by apparent pulses of speciation in

[1] University of Alaska Museum and Institute of Arctic Biology, 907 Yukon Drive, Fairbanks, Alaska 99775-6960.
[2] Present address: Department of Biological Sciences, Stanford University, Stanford, California 94305-5020.
[3] To whom correspondence should be addressed. e-mail: chris.conroy@stanford.edu

0164-7554/99/0900-0221$16.00/0 © 1999 Plenum Publishing Corporation

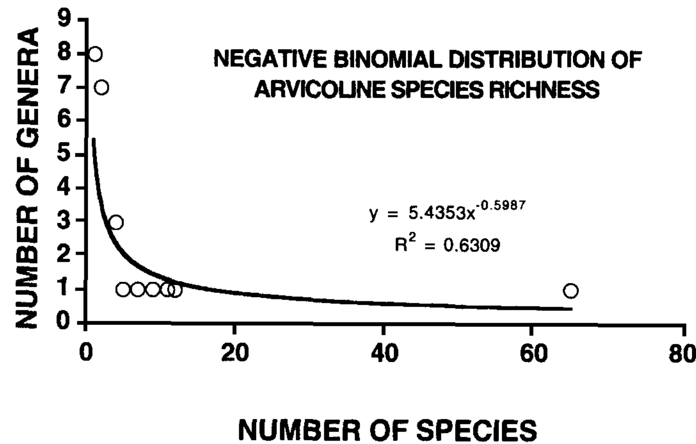

Fig. 1. The hollow curve distribution of species across the 25 genera of the Arvicolinae recognized by Musser and Carleton (1993), but including *Volemys* within *Microtus*. The negative binomial relationship between the number of species within genera and the number of genera with that frequency is modeled with the adjacent equation. The genus with 65 species is *Microtus*.

the fossil record. The earliest arvicoline fossils date to the Late Miocene (Chaline, 1990). Modern arvicoline genera appeared in the Mid- to Late Pliocene (Repenning *et al.*, 1990) and radiated into diverse habitats including tundra, taiga, deciduous and coniferous forest, prairie, and steppe (Gromov and Polyakov, 1977). The radiation of *Microtus* is thought to have occurred within the last 2 million years (Chaline and Graf, 1988). Due to temporal and spatial gaps in the fossil record, the origin of specific lineages has remained obscure (Repenning *et al.*, 1990).

In this paper, we focus on diversification within the Arvicolinae and provide preliminary data on two other apparent cases of rapid diversification at the level of murid subfamilies and among species of *Microtus*. Muridae, the most speciose family of mammals (Musser and Carleton, 1993), also exhibits a hollow curve distribution. A few murid subfamilies are diverse [e.g., Murinae with 122 genera, 529 species (Wilson and Reeder, 1993)], while others are monotypic, suggesting unequal rates of diversification.

We lack a phylogenetic framework to begin exploring diversification in these groups. In particular, a phylogenetic approach would help (1) to address relationships among arvicoline genera which have remained problematic, (2) to characterize the tempo of diversification, (3) to date certain cladogenic events, and (4) to identify sister clades for investigations of the hollow curve distribution of species. A resolved phylogeny would address relationships among taxa. Unresolved branches (e.g., a multifurcation), although obscuring relationships, might best characterize the tempo of diversification.

We used sequences from two mitochondrial genes and expanded the number of taxa previously examined (e.g., DeBry, 1992; Modi, 1987, 1996; Nadler *et al.*, 1978) to test phylogenetic hypotheses among seven of the eight arvicoline tribes and examine further the complex history of diversification within the Muridae.

MATERIALS AND METHODS

Nucleotide Sequences

DNA sequences of the mitochondrial cytochrome *b* (*cyt-b*) gene and a portion of the ND4 gene were generated for 11 genera and 17 species of arvicolines (92 individuals), representing seven tribes (Table 1). These genes efficiently retrieve known phylogenies for this and deeper levels of divergence and they have been sequenced for a substantial number of related taxa (Russo *et al.*, 1996; Zardoya and Meyer, 1996). The *cyt-b* genes of 15 outgroup taxa (4 murine genera, 2 cricetine genera, 3 sigmodontine genera, 1 gerbilline, 1 dipodid, 1 sciurid, and 3 hystricognath genera) were sequenced, retrieved from GenBank, or obtained from J. Salazar (Table I). In related studies, we examined complete sequences of *cyt-b* from 26 species of *Microtus* (Conroy and Cook, 1999).

DNA was extracted from heart or liver (Table I) via a modified salt method (Medrano *et al.*, 1990). Symmetric PCR (Saiki *et al.*, 1988) was used to amplify the complete *cyt-b* gene [*Mus* bp 14139–15282 of the complete mitochondrial genome (Bibb *et al.*, 1981)] using primer pairs MVZ04–MVZ05 and MVZ23–MVZ14 (Smith and Patton, 1993) and arvicoline-specific primer pairs CLETH-16 (5′-AGAAARTAYCATTCTGGYTTAAT; *Mus* bp 14940 is the 3′ end of the primer), CLETH-37 (5′-TAYAAYATAATYGAAACHTGAA; *Mus* bp 14457), VOLE-23 (5′-TACAAGAAACAGGATCAAACAACC; *Mus* bp 14752), and VOLE-14 (5′-TTTCATTACTGGTTTACAAGAC; *Mus* bp 15309). A portion of the mitochondrial ND4 gene (*Mus* bp 832–1377) was sequenced for at least one representative of each arvicoline genus and the sigmodontine *Peromyscus* (Table I) using primers ND4 and LEU (Arévalo *et al.*, 1994). PCR reactions generally included a denaturation step at 94°C for 5 min, followed by 35 cycles at 94°C for 15 sec, 45–50°C for 15 sec, and 72°C for 1 min, followed by a final extension step at 72°C for 5 min. Double-stranded PCR products were precipitated with polyethylene glycol and sodium chloride and pellets were rinsed with 75% ethanol prior to cycle sequencing. Cycle sequencing consisted of a denaturation step at 96°C for 1 min, followed by 35 cycles at 96°C for 10 sec, 50°C for 5 sec, and 60°C for 4 min. Cycle sequencing products [Perkin–Elmer Prism dye terminator kit (Fst-RR, 402119)] were purified with Sephadex G-50 (Sigma) and dried under vacuum. Sequences for both strands were determined on an ABI 373a Stretch DNA sequencer. Alignment was done by eye. Sequences have been deposited in Genbank with Accession Numbers AF119259–119280, AF126430, and AF128932–AF128946.

We sequenced multiple species for four polytypic genera to attempt to break up long branches of the phylogeny (Hillis *et al.*, 1996) and to identify representatives of each group. For higher-level analysis, we used *cyt-b* sequences from the rodents *Phodopus sungorus*, *P. campbelli*, and *Mesocricetus auratus* (Cricetinae); *Apodemus flavicollis*, *Rattus rattus*, *Mus musculus*, and *Acomys airensis* (Murinae); *Meriones unguiculatus* (Gerbillinae; 827 bp cyt b); and *Bolomys amoenus*, *Calomys callosus*, and *Peromyscus keeni* (Sigmodontinae). *Zapus trinotatus* (Dipodidae) and *Sciurus aberti* (Sciuridae) were included as outgroups to Muridae. Hystricognath sequences (Echimyidae: *Makalata didelphoides*, *Proechimys amphichoricus*, and *Mesomys hispidus*) rooted the initial sciurognath tree.

Table I. Taxa Used in Phylogenetic Analysis with cyt b[a]

Family	Subfamily	Arvicoline tribe	Species	Common name	Reference No.
1. Muridae	Arvicolinae	Microtini	*Arvicola terrestris*[b]	Water vole	AF22737
2. Muridae	Arvicolinae	Microtini	*Microtus agrestis*[b]	Common vole	AF3131
3. Muridae	Arvicolinae	Microtini	*Microtus pennsylvanicus*[b]	Meadow vole	NK11205
4. Muridae	Arvicolinae	Microtini	*Microtus montanus*[b]	Montane vole	NK55041
5. Muridae	Arvicolinae	Microtini	*Microtus longicaudus*[b]	Long-tailed vole	AF2031
6. Muridae	Arvicolinae	Clethrionomyini	*Alticola macrotis*[b]	Lemming vole	AF3791
7. Muridae	Arvicolinae	Clethrionomyini	*Clethrionomys glareolus*[b]	Bank vole	AF3133
8. Muridae	Arvicolinae	Clethrionomyini	*Clethrionomys rutilus*	Red-backed vole	AF4853
9. Muridae	Arvicolinae	Dicrostonichini	*Dicrostonyx groenlandicus*[b]	Collared lemming	AF2246
10. Muridae	Arvicolinae	Dicrostonichini	*Dicrostonyx torquatus*	" "	AF5430
11. Muridae	Arvicolinae	Ellobiini	*Ellobius tancrei*[b]	Mole vole	VF224
12. Muridae	Arvicolinae	Ellobiini	*Ellobius fuscocapillus*	" "	VF226
13. Muridae	Arvicolinae	Lemmini	*Lemmus trimucronatus*[b]	Arctic lemming	AF7421
14. Muridae	Arvicolinae	Lemmini	*Myopus schisticolor*[b]	Wood lemming	AF1939
15. Muridae	Arvicolinae	Lemmini	*Synaptomys borealis*[b]	Bog lemming	AF1196
16. Muridae	Arvicolinae	Ondatrini	*Ondatra zibethicus*[b]	Muskrat	AF7445
17. Muridae	Arvicolinae	Phenacomyini	*Phenacomys intermedius*[b]	Heather vole	AF12726
18. Muridae	Cricetinae		*Mesocricetus auratus*	Golden hamster	AF19870
19. Muridae	Cricetinae		*Phodopus sungorus*	Djungarian hamster	AF20111
20. Muridae	Cricetinae		*Phodopus campbelli*	Hamster	AF774
21. Muridae	Gerbillinae		*Meriones unguiculatus*	Jird	AF19868
22. Muridae	Murinae		*Mus musculus*[b]	House mouse	Genbank V00711
23. Muridae	Murinae		*Rattus rattus*[b]	Rat	Genbank X14848
24. Muridae	Murinae		*Acomys airensis*	Spiny rat	Genbank X96996
25. Muridae	Murinae		*Apodemus flavicollis*	Yellow-necked mouse	AF1943
26. Muridae	Sigmodontinae		*Bolomys amoenus*	—	J. Salazar
27. Muridae	Sigmodontinae		*Calomys callosus*	Vesper mice	J. Salazar
28. Muridae	Sigmodontinae		*Peromyscus keeni*[b]	Deer mouse	AF17750
29. Sciuridae	Sciurinae		*Sciurus aberti*	Abert's squirrel	Genbank U10163
30. Zapodidae	Zapodinae		*Zapus trinotatus*	Jumping mouse	AF18534
31. Echimyidae	Echimyinae		*Makalata didelphoides*	Tree rat	Genbank U35413
32. Echimyidae	Eumyopsinae		*Proechimys amphichoricus*	Spiny rat	Genbank L23363
33. Echimyidae	Eumyopsinae		*Mesomys hispidus*	Tree rat	Genbank L23385

[a]Complete data on all specimens are available from C.J.C.
[b]Taxa include ND4 sequences.

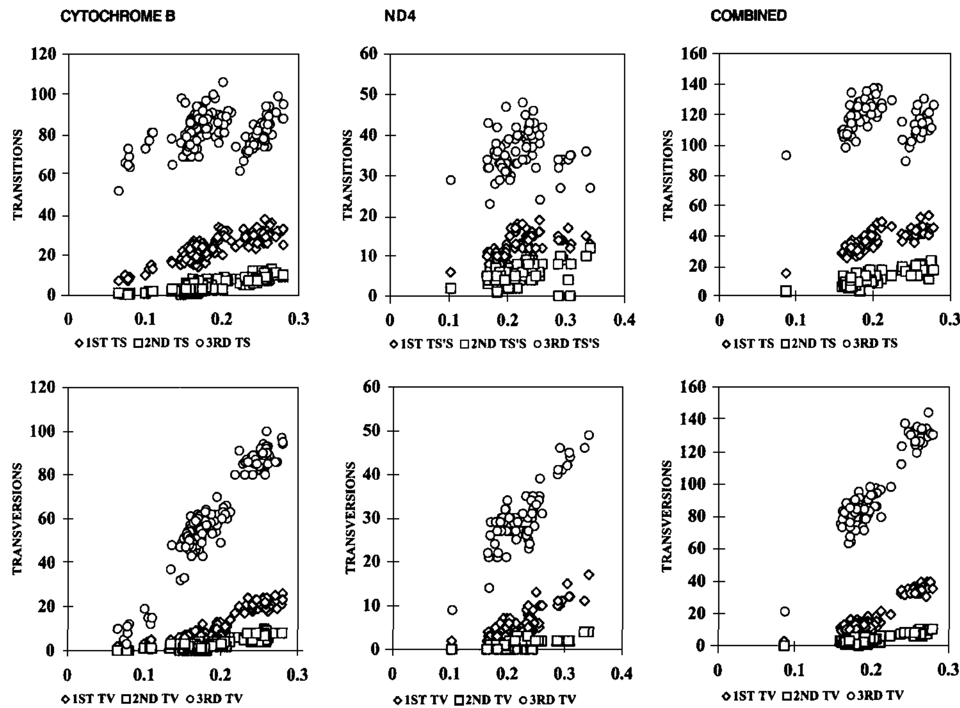

Fig. 2. Comparison of the number of pairwise TS (above) and TV (below) mutations (ordinate) plotted against the maximum-likelihood distance (abscissa) between arvicolines and *Mus* and *Rattus*.

Data Analyses

Nucleotide variation and amino acid variation were examined for both genes [MEGA version 1.02 (Kumar *et al.*, 1993)] across arvicoline genera and between arvicolines and murines (*Mus musculus* and *Rattus rattus*). Pairwise transitions (TS) and transversions (TV) were plotted against maximum-likelihood distance [DNADIST in PHYLIP version 3.572 (Felsenstein, 1993)] to examine saturation for TSs and TVs at each codon position (Fig. 2). To estimate the time of divergence, we tabulated pairwise TVs in codon third positions for several levels of taxonomic divergence and adjusted them with a sigmodontine-based rate of 2.3% substitution per million years (Smith and Patton, 1993).

The random trees option on test version 4.0d59 of PAUP* (Swofford, 1998) constructed 100,000 trees to examine phylogenetic signal at different taxonomic levels using g_1 statistics (Hillis and Huelsenbeck, 1992). All g_1 values were significant ($\alpha = 0.05$), except ND4 third positions. Tajima's (1993) relative rate test was applied within the Arvicolinae. We also tested for a molecular clock by comparing trees built under the F84 model, which allows variation in root to tip path lengths [DNAML (Felsenstein, 1993)] and under a model which assumed a molecular clock (DNAMLK) under three TS/TV ratios (2/1, 10/1, 20/1).

Phylogenetic reconstruction was done with three methods. Weighted maximum-parsimony (MP) searches were completed on PAUP*, with weights based on empirical variation in codon position in pairwise comparisons between arvicolines and murines. A

TS/TV ratio of 10 : 1 (Wakeley, 1996) was also used at deeper levels. Trees were initially rooted with three hystricognath sequences (Lara *et al.*, 1996) to polarize the sciurognath characters (*cyt-b* only). The sigmodontine *Peromyscus* was identified as the closest outgroup to the arvicolines by successively removing basal taxa (Kitching, 1994). Due to the large number of taxa, a heuristic search with default search options was implemented. Random starting options (n = 100) were also implemented to minimize the potential of islands-of-trees problem (Maddison, 1991). To avoid the use of saturated characters in such a taxonomically deep analysis, we also constructed unweighted parsimony trees with amino acids. We used only the *cyt-b* amino acids with deeper murid representatives and *cyt-b* and ND4 for arvicolines with *Peromyscus* as an outgroup.

We also used PAUP* for neighbor-joining [NJ; Kimura's (1980) two-parameter distance] (Saitou and Nei, 1987) and maximum-likelihood (ML; F84 model of substitution, TI/TV = 2, empirical base frequencies, four rate categories, gamma distributed with shape = 0.296) trees. The MP and NJ trees were bootstrapped 250 times, maintaining the original distance schemes, and all were rooted for presentation. Bremer decay indices (Bremer, 1988) were constructed with TreeRot (Sorenson, 1996). These values are not meant for comparison across studies, particularly since weighted parsimony makes these values appear larger than normal. Decay values divided by the length of the tree (Fig. 5) are presented solely for visual purposes and are not meant to be compared across studies.

The topologies of the MP and NJ trees were tested against the ML tree with a likelihood-ratio test (Kishino and Hasegawa, 1989). We also tested four particular relationships based on morphology and karyology. (1) Is *Arvicola* sister to *Microtus* (Bailey, 1900; Hooper and Hart, 1962; Miller, 1896; Nadler *et al.*, 1978)? *Arvicola* displays a striking morphological resemblance to *Microtus*, except that it is more semiaquatic, somewhat like the muskrat *Ondatra*, and it is larger than most species of *Microtus*. (2) Is *Dicrostonyx*, the collared lemming, a member of the true lemmings (Hinton, 1926; Matthey, 1957)? *Dicrostonyx* is a common small mammal of high-latitude tundra habitats. However, despite similarities in habitat and morphology with other "true" lemmings, particularly *Lemmus* and *Myopus*, the tooth structure and karyology of *Dicrostonyx* suggest a deeper historical split. (3) Is *Phenacomys*, the heather vole, sister to *Microtus* (Hinton, 1926; Miller, 1896)? The systematic position of *Phenacomys* has never been clear. (4) Is *Ellobius* sister to *Microtus?* This relationship was hypothesized based on similarity in karyotypes (Matthey, 1957). The phyletic position of *Ellobius*, the mole vole, has been unclear because of its exceptional subterranean adaptations. Repenning (1968) suggested that *Ellobius* be excluded from Arvicolinae based on mandibular musculature. Maximum-likelihood searches were constrained to satisfy these four relationships and evaluated under the F84 ML model with a likelihood-ratio test (Kishino and Hasegawa, 1989).

Trees based on either *cyt-b* or ND4 differed only at branches with weak bootstrap support. We combined these data (Zardoya and Meyer, 1996) because the genes are linked within the nonrecombining mitochondrial genome and appear to be evolving at similar rates (Fig. 2). Increasing sequence length may resolve suspected rapid radiations by increasing the number of synapomorphies (Kraus and Miyamoto, 1991). Although phylogenetic investigations can be flawed if large biases in base and codon composition exist (e.g., Naylor and Brown, 1998), we suggest that this issue is limited within the relatively limited taxonomic scope of this study.

RESULTS

Sequence Variation

This study expands the only other paper describing mtDNA variation in arvicolines, which was limited to two closely related species of *Microtus* over a narrow geographic area (Baker *et al.*, 1996). Arvicolines share a *cyt-b* gene of 1143 bp (381 codons). Among mammals, the number of codons in the *cyt-b* gene varies from 379 [carnivorans, perissodactyls, proboscideans, artiodactyls (Jermiin *et al.*, 1994)] to 388 codons [marsupials (Patton *et al.*, 1996)]. Overall, the nucleotide composition in arvicolines is similar to that in other mammals: adenine (31.5%), cytosine (28.5%), thymine (27.7%) and guanine (12.4%). Third positions exhibited an extreme deficiency of guanine (3.3%), similar to other mammals (e.g., Irwin *et al.*, 1991; Patton *et al.*, 1996). Third-position transitions appeared to saturate more rapidly than first and second positions due to a faster rate of evolution (Fig. 2).

Variation in nucleotides and amino acids for *cyt-b* was similar to that found in other mammals. Of the 469 variable nucleotide sites, 339 were phylogenetically informative among 11 arvicoline and 2 murine genera. The distribution of amino acid replacements among transmembrane, outer surface, and inner surface regions was equivalent ($\chi^2 = 0.87$, $P < 0.1$) to that identified by Irwin *et al.* (1991) for hypervariable sites. For cyt b (n = 32), there were 380 total amino acids (plus a stop codon in arvicolines). Of these, 168 were variable, of which 115 were parsimony informative.

Structural models of the ND4 gene comparable to that for *cyt-b* (Irwin *et al.*, 1991) are not available. Variation across codon positions for ND4 (2.4 : 1 : 8.4) was not statistically different (χ^2 test: $0.90 < p < 0.75$) from *cyt-b* (4 : 1 : 17). Saturation of third-position TSs (Fig. 2), overall base composition, and guanine deficiency (i.e., guanines were present in 10% of bases overall and 4% in third positions) were similar between ND4 and *cyt-b*. Across ND4 sequences (n = 15), there were 181 amino acids, and of these, 55 were variable and 20 parsimony informative.

Of 1689 nucleotide sites from the combined *cyt-b* (1143) and ND4 (546) sequences, 723 were variable (966 invariant) and 529 were parsimony informative among arvicolines and murines (*Mus* and *Rattus*). Variation across codon positions was 3 : 1 : 13 (3 : 1 : 10 for TS and 4 : 1 : 22 for TV) across all taxa. For MP trees, positions were weighted inversely (4 : 13 : 1) to the observed variation (Chippendale and Weins, 1994; Huelsenbeck *et al.*, 1994). The overall TS/TV ratio was 1.4. Varying the TS/TV parameter between 1 and 10 did not significantly alter the likelihood. Third-position TSs appeared saturated beyond a likelihood distance of 0.2 whereas slopes for first- and second-position TSs and all TVs appeared to be constant (Fig. 2). We used the F84 likelihood distance (e.g., Lara *et al.*, 1996; Tan and Wake, 1995), though a similar relationship was found with other genetic distances [e.g., Kimura's two-parameter, calculated with DNADIST (Felsenstein, 1993)]. The combined sequences were 561 amino acids long, of which 223 were variable and 135 were parsimony informative.

Tajima's (1993) relative rate test indicated that branches were not significantly different from expectations under equal rates of evolution. Tree likelihood and topology did not differ significantly when a molecular clock (DNAMLK) was assumed (Felsenstein, 1993), further suggesting similar rates of nucleotide evolution.

Phylogenetic Implications

Similar topologies were found across methods, and three pulses of diversification were identified. Support for monophyly of the Muridae, subfamilies Arvicolinae and Cricetinae, several arvicoline groups (e.g., true lemmings, *Microtus*, *Dicrostonyx*, *Ellobius*), and some sister species of *Microtus* agrees with previous morphological analyses and indicates that the molecular data can resolve relationships across these taxonomic levels (Figs. 3 and 4). For instance, Muridae is supported by numerous dental and

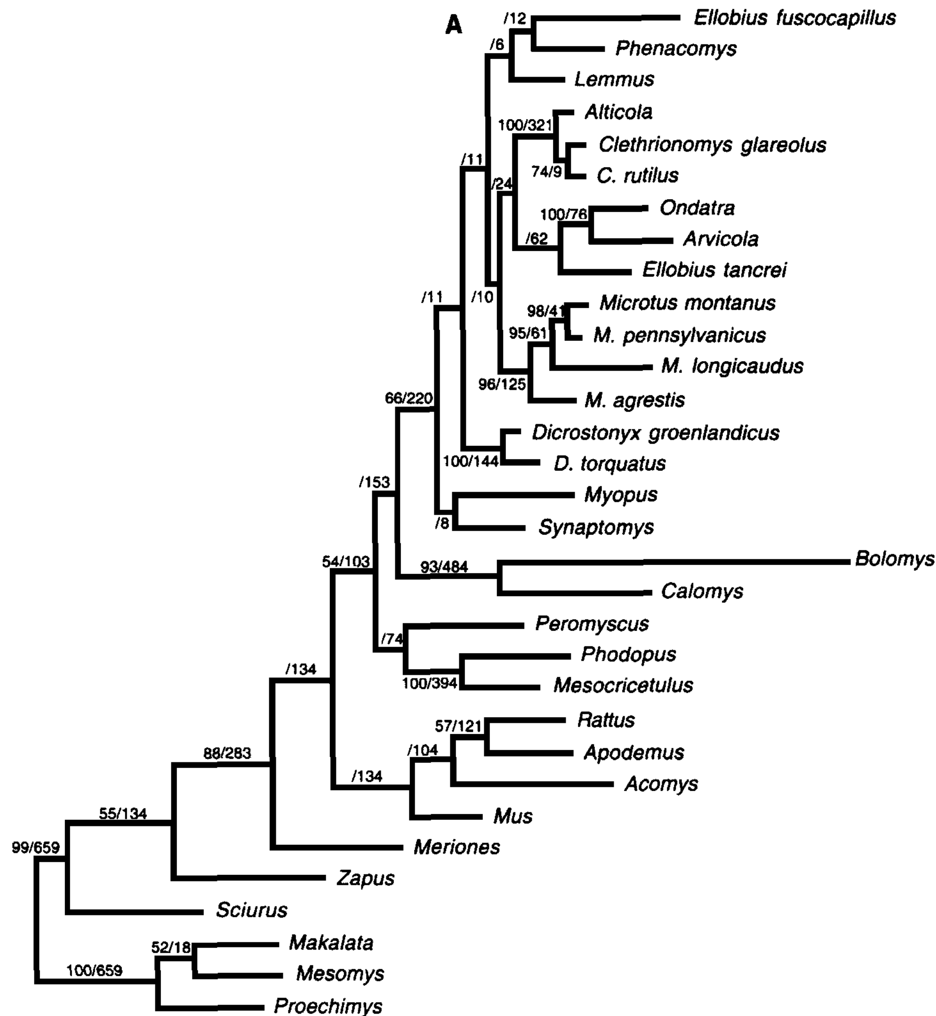

Fig. 3. (A) Maximum-parsimony tree from *cyt-b* sequences rooted with three histricognath sequences, 1 : 10 transition-to-transversion weighting, and 4 : 13 : 1 codon position weighting. (B) Neighbor-joining topology based on Kimura two-parameter distances. Both topologies indicate that *Sciurus* is basal among the sciurognaths examined. Value to the left of the slash is the bootstrap support from 250 replicates where it is 50% or higher; value to the right of the slash is the Bremer decay index.

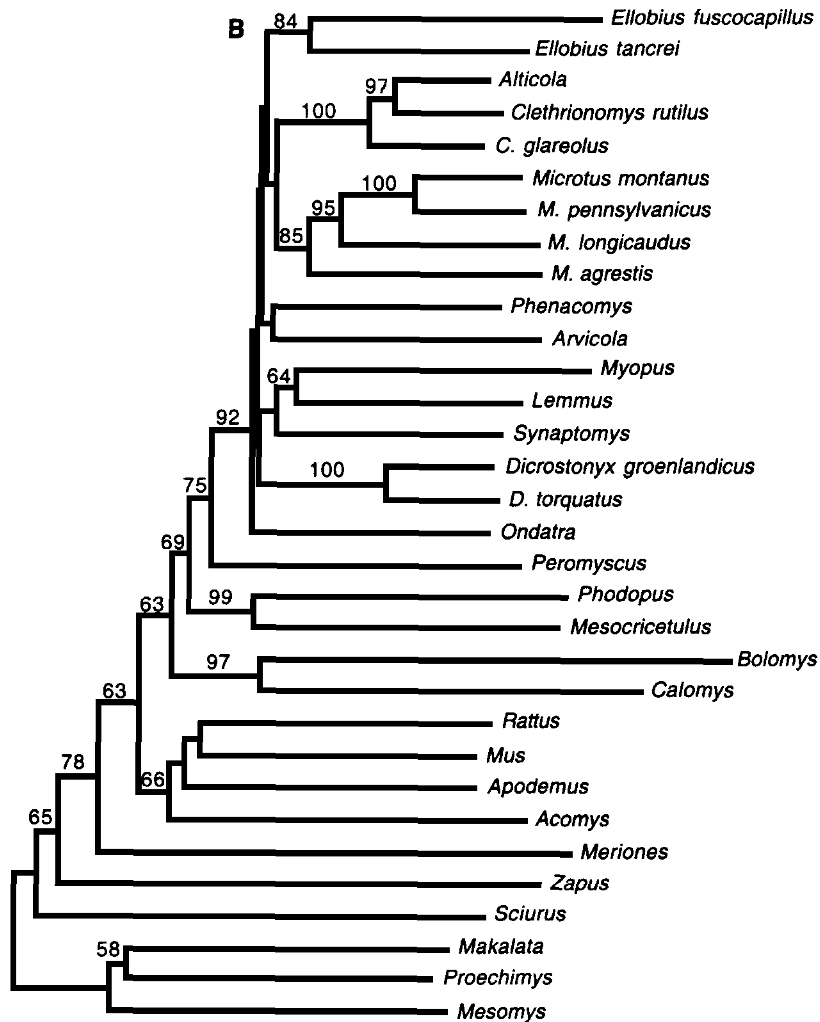

Fig. 3. Continued.

skeletal synapomorphies (Carleton and Musser, 1984) such as uniserial enamel (Flynn *et al.*, 1985). Arvicolinae has been recognized for more than 100 years (Alston, 1876) based on its prismatic tooth pattern. True lemmings (*Lemmus*, *Myopus*, *Synaptomys*) are usually recognized as a monophyletic group, to the exclusion of *Dicrostonyx* (Jarrell and Fredga, 1993). *Microtus*, though possibly paraphyletic with other arvicoline genera (Carleton, 1985), exhibits some derived morphological features such as the way the teeth are rooted and the numbers of triangles on the occlusal surface (Miller, 1896). The monophyly of the genera *Dicrostonyx* and *Ellobius* is supported by the morphological synapomorphies that define those genera (e.g., winter claws in *Dicrostonyx* and fossorial adaptations in *Ellobius*).

A MP tree of *cyt-b* sequences rooted with hystricognath sequences (Fig. 3) placed

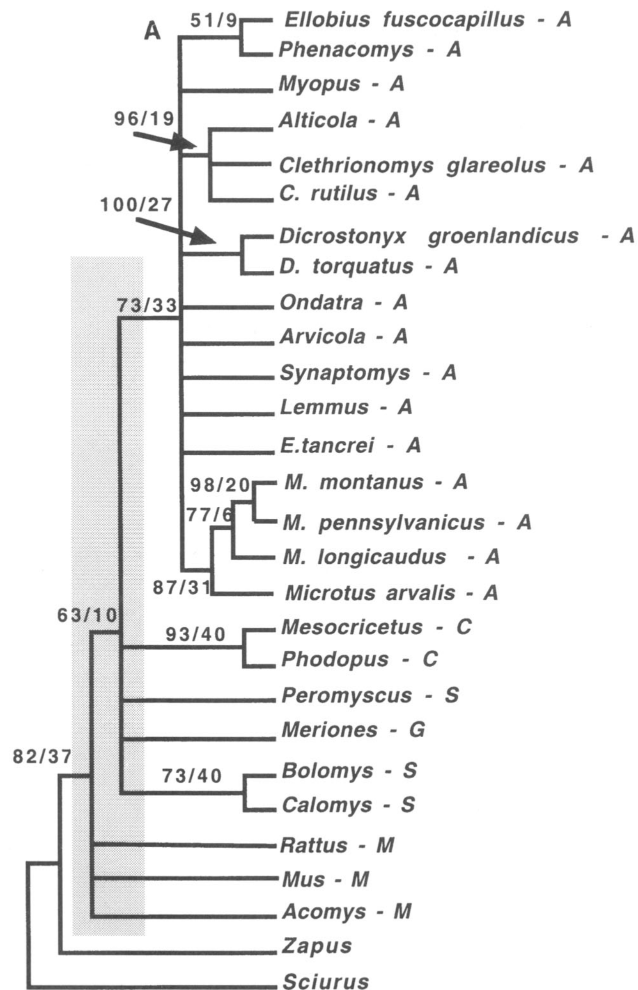

Fig. 4. Phylogenetic analysis of cyt b sequences with *Sciurus aberti* (Sciuridae) as an outgroup to *Zapus trinotatus* (Dipodidae) and murid representatives: (A) maximum parsimony, (B) neighbor-joining with Kimura two-parameter weighting, and (C) maximum-likelihood using the F84 model. Values on branches are bootstrap percentages from 250 iterations, followed by the Bremer decay index. The MP tree (7419 steps) had CI = 0.3937, HI = 0.6649, and RI = 0.4807. Subfamilies: A, Arvicolinae; S, Sigmodontinae; G, Gerbillinae; C, Cricetinae; M, Murinae. Gray shading indicates the area of the inferred pulse.

the sciurid basal to the dipodid and all murids within Sciurognathi. The sciurid sequence was used to root subsequent analyses of higher level murid relationships (Fig. 4) and these indicated that the family Muridae was monophyletic (82% MP and 80% NJ bootstrap support). Two MP trees were obtained. Not all murid subfamilies were monophyletic and bootstrap support for relationships among subfamilies was generally weak (<50%).

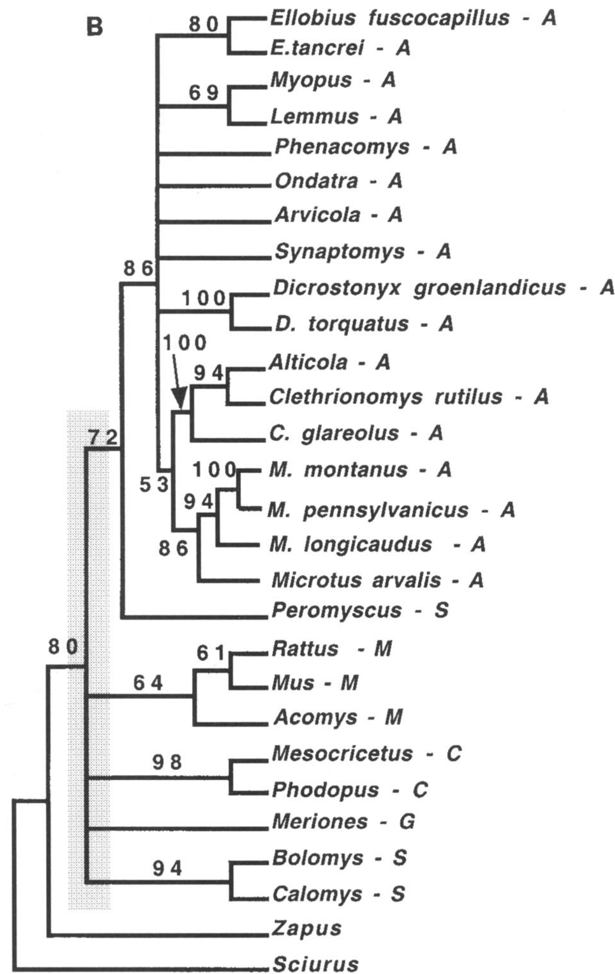

Fig. 4. Continued.

The NJ and ML trees also exhibited weak support for relationships among subfamilies, suggesting a pulse of diversification among the murid subfamilies. However, the Arvicolinae was monophyletic across all methods (bootstrap support, 67% MP and 87% NJ).

The second pulse was among arvicoline genera. Monophyly of the genera *Microtus* and *Dicrostonyx* was well supported in MP analyses. However, the genus *Clethrionomys* was paraphyletic because *Alticola macrotis* was sister to *Clethrionomys rutilus* when arvicolines were rooted with *Peromyscus* (Figs. 5B and C) and in higher-level NJ and ML trees (Figs. 5A–C). One tree island of shortest length was found when MP searches were limited to arvicolines with a *Peromyscus* outgroup. The NJ and ML trees (Figs. 5B and C) supported the monophyly of *Ellobius*, true lemmings (*Myopus*, *Lemmus*, and *Synaptomys*) and a weak but consistent sister-group relationship between the Clethrionomyini

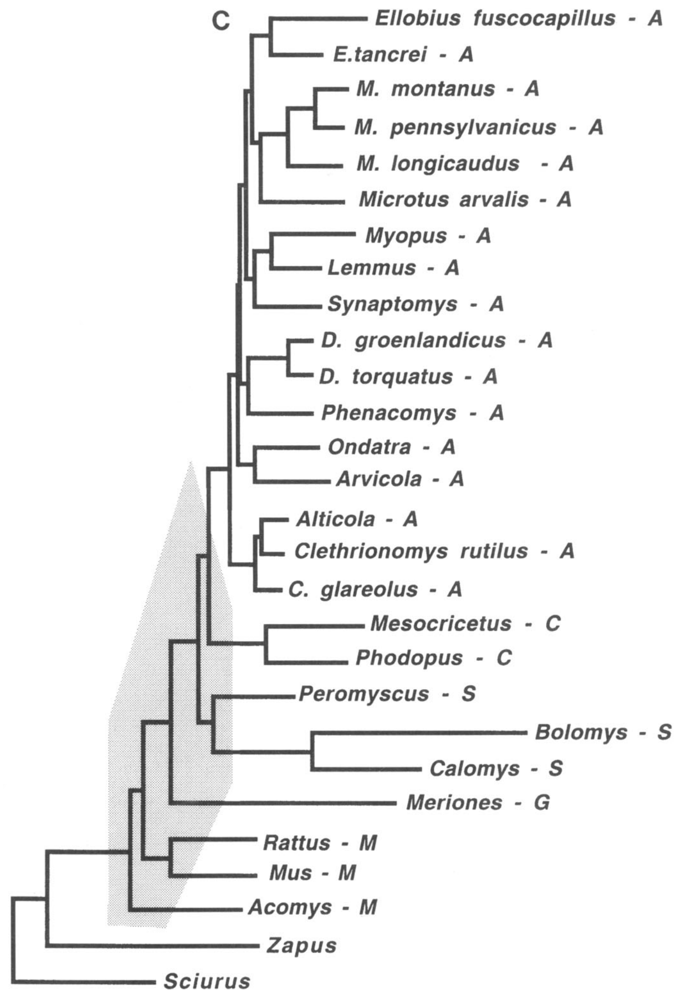

Fig. 4. Continued.

and *Microtus* (69% bootstrap support in the *Peromyscus*-rooted NJ; Fig. 5B). Although nucleotide analysis does not support monophyly of the Clethrionomyini, the amino acid analysis does (Fig. 6). However, this relationship needs to be tested further due to weak sampling of the Clethrionomyini. Otherwise, relationships among genera of the Arvicolinae (e.g., *Dicrostonyx*, *Phenacomys*, *Ondatra*, and *Ellobius*) were poorly resolved. This was corroborated by likelihood-ratio tests which did not reject any of the topologies we tested (Table II). MP and NJ trees constructed with amino acids only (Fig. 6) also suggested that these pulses are not due to saturation, but to rapid branching.

A third most recent pulse led to a rapid diversification among species of *Microtus*, and these details are presented elsewhere (Conroy and Cook, 1999). Evidence for that pulse was apparent only when larger numbers of species of *Microtus* were included. Short branches were found at the base of the radiation of *Microtus*, but a number of previously

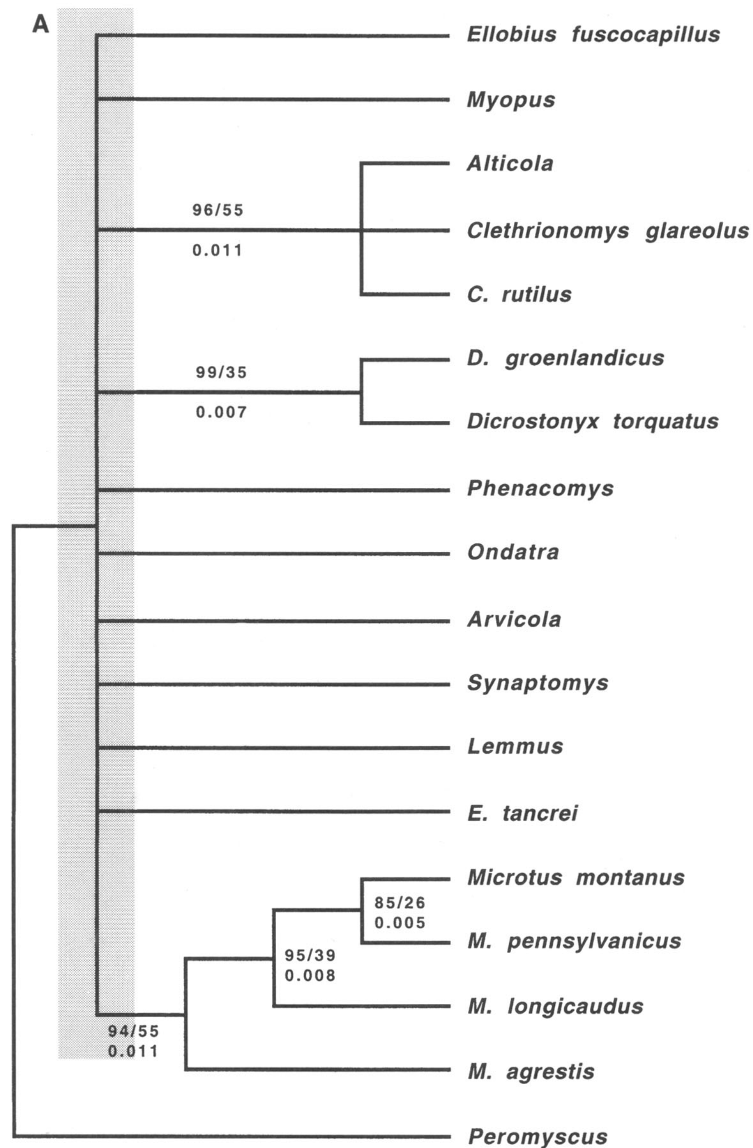

Fig. 5. Phylogenetic analysis with *Peromyscus* as an outgroup to arvicoline representatives and including *cyt-b* and ND4 sequences. (A) One of two maximum-parsimony trees, (B) neighbor-joining with Kimura two-parameter weighting, and (C) maximum-likelihood using the F84 model. Values above MP and NJ branches are bootstrap percentages greater than 50% from 250 iterations, followed by the Bremer decay index. Values below branches are relative Bremer decay indices, calculated by dividing the decay index above the branch by the length of the tree. The MP tree (4862 steps) had CI = 0.5197, HI = 0.4803, and RI = 0.3951. Gray shading indicates the area of the inferred pulse.

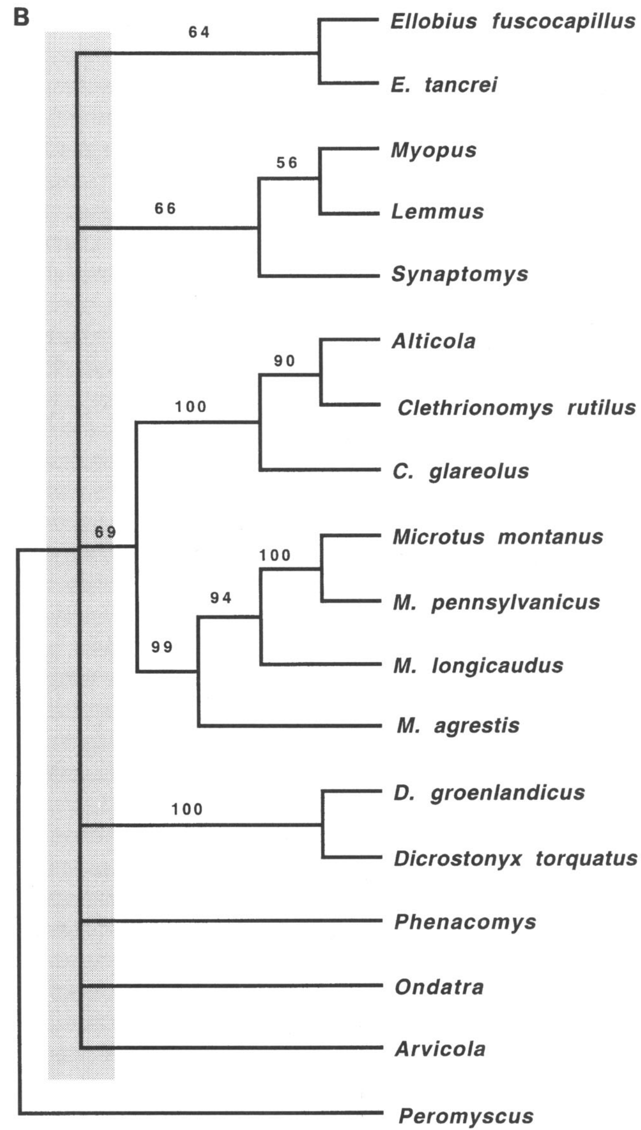

Fig. 5. Continued.

identified sister species (e.g., *M. montanus* and *M. pennsylvanicus*, *M. miurus* and *M. abbreviatus*) were also supported.

DISCUSSION

Polytomies may be the result of homoplasy due to saturation [i.e., "soft" rather than "hard" (Maddison and Maddison, 1992)] or a paucity of synapomorphies along short

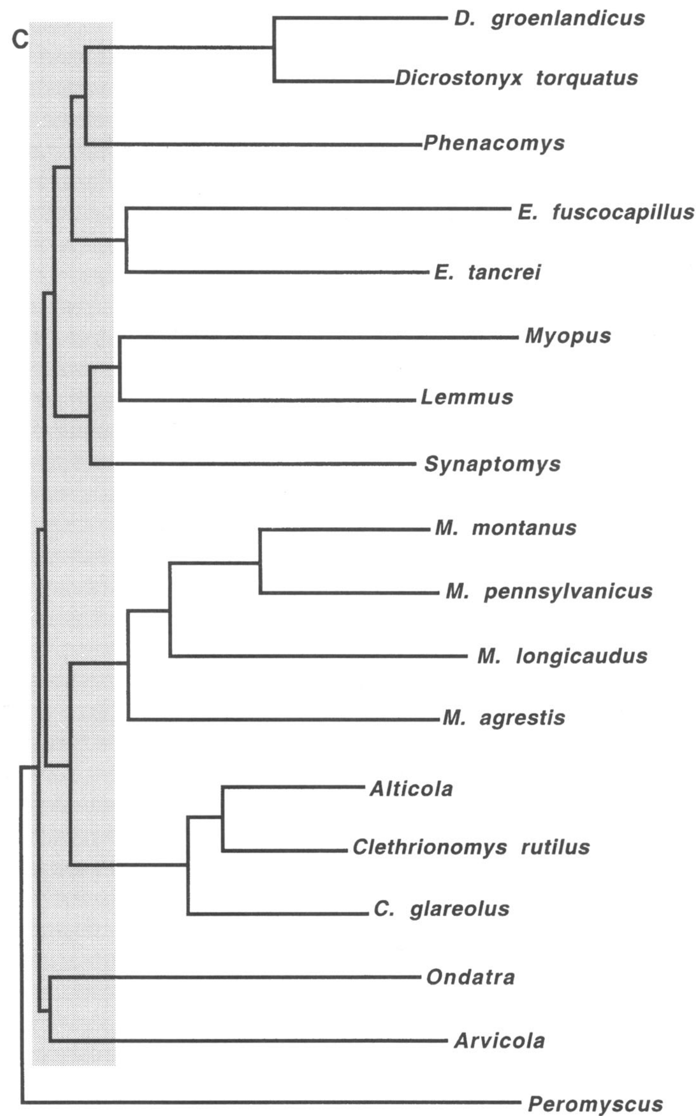

Fig. 5. Continued.

internodal branches due to rapid pulses of speciation. Our data have several characteristics that argue for hard polytomies (Lara *et al.*, 1996; Lessa and Cook, 1998). First, significant g_1 statistics for all but ND4 third positions suggest that the data have phylogenetic signal. Second, consistency across methods suggests that short branches are not an artifact of the phylogenetic analysis. Third, relative rate tests demonstrated that rate heterogeneity among lineages was not a factor affecting tree construction. Fourth, although third positions may exhibit excess homoplasy at deeper branches, when they are removed from analyses we recover a similar tree topology (i.e., the same areas of low support).

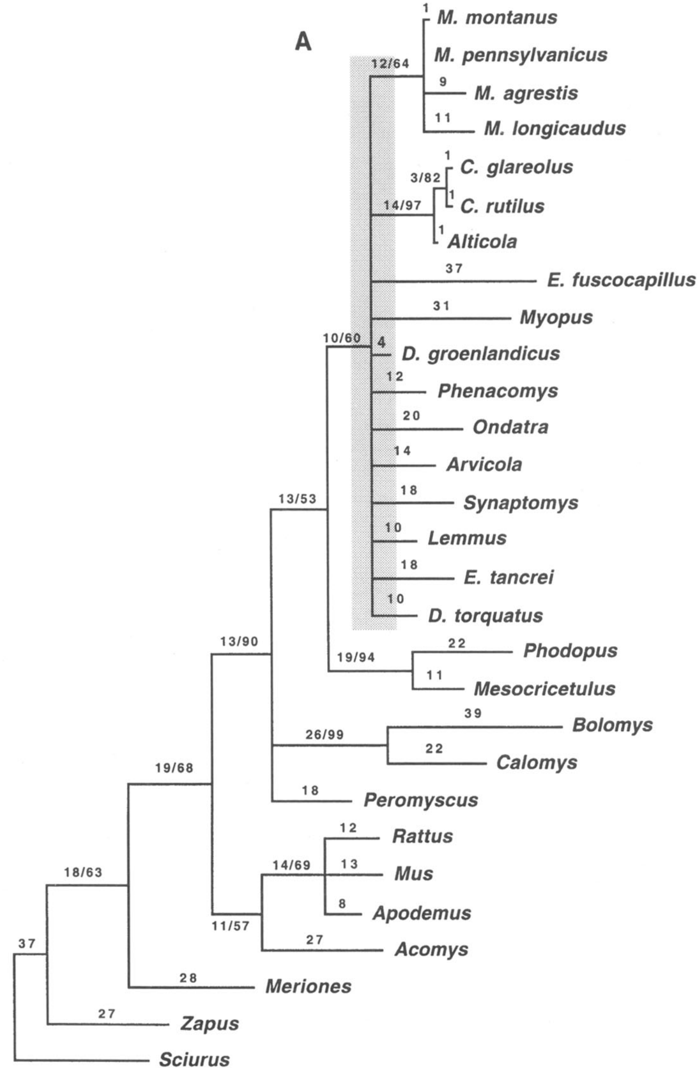

Fig. 6. Maximum-parsimony trees constructed only with amino acids. (A) This tree was constructed with only *cyt-b* amino acids. (B) This tree was constructed from both *cyt-b* and ND4 amino acids as described for the nucleotide analyses. Branch lengths are indicated on all branches, and bootstrap values greater than 50% are shown after the slash.

Amino acid parsimony, which should identify the effects of saturation, also resulted in a similar tree topology (Fig. 6). Finally, bootstrap support at nodes above and below polytomies indicate diversification over a short period of time, rather than saturation effects.

These polytomies may be partially responsible for the problematic taxonomic history of this clade. The number and constitution of tribes have changed repeatedly. Currently

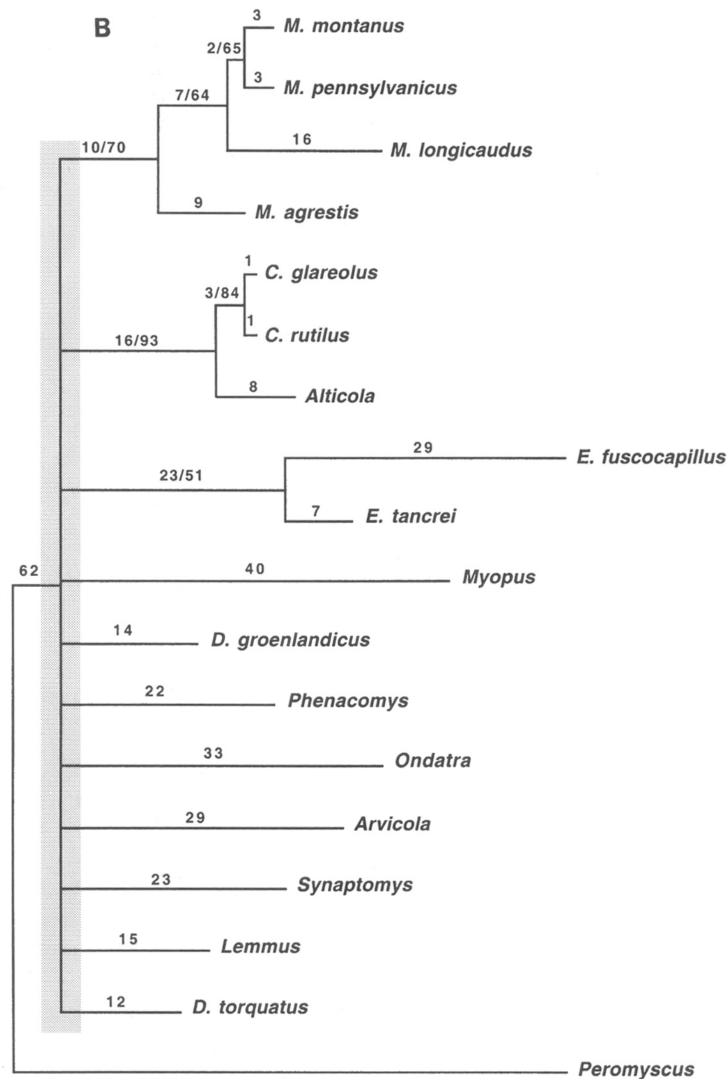

Fig. 6. Continued.

Lemmini, Lagurini, Clethrionomyini, Microtini, Ondatrini, Ellobiini, Phenacomyini, and Dicrostonychini are recognized (Musser and Carleton, 1993). Fossils are abundant for some taxa, but evolutionary relationships among tribes and genera remain poorly resolved (Chaline, 1990; Chaline and Graf, 1988). Morphological convergence is widespread in the subfamily and may have obscured phylogenetic relationships (Courant *et al.*, 1997).

Well-corroborated phylogenies may elucidate aspects of the history of speciation (Mooers and Heard, 1997), such as tempo. Fluctuations between high and low rates of speciation might be reflected in a phylogenetic tree by polytomies (pulses) interspersed with periods of anagenesis versus more regularly spaced bifurcations. Because we did not

Table II. Kishino and Hasegawa (1989) Test of the ML Tree Against the NJ and MP Trees, in Addition to Hypothesized Relationships Derived from the Literature (See Text for Order of Hypotheses)

Tree	−ln*L*	Diff −ln*L*	SD (diff)	*t*	*P*[a]
ML	11821.88	(best)			
NJ, Kimura 2-parameter	11836.49	14.61	11.80	1.24	0.216
MP, no constraint	11839.82	17.94	10.00	1.79	0.073
(1) *Arvicola–Microtus* monophyletic	11838.53	16.65	15.24	1.09	0.275
(2) *Dicrostonyx* & *Lemmini* monophyletic	11826.04	4.16	10.49	0.40	0.692
(3) *Phenacomys* & *Microtus* monophyletic	11830.76	8.88	13.12	0.68	0.499
(4) *Ellobius* & *Microtus* monophyletic	11825.62	3.74	13.48	0.28	0.782

[a]Probability of finding a more extreme *t* value under the null hypothesis of no difference between the two trees (two-tailed test).

reject a molecular clock, branch length may be equated with time (e.g., short branches indicate short periods of time). Below we discuss each of the pulses in conjunction with their systematic implications.

Murid Subfamily Relationships

These data are consistent with other molecular studies that have also suggested a rapid cladogenesis among murid subfamilies [DNA–DNA hybridization (Catzeflis *et al.*, 1995), mtDNA sequences (Engel *et al.*, 1998), nDNA sequences (Robinson *et al.*, 1997)] or their problematic systematics (Flynn *et al.*, 1985). However, no phylogenetic studies have comprehensively considered all 17 subfamilies (Musser and Carleton, 1993). Although the shared polytomous relationships across studies and markers suggest that this pulse may be genuine, it is also possible that the methods used to date have been inappropriate for addressing questions at this taxonomic level. We recommend that markers other than *cyt-b* be used to address this question further. Other subfamilies also need to be sampled for a robust test.

Genera of the Arvicolinae

The phylogenetic relationships among genera in this subfamily were not strongly supported. A hard basal polytomy would suggest that systematic inferences at this level may be flawed. However, relationships that were consistent across methods, that had strong bootstrap support, and that were consistent with previous morphological analyses deserve further attention. For instance, monophyly of Arvicolinae, true lemmings, and *Microtus* reflects relationships identified previously. Paraphyly of *Clethrionomys* with respect to *Alticola* has been reported previously based on DNA–DNA hybridization (Gilëva *et al.*, 1990). Other genera (e.g., *Eothenomys* and *Hyperacrius*) should be included to test monophyly. Nuclear repetitive elements (Modi, 1996) supported the sister relationship between *Microtus* and *Clethrionomys*, the monophyly of lemmings excluding *Dicrostonyx*, and the paucity of synapomorphies among basal nodes within the Arvicolinae. A hard basal polytomy would explain why relationships among arvicoline tribes have remained intractable.

Species of *Microtus*

Our preliminary data for *Microtus* (Conroy and Cook, 1999) support a rapid diversification in this lineage. The tribe Microtini originated in the Late Pliocene, with the earliest fossils assignable to *Microtus* dating to about 2.2 million years ago (Repenning *et al.*, 1990). Fossils for many extant species appeared about 1 to 0.5 million years ago (Zakrzewski, 1985). Despite the abundant fossil record for *Microtus*, phylogenetic reconstruction of its diversification has proved difficult (Chaline and Graf, 1988). Cladogenesis among lineages during Pleistocene glacial cycles has been invoked to explain species diversity in *Microtus* (Hoffmann and Koeppl, 1985). However, the rapid appearance of fossils, lack of morphological synapomorphies that define clades within the genus (e.g., see Carleton, 1985), and molecular phylogenies (Conroy and Cook, 1999) argue instead for a single, major pulse of diversification.

Macroevolution

Molecular phylogenies, by explicitly establishing sister-group relationships and estimating the duration of lineages, provide an opportunity to characterize further the hollow curve distribution of species richness. For example, the consistent sister relationship between *Microtus* and *Clethrionomys* (including *Alticola*) that we have identified will allow us to test whether the increased species diversity in *Microtus* is significantly greater than expected under null models (Sanderson and Donoghue, 1996). If we assume that they are sister taxa, then by definition they are of equivalent age. We can begin investigating differences in diversification rate once a phylogeny is developed that includes more of the extant species of these lineages (Clethrionomyini and *Microtus*).

Periods in which taxonomic diversity has accumulated in these murids have been brief (as identified by short internodal branch lengths). Extant lineages have arisen abruptly at the level of family, subfamily, and genus and subsequently have undergone gradual morphological diversification (Barnosky, 1987; Chaline, 1987). Whether this pattern poses a statistically significant challenge to a gradualistic model of macroevolution will require further testing. Molecular and paleontological data support rapid diversifications in other Rodentia [e.g., Echimyidae (Lara *et al.*, 1996) and Ctenomyinae (Lessa and Cook, 1998)]. However, the mechanisms underlying those pulses remain obscure.

There has been much debate over the causes of pulses and radiations (Givnish and Sytsma, 1997; Stanley, 1979). Vrba (1993, 1995) noted that pulses in speciation may be correlated with abiotic factors [e.g., Milankovitch cycles (Bennett, 1990)]. Repenning *et al.* (1990) and Chaline *et al.* (1993) have suggested that arvicoline evolution and distribution are strongly tied to periodic fluctuations in global climate, with arvicoline dispersals from northern to southern regions tied to regular intervals of roughly every 500,000 years over the last 5.5 million years. We did not find evidence for speciation at regular intervals. Alternatively, our data support Chaline and Graf (1988), who suggested two main radiations in the Arvicolinae: the first, 3–5 million years ago, resulted in diversification among genera, and another, about 2 million years ago, involved the radiation of species of *Microtus*. The specific climatic episodes or other circumstances potentially responsible for these pulses need further investigation.

Age of Divergence and Evolutionary Rates

The paleontological record and other molecular data have established dates and phylogenetic relationships that may be tested with these data. For example, North American sigmodontines may be more closely related to the arvicolines than to other murid subfamilies [DNA–DNA hybridization (Catzeflis *et al.*, 1989, Fig. 12.4), mtDNA sequences (Engel *et al.*, 1998), and fossils, such as *Copemys* (Martin, 1975)]. Arvicolines are found first in the late Miocene, with *Prosomys mimus* in North America and *P. insuliferus* in Eurasia (Chaline, 1987, and references therein). The earliest age of species of *Mimomys* and other ancient arvicolines (*Microtoscoptes* and *Goniodontomys*) has been estimated as 8 million years old (Repenning *et al.*, 1990). Other testable dates include the first appearance of modern genera (3 to 5 million years ago) and the diversification among species of *Microtus* about 2 million years ago (Chaline and Graf, 1988).

To test these dates we averaged the divergence of third position TVs in *cyt-b* between taxa and calibrated a clock at 2.3% change per million years (Smith and Patton, 1993). This rate would place the divergence between Murinae (*Mus* and *Rattus*) and the lineage leading to arvicolines at 9.8 ± 0.5 (1 SD) million years ago. The pulse of diversification among arvicoline genera would have occurred at about 5.7 ± 0.6 million years ago (Fig. 7). This date is much closer to the diversification among genera recognized by paleontologists [e.g., 3 to 5 million years ago (Chaline and Graf, 1988)]. This rate places our estimate of divergence among species of *Microtus* at 3.5 ± 0.95 million years ago. Thus, cladogenesis within *Microtus* or within its putative ancestor *Allophaiomys* (Repenning *et al.*, 1990; Gromov and Polyakov, 1977) may have occurred much earlier than implied by the existing fossil record.

Estimates from Other Molecular Data

Other estimates of divergence do not coincide with the dates based on the mitochondrial data. Using DNA–DNA hybridization data, Catzeflis *et al.* (1989) placed the divergence between Arvicolinae and Murinae at 15.6 (± 3.3) million years ago, which is much older than our estimate of 9.8 million years ago. Estimates from other DNA–DNA hybridization and immunological distance studies (reviewed by Nikoletopoulos *et al.*, 1992) are also more ancient, suggesting that this divergence may have been 20 to 58 million years ago. The *Clethrionomys–Microtus* divergence, which Catzeflis *et al.* (1989) dated at 4.2 (3.2–5.5) million years ago, falls near our estimate of 5.76 million years ago. These are less than estimates from nuclear LCAT sequences [7 to 12 million years ago (Robinson *et al.*, 1997)].

Calibration of a molecular clock for sequence evolution in murids might benefit from additional comparative molecular studies and refinement of the fossil record. Rodents may have higher rates of molecular evolution than other mammals due to their small body size, short generation time, and high metabolic rate (Martin and Palumbi, 1993; Wu and Li, 1985). Although a rate based on sigmodontine divergence (Smith and Patton, 1993) helps to reconcile fossil and molecular estimates in arvicolines, a faster rate may improve the fit. However, we lack independent evidence to corroborate third-position transversion rates faster than 2.3%/MY.

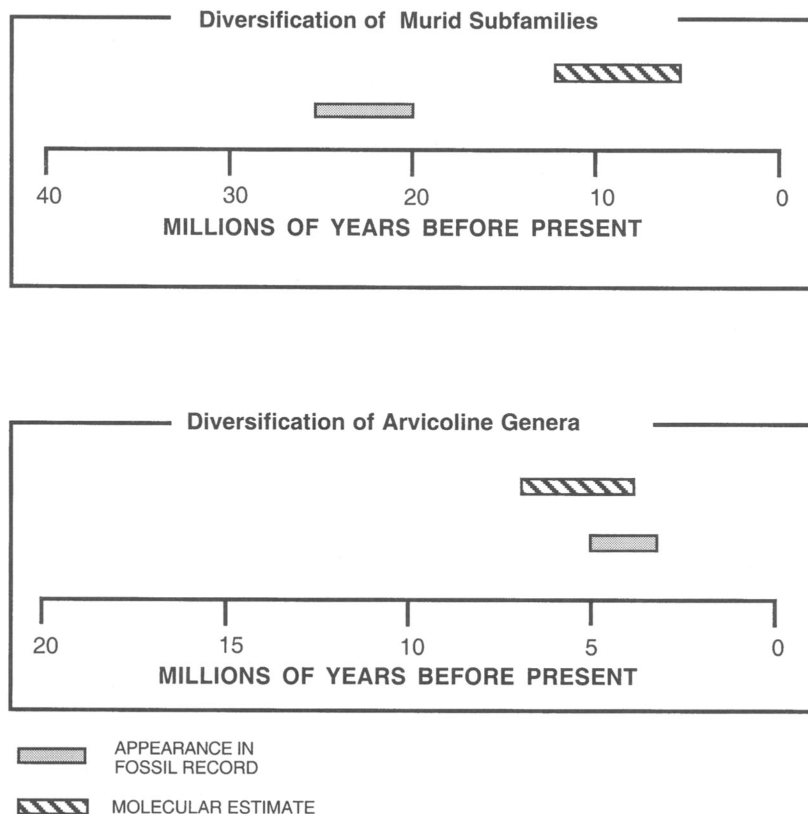

Fig. 7. Estimates of divergence from fossil and molecular data. Molecular estimates are based on change in third-position transversions and assume a rate of 2.3% per million years (Smith and Patton, 1993). Fossil estimates are from Chaline and Graf (1986) for arvicolines (3 to 5 million years ago) and from Catzeflis *et al.* (1992) for murid subfamily diversification (≈25 to 20 million years ago).

Fossil vs Molecular Estimates

A potential source of error in calibrating a molecular clock for rodents is the estimation of the dates of divergence between fossil taxa (O'hUigin and Li, 1992; Robinson *et al.*, 1997; Ruedas and Kirsch, 1997; Catzeflis *et al.*, 1992). Fossils provide a minimum estimate for divergence times among taxa (Springer, 1995; Novacek, 1992) and molecular data often estimate branching events that predate the fossil record. For example, Riddle (1995) attributed the diversification of arid land rodents in North America to the Mid-Miocene climate change, rather than Pleistocene glacial cycles, as suggested previously from the fossil record. Similarly, Klicka and Zink (1997) placed much of the highly diverse North American passerine birds divergence in the Pliocene. Cooper and Fortey (1998) noted that several taxonomic explosions (e.g., the Cambrian) may have been preceded by millions of years of molecular evolution that were not identified in the fossil record. The late Miocene appearance of some arvicolines (*Microtoscoptes*, *Mimomys*,

Goniodontomys) suggests an earlier arvicoline diversification than generally recognized [e.g., 3 to 5 million years ago (Chaline and Graf, 1988)] and is more ancient than our molecular estimates suggest. However, an earlier and additional diversification is compatible with our analysis since the extant taxa in our study may be derived from one survivor of this earlier event.

CONCLUSIONS

This paper is another step toward recovering and refining the history of arvicoline rodents. We found two rapid pulses of speciation in this clade, one among genera and another among species of *Microtus*. An additional pulse earlier in the history of the Muridae (i.e., radiation of subfamilies) may be the result of saturation of our DNA data (i.e., among third-position transitions). These repeated pulses of speciation challenge a gradualistic model of speciation in the Muridae, and help to interpret the uncertain systematics that have plagued this group.

A molecular estimate based on sigmodontines (Smith and Patton, 1993) indicates that the major diversification of the modern arvicoline genera occurred much more recently than the origin of the clade, as suggested by paleontologists.

Further investigation of rate heterogeneity among rodent lineages and more attention to cladogenesis among fossils would improve our understanding of nested diversification in the Muridae, the most speciose family of mammals. Vrba (1995) suggested that because climatic phenomena may be cyclical, their effects on pulses in speciation should be hierarchical. A profitable area of research may be to relate the nested nature of the pulses we have identified with nested climatic phenomena.

ACKNOWLEDGMENTS

Many individuals and institutions provided material for this study, including T. Yates, J. Salazar, and W. Gannon, Museum of Southwestern Biology; J. Patton and A. H.-T. Yu, Museum of Vertebrate Zoology, University of California, Berkeley; V. Fedorov and K. Fredga, Uppsala University; H. Hentonnen, Finnish Forest Research Institute; G. Jarrell, University of Alaska Museum; N. Dokuchaev and A. Tsvetkova, Institute for Biological Problems of the North, Magadan, Russia; H. Anchordoquy, University of Colorado, Boulder; and F. Jannett, Science Museum of Minnesota, St. Paul. We thank S. Anderson, S. Armbruster, J. Demboski, A. Dickerman, M. Fleming, R. D. Guthrie, D. Hopkins, E. Lessa, W. Maddison, S. Nichol, G. Shields, and K. Stone for valuable input. This research was supported by a Graduate Resource Fellowship from UAF and grants from the USFWS, USDA Forest Service, UA Museum Endowment, and Hayward Fund (UA Foundation).

LITERATURE CITED

Alston, E. R. (1876). On the classification of the Order Glires. *Proc. Zool. Soc. London* **1876:** 61–98.

Anderson, S. (1974). Patterns of faunal evolution. *Q. Rev. Biol.* **49:** 311–332.

Arévalo, E., Davis, S. K., and Sites, J. W., Jr. (1994). Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of the *Sceloporus grammicus* complex (Phrynosomatidae) in central Mexico. *Syst. Biol.* **43:** 387–418.

Bailey, V. (1900). Revision of the American voles of the genus *Microtus. North Am. Fauna* **17:** 1–88.

Baker, R. J., Van Den Bussche, R. A., Wright, A. J., Wiggins, L. E., Hamilton, M. J., Reat, E. P., Smith, M. H., Lomakin, M. D., and Chesser, R. K. (1996). High levels of genetic change in rodents of Chernobyl. *Nature* **380:** 801–802.

Barnosky, A. D. (1987). Punctuated equilibrium and phyletic gradualism. In: *Current Mammalogy, Vol. 1*, H. H. Genoways, ed., pp. 109–147, Plenum Press, New York.

Bennett, K. D. (1990). Milankovitch cycles and their effects on species in ecological and evolutionary time. *Paleobiology* **16:** 11–21.

Bibb, M. J., Van Etten, R. A., Wright, C. T., Walberg, N. W., and Clayton, D. A. (1981). Sequence and gene organization of mouse mitochondrial DNA. *Cell* **26:** 167–180.

Bremer, K. (1988). The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* **42:** 795–803.

Carleton, M. D. (1985). Macroanatomy. In: *Biology of New World Microtus*, R. H. Tamarin, ed., pp. 116–175, Special Publication, American Society of Mammalogists.

Carleton, M. D., and Musser, G. G. (1984). Muroid rodents. In: *Order and Families of Recent Mammals of the World*, S. Anderson and J. K. Jones, Jr., eds., pp. 289–379, John Wiley & Sons, New York.

Catzeflis, F. M., Nevo, E., Ahlquist, J. E., and Sibley, C. G. (1989). Relationships of the chromosomal species in the Eurasian mole rats of the *Spalax ehrenbergi* group as determined by DNA-DNA hybridization, and an estimate of the spalacid-murid divergence time. *J. Mol. Evol.* **29:** 223–232.

Catzeflis, F. M., Aguilar, J.-P., and Jaeger, J.-J. (1992). Muroid rodents: Phylogeny and evolution. *TREE* **7:** 122–126.

Catzeflis, F. M., Hänni, C., Sourrouille, P., and Douzery, E. (1995). Re: Molecular systematics of hystricognath rodents: The contribution of sciurognath mitochondrial 12S rRNA sequences. *Mol. Phyl. Evol.* **4:** 357–360.

Chaline, J. (1987). Arvicolid data (Arvicolidae, Rodentia) and evolutionary concepts. In: *Evolutionary Biology, Vol. 21*, M. K. Hecht, B. Wallace, and G. T. Prance, eds., pp. 237–310, Plenum Press, New York.

Chaline, J. (1990). An approach to studies of fossil arvicolids. In: *International Symposium on Evolutionary Phylogenetics and Biostratigraphy of Arvicolids*, O. Fejfar and W. Heinrich, eds., pp. 45–84, Pfeil-Verlag, Prague.

Chaline, J., and Graf, J.-D. (1988). Phylogeny of the Arvicolidae (Rodentia): Biochemical and paleontological evidence. *J. Mammal.* **69:** 22–33.

Chaline, J., Laurin, B., Brunet-Lecomte, P., and Viriot, L. (1993). Morphological trends and rates of evolution in arvicolids (Arvicolidae, Rodentia): Towards a punctuated equilibrium/disequlibrium model. *Quaternary Int.* **19:** 27–39.

Chippendale, P. T., and Weins, J. J. (1994). Weighting, partitioning, and combining characters in phylogenetic analysis. *Syst. Biol.* **43:** 278–287.

Conroy, C. J., and Cook, J. A. (1999). Molecular systematics of a holarctic rodent (*Microtus:* Muridae). *J. Mammal.* (in press).

Cooper, A., and Fortey, R. (1998). Evolutionary explosions and the phylogenetic fuse. *TREE* **13:** 151–156.

Courant, F., David, B., Laurin, B., and Chaline, J. (1997). Quantification of cranial convergences in arvicolids (Rodentia). *Biol. J. Linn. Soc.* **62:** 505–517.

DeBry, R. W. (1992). Biogeography of New World taiga-dwelling *Microtus* (Mammalia: Arvicolidae): A hypothesis test that accounts for phylogenetic uncertainty. *Evolution* **46:** 1347–1357.

Engel, S. R., Hogan, K. M., Taylor, J. F., and Davis, S. K. (1998). Molecular systematics and paleobiogeography of the South American sigmodontine rodents. *Mol. Biol. Evol.* **15:** 35–49.

Felsenstein, J. (1993). *PHYLIP (Phylogeny Inference Package), Version 3.57c*, University of Washington, Seattle.

Flynn, L. J., Jacobs, L. L., and Lindsay, E. H. (1985). Problems in muroid phylogeny: Relationship to other rodents and origin of major groups. In: *Evolutionary Relationships Among Rodents: A Multidisciplinary Analysis*, W. P. Luckett and J.-L. Hartenberger, eds., pp. 589–616, Plenum Press, New York.

Gilëva, E. A., Rybnikov, D. E., and Miroshnichenko, G. P. (1990). DNA-DNA hybridization and phylogenetic relationships in two genera of voles, *Alticola* and *Clethrionomys* (Microtinae: Rodentia). *Doklady Nauk SSSR* **311:** 477–480.

Givnish, T. J., and Sytsma, K. J. (eds.) (1997). *Molecular Evolution and Adaptive Radiation*, Cambridge University Press, Cambridge and New York.

Gromov, I. M., and Polyakov, I. Ya. (1977). *Fauna SSSR, Mlekopitayushchie, tom 3, vyp. 8 [Fauna of the USSR, Vol. 3, Part 8. Mammals]. Polevki [Voles (Microtinae)].* Nauka, Moscow–Leningrad. (English translation, Smithsonian Institution, Washington, DC.)

Hillis, D. M., and Huelsenbeck, J. P. (1992). Signal, noise, and reliability in molecular phylogenetic analyses. *J. Hered.* **83:** 189–195.

Hillis, D. M., Moritz, C., and Mable, B. K. (1996). Applications of molecular systematics. In: *Molecular System-*

atics, 2nd ed., D. M. Hillis, C. Moritz, and B. K. Mable, eds., pp. 515–544, Sinauer Associates, Sunderland, MA.

Hinton, M. A. C. (1926). *Monograph of the Voles and Lemmings (Microtinae) Living and Extinct, Vol. 1*, Trust. Br. Mus., London.

Hoffmann, R. S. and Koeppl, J. W. (1985). Zoogeography. In: *Biology of New World Microtus*, R. H. Tamarin, ed., pp. 84–115, Special Publication, American Society of Mammalogists.

Hooper, E. T., and Hart, B. S. (1962). A synopsis of Recent North American microtine rodents. *Misc. Publ. Mus. Zool. Univ. Mich.* **120:** 1–68.

Huelsenbeck, J. P., Swofford, D. L., Cunningham, C. W., Bull, J. J., and Waddell, P. J. (1994). Is character weighting a panacea for the problem of data heterogeneity in phylogenetic analysis? *Syst. Biol.* **43:** 288–291.

Huston, M. A. (1995). *Biological Diversity: The Coexistence of Species on Changing Landscapes*, Cambridge University Press, Cambridge.

Irwin, D. M., Kocher, T. D., and Wilson, A. C. (1991). Evolution of the cytochrome b gene of mammals. *J. Mol. Evol.* **32:** 128–144.

Jarrell, G. H., and Fredga, K. (1993). How many kinds of lemmings? In: *The Biology of Lemmings*, pp. 45–57, Linnean Society of London, London.

Jermiin, L. S., Graur, D., Lowe, R. M., and Crozier, R. H. (1994). Analysis of directional mutation pressure and nucleotide content in mitochondrial cytochrome *b* genes. *J. Mol. Evol.* **39:** 160–173.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative study of nucleotide sequences. *J. Mol. Evol.* **16:** 111–120.

Kishino, H., and Hasegawa, M. (1989). Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. *J. Mol. Evol.* **29:** 170–179.

Kitching, I. J. (1994). The determination of character polarity. In: *Cladistics: A Practical Course in Systematics*, P. L. Forey, C. J. Humphries, I. J. Kitching, R. W. Scotland, D. J. Siebert, and D. M. Williams, eds., pp. 22–43, Oxford Science, Systematics Association Publication No. 10, Oxford.

Klicka, J., and Zink, R. M. (1997). The importance of recent ice ages in speciation: A failed paradigm. *Science* **277:** 1666–1669.

Kraus, F., and Miyamoto, M. M. (1991). Rapid cladogenesis among the pecoran ruminants: evidence from mitochondrial DNA sequences. *Syst. Zool.* **40:** 117–130.

Kumar, S., Tamura, K., and Nei, M. (1993). *MEGA: Molecular Evolutionary Genetics Analysis, Version 1.02*, Pennsylvania State University, University Park.

Lara, M. C., Patton, J. L., and Da Silva, M. N. F. (1996). The simultaneous diversification of South American echimyid rodents (Hystricognathi) based on complete cytochrome b sequences. *Mol. Phyl. Evol.* **5:** 403–413.

Lessa, E. P., and Cook, J. A. (1998). The molecular phylogenetics of tuco-tucos (genus *Ctenomys*, Rodentia: Octodontidae) suggests an early burst of speciation. *Mol. Phyl. Evol.* **9:** 88–99.

Maddison, D. R. (1991). The discovery and importance of multiple islands of most-parsimonious trees. *Syst. Zool.* **40:** 315–328.

Maddison, W. P., and Maddison, D. R. (1992). *MacClade 3.0: Interactive Analysis of Phylogeny and Character Evolution*, Sinauer Associates, Sunderland, MA.

Martin, A. P., and Palumbi, S. R. (1993). Body size, metabolic rate, generation time and the molecular clock. *Proc. Natl. Acad. Sci. USA* **90:** 4087–4091.

Martin, L. D. (1975). Microtine rodents from the Ogallala Pliocene of Nebraska and the early evolution of the Microtinae in North America. *Univ. Mich. Pap. Paleontol.* **12:** 101–110.

Matthey, R. (1957). Cytologie comparée, systématique et phylogénie des Microtinae (Rodentia–Muridae). *Rev. Suisse Zool.* **64:** 39–71.

Medrano, J. F., Aasen, E., and Sparrow, L. (1990). DNA extraction from nucleated red blood cells. *Biotechniques* **8:** 43.

Miller, G. S., Jr. (1896). The genera and subgenera of voles and lemmings. *North Am. Fauna* **12:** 1–84.

Modi, W. S. (1987). Phylogenetic analyses of the chromosomal banding patterns among the Nearctic Arvicolidae. *Syst. Zool.* **36:** 109–136.

Modi, W. S. (1996). Phylogenetic history of LINE-1 among arvicolid rodents. *Mol. Biol. Evol.* **13:** 633–641.

Mooers, A. Ø., and Heard, S. B. (1997). Inferring evolutionary process from phylogenetic tree shape. *Q. Rev. Biol.* **72:** 31–54.

Musser, G. G., and Carleton, M. D. (1993). Family Muridae. In: *Mammal Species of the World: A Taxonomic and Geographic Reference*, 2nd ed., D. E. Wilson and D. M. Reeder, eds., pp. 501–755, Smithsonian Institution Press, Washington, DC, and London.

Nadler, C. F., Zhurkevich, N. M., Hoffmann, R. S. Kozlovskii, A. I., Deutsch, L., and Nadler, C. F., Jr. (1978). Biochemical relationships of the Holarctic vole genera (*Clethrionomys*, *Microtus*, and *Arvicola* (Rodentia: Arvicolinae)). *Can. J. Zool.* **56:** 1564–1575.

Naylor, G. J. P., and Brown, W. M. (1998). Amphioxus mitochondrial DNA, chordate phylogeny, and the limits of inference based on comparisons of sequences. *Syst. Biol.* **47:** 61–76.

Nikoletopoulos, N. P., Chondropoulos, B. P., and Fraguedakis-Tsolis, S. E. (1992). Albumin evolution and phylogenetic relationships among Greek rodents of the families Arvicolidae and Muridae. *J. Zool. Lond.* **228:** 445–453.

Novacek, M. J. (1992). Fossils, topologies, missing data, and the higher level phylogeny of eutherian mammals. *Syst. Biol.* **41:** 58–73.

O'hUigin, C., and Li, W.-H. (1992). The molecular clock ticks regularly in muroid rodents and hamsters. *J. Mol. Evol.* **35:** 377–384.

Patton, J. L., dos Reis, S. F., and da Silva, M. N. F. (1996). Relationships among didelphid marsupials based on sequence variation in the mitochondrial cytochrome b gene. *J. Mammal. Evol.* **3:** 3–29.

Reig, O. A. (1989). Karyotypic repatternings as one triggering factor in cases of explosive speciation. In: *Evolutionary Biology of Transient Unstable Populations*, A. Fontdevila, ed., pp. 246–289, Springer-Verlag, Berlin.

Repenning, C. A. (1968). Mandibular musculature and the origin of the subfamily Arvicolinae (Rodentia). *Acta Zool. Cracov* **13:** 29–72.

Repenning, C. A., Fejfar, O., and Heinrich, W.-D. (1990). Arvicolid rodent biochronology of the Northern Hemisphere. In: *International Symposium on the Evolution and Phylogenetic Biostratigraphy of Arvicolids*, O. Fejfar and W.-D. Heinrich, eds., pp. 385–418, Pfeil-Verlag, Prague.

Riddle, B. R. (1995). Molecular biogeography in the pocket mice (*Perognathus* and *Chaetodipus*) and grasshopper mice (*Onychomys*): The late Cenozoic development of a North American aridlands rodent guild. *J. Mammal.* **76:** 283–301.

Robinson, M., Catzeflis, F., Briolay, J., and Mouchiroud, D. (1997). Molecular phylogeny of rodents, with special emphasis on murids: Evidence from nuclear gene LCAT. *Mol. Phyl. Evol.* **8:** 423–434.

Ruedas, L. A., and Kirsch, J. A. W. (1997). Systematics of *Maxomys* Sody, 1936 (Rodentia: Muridae: Murinae): DNA/DNA hybridization studies of some Borneo-Javan species and allied Sundaic and Australo-Papuan genera. *Biol. J. Linn. Soc.* **61:** 385–408.

Russo, C. A. M., Takezaki, N., and Nei, M. (1996). Efficiencies of different genes and different tree-building methods in recovering a known phylogeny. *Mol. Biol. Evol.* **13:** 525–536.

Saiki, R. K., Gefland, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B., and Erlich, H. A. (1988). Primer-directed enzymatic amplifications of DNA with thermostable DNA polymerase. *Science* **239:** 487–491.

Saitou, N., and Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4:** 406–425.

Sanderson, M. J., and Donoghue, M. J. (1996). Reconstructing shifts in diversification rates on phylogenetic trees. *TREE* **11:** 15–20.

Smith, M. F., and Patton, J. L. (1993). The diversification of South American murid rodents: Evidence from mitochondrial DNA sequence data for the akodontine tribe. *Biol. J. Linn. Soc.* **50:** 149–177.

Sorenson, M. D. (1996). *TreeRot*, University of Michigan, Ann Arbor.

Springer, M. S. (1995). Molecular clocks and the incompleteness of the fossil record. *J. Mol. Evol.* **41:** 531–538.

Stanley, S. M. (1979). *Macroevolution: Pattern and Process*, W. H. Freeman, San Francisco.

Swofford, D. L. (1997). *PAUP*4.0d59: Phylogenetic Analysis Using Parsimony*, distributed by the author.

Tajima, F. (1993). Simple methods for testing the molecular evolutionary clock hypothesis. *Genetics* **135:** 599–607.

Tan, A.-M., and Wake, D. B. (1995). MtDNA phylogeography of the California newt, *Taricha torosa* (Caudata, Salamandridae). *Mol. Phyl. Evol.* **4:** 383–394.

Vrba, E. S. (1993). Turnover-pulses, the Red Queen, and related topics. *Am. J. Sci.* **293-A:** 418–452.

Vrba, E. S. (1995). On the connection between paleoclimate and evolution. In: *Paleoclimate and Evolution, with Emphasis on Human Origins*, E. S. Vrba, G. H. Denton, T. C. Partridge, and L. H. Burckle, eds., pp. 24–45, Yale University Press, New Haven, CT.

Wakeley, J. (1996). The excess of transitions among nucleotide substitutions: New methods of estimating transition bias underscore its significance. *TREE* **11:** 158–163.

Wilson, D. E., and Reeder, D. M. (1993). *Mammal Species of the World: A Taxonomic and Geographic Reference*, 2nd ed., Smithsonian Institution Press, Washington, DC.

Wu, C.-I., and Li, W.-H. (1985). Evidence for higher rates of nucleotide substitutions in rodents than in man. *Proc. Natl. Acad. Sci. USA* **82:** 1741–1745.

Zakrzewski, R. J. (1985). The fossil record. In: *Biology of New World Microtus*, R. H. Tamarin, ed., pp. 1–51, Special Publication No. 8, American Society of Mammalogists.

Zardoya, R., and Meyer, A. (1996). Phylogenetic performance of mitochondrial protein-coding genes in resolving relationships among vertebrates. *Mol. Biol. Evol.* **13:** 933–942.