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# MOLECULAR PHYLOGENETICS OF ARVICOLINE RODENTS

A

THESIS

Presented to the Faculty

of the University of Alaska Fairbanks

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for the Degree of

DOCTOR OF PHILOSOPHY

By

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Fairbanks, Alaska

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MOLECULAR PHYLOGENETICS OF ARVICOLINE RODENTS

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Abstract:

The impetus for this dissertation was an interest in geographic variation in *Microtus longicaudus* with a particular focus on populations in the Alexander Archipelago of southeastern Alaska. To establish a framework for interpreting intraspecific variation in *M. longicaudus*, I examined the phylogenetics of 28 species of the genus *Microtus*, including all North American species (Chapters 2 and 4). That study, which corroborates a rapid pulse of diversification noted in the fossil record, necessitated a deeper phylogenetic perspective. Thus, a third objective of the dissertation was to investigate relationships among genera of arvicolines within the framework of other murid rodents. I examined variation in the mitochondrial cytochrome b and ND4 genes using maximum parsimony, distance, and maximum likelihood phylogenetic analyses. Relationships at several taxonomic levels appear intractable due to rapid accumulation and survival of genetic lineages. These rapid radiations were found among species, genera, and possibly subfamilies; however, strong support at these levels for other taxa (e.g., the monophyly of *Microtus*) suggests these genes have strong phylogenetic signal.

Many of the well-supported sister species pairs within *Microtus* (Chapters 2 and 4) had been previously identified based on morphologic or allozyme work (e.g., *M. pennsylvanicus* and *M. montanus*, *M. pinetorum* and *M. quasiater*). The sequence data supported a clade of taiga dwelling species in North America and a clade of eastern and central Asian species. The southernmost arvicoline species of Mexico and Guatemala,

though previously suggested to be derived from a single ancient invasion, did not appear to be either ancient or monophyletic.

Within *M. longicaudus*, a large east-west phylogeographic break was detected that is equivalent in genetic distance to other sister species pairs in the genus. This break may indicate mid to late-Pleistocene differentiation (Chapter 3) within the genus. At higher latitudes, populations of *M. longicaudus* exhibited evidence of recent range expansion including absence of correlation between geographic and genetic structure; and pairwise mismatches among DNA sequences with a single peak and few differences.

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# VI. INTRODUCTION:

Arvicoline rodents have provided model systems for studies in disparate areas of biology. Because of their morphological, physiological, behavioral, and ecological variation, voles of the genus *Microtus* are commonly used as models in mammalian biology (Tamarin, 1985). Species of *Microtus* are found in numerous habitats at the higher latitudes of the Northern Hemisphere (Rose and Birney, 1985) and are common components of mammalian species assemblages from temperate to arctic biomes (Gromov and Polyakov, 1977; Hoffmann and Koeppl, 1985). They have been essential to exploring such phenomena as cycling in mammalian populations (Krebs and Myers, 1974).

Arvicolines have also been useful as biostratigraphic markers for establishing paleoclimatic events (Repenning et al., 1990). The occlusal surface of arvicoline teeth has increased in complexity over geological time scales (Chaline, 1987). Therefore, the complexity of arvicoline teeth and abundance in the fossil record make arvicolines useful paleoclimatic indicators. The synchronicity of climatic events that affect whole continents has been identified from the appearance of arvicolines with similarly complex tooth structure in the fossil record.

Despite their importance to neontology and paleontology, many aspects of arvicoline evolution are understudied. Paleontological studies have documented the first

appearance and subsequent transformation of taxa over time (anagenesis), but cladogenic events and evolutionary relationships have been difficult to identify.

Two major radiations have been recognized within the arvicolinae (Chaline and Graf, 1988). The first radiation occurred among the basal lineages of the subfamily. Several tribes or suprageneric groups are recognized (e.g., collared lemmings, Dichrostonychini; true lemmings, Lemmi; muskrats, Ondatrini), but the relationships among them are unclear due to a nearly simultaneous appearance in the fossil record and few shared characteristics among extant members. Because many species of *Microtus* have a shallow or non-existent fossil record, phylogenetic hypotheses have been difficult to construct from fossil data.

This dissertation adds to the evolutionary framework for arvicoline rodents using molecular techniques. Most molecular studies so far in this group have been biased with respect to taxonomic sampling, or have made use of data that are difficult to compare across studies (e.g., allozymes). This dissertation uses molecular markers that are well-characterized by comparative studies (cytochrome b, ND4) and contribute an independent perspective on the evolutionary history of this group from that interpreted from their morphology. Each chapter evaluates interpretations of the fossil, taxonomic, and molecular data.

The chapters in this dissertation are organized in an effort to uncover emergent properties of evolution that span populations, species, and higher levels within a clade

(Fig. 1). To begin to understand speciation in this group, it is necessary to investigate variation within species, such as genetic divergence over geography. However, to understand macroevolutionary phenomena such as species sorting, higher level studies are necessary. Each level requires a perspective deeper than its primary focus. Unfortunately, few studies exist that have addressed relationships at the family level within the Order Rodentia.

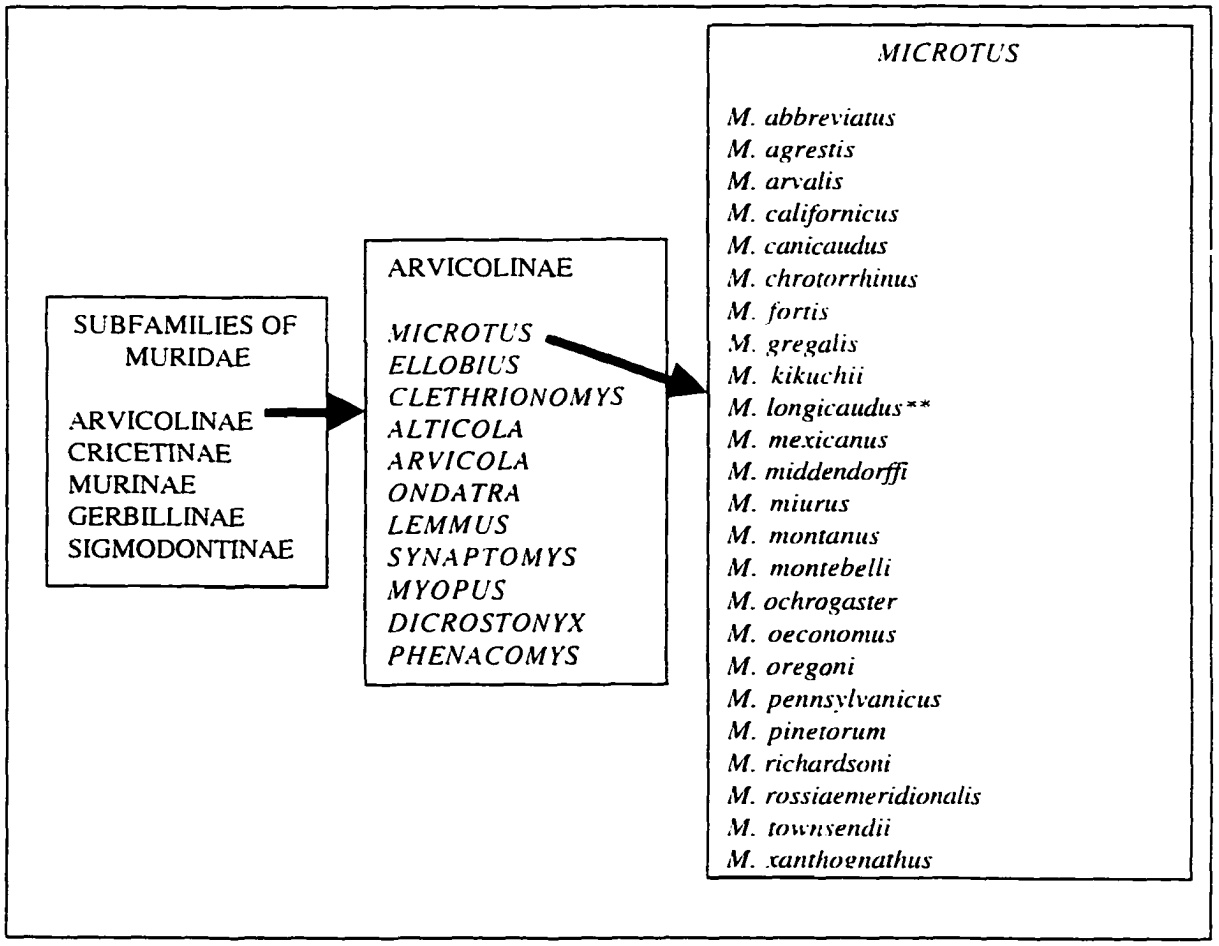


Fig. 1. The chapters in this dissertation are nested in this arrangement. The initial focus is among murid subfamilies and genera of Arvicolinae (Chapter 1), then on species within the genus *Microtus* (Chapter 2). Chapter 3 focuses on populations of one species, *Microtus longicaudus*.

Although molecular data have frequently resolved dichotomous branching relationships among lineages, non-bifurcating or polytomous relationships also have been revealed. This may be due to either saturation of characters for phylogenetic inference (soft polytomy), or might reflect truly rapid speciation (hard polytomy). Inferences from the fossil record and from taxonomy suggest a combination of both dichotomous branching and polytomous relationships might be found among lineages of arvicoline rodents at several levels.

I conducted all the molecular work for these projects, with the occasional assistance of other students in Joe Cook's lab and staff in the Institute of Arctic Biology Core Sequencing Facility. Joe Cook, my advisor and coauthor on all chapters, provided funding for the bulk of the research and provided technical support in methods and assisted in expressing the ideas presented. Fernando Cervantes and Yolanda Hortelano, coauthors on the fourth chapter, provided the specimens and are also contributing their knowledge of the biology of the southern species of arvicolines found in Mexico.

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# Chapter 1

## MtDNA Evidence for Repeated Pulses of Speciation within Arvicoline and Murid Rodents[1]

**Abstract**: We examined temporal aspects of phylogenetic relationships among five 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 three and five 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.

[1] Conroy, C. J., and J. A. Cook. Accepted. MtDNA evidence for repeated pulses of speciation within arvicoline and murid rodents. Journal of Mammalian Evolution

## INTRODUCTION

The hollow curve distribution of species richness (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 in 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 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 to 1) address relationships among arvicoline genera which have remained problematic. 2) characterize the tempo of diversification. 3) date certain cladogenic events. and 4) identify sister clades for investigations of the hollow curve distribution of species. A resolved phylogeny would best aid in address relationships among taxa. However. unresolved branches (e.g.. a multifurcation) might obscure relationships. but they 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 previous 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 I). 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 fourteen outgroup taxa (three murine genera, two cricetine genera, three sigmodontine genera, one gerbilline, one dipodid, one sciurid, and three 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, submitted).

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 minutes, followed by 35 cycles of 94°C for 15 seconds, 45-50°C for 15 seconds, and 72°C for 1 minute, followed by a final extension step of 72°C for 5 minutes. 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 of 96° for one minute followed by 35 cycles of 96°C for 10 seconds, 50°C for 5 seconds, and 60°C for 4 minutes. 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 on Genbank with Accession Numbers XXXX through XXXX.

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); *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.

## 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 TS's and TV's at each codon position (Fig. 2). To estimate time of divergence, we tabulated pairwise TV's 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, 1997) constructed 100,000 trees to examine phylogenetic signal at different taxonomic levels using $g_1$ statistics (Table II; 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 used amino acid parsimony.

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).

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 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 non-recombining 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 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 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 (carnivores, perissodactyls, proboscideans, artiodactyls; Jermiin *et al.*, 1994) to 388 codons (marsupials; Patton *et al.*, 1996). Overall, nucleotide composition in arvicolines is similar to 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 faster 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 two murine genera. The distribution of amino acid replacements among transmembrane, outer surface, and inner surface regions was

equivalent ($X^2 = 0.87$, $p < 0.1$) 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 ($X^2$ test: $0.90 < p < 0.75$) from *cyt-b* (4 : 1 : 17). Saturation of third position TS's (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. 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 TS's appeared saturated beyond a likelihood distance of 0.2, whereas slopes for first and second position TS's and all TV's appeared 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 amino acid sequences were 561 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 the molecular data can resolve relationships across these taxonomic levels (Fig. 4). For instance, Muridae is supported by numerous skeletal synapomorphies (Carleton and Musser, 1984) such as uniserial enamel (Flynn *et al.*, 1984). Arvicolinae has been recognized for more than 100 years (Alston, 1876) based on their 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 the sciurid basal to the dipodid and all murids. 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, 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 %). 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, 87 % NJ).

The second pulse was among arvicoline genera. Monophyly of the genera *Microtus* and *Dicrostonyx* were 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* (Fig. 5-b, c) and in higher level NJ and ML trees (Fig. 4-a, b, 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 (Fig. 5-b, c) supported the monophyly of *Ellobius*, true lemmings (*Myopus*, *Lemmus*, and *Synaptomys*), and a weak but consistent sister-group relationship between the Clethrionomyini and *Microtus* (69 % bootstrap support in *Peromyscus* rooted NJ, Fig. 5-

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

## 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 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 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 removed from analyses we recover similar tree topology (i.e. the same areas of low support). Amino acid parsimony, which should identify the effects of saturation, also resulted in 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 responsible partially for the problematic taxonomic history of this clade. The number and constitution of tribes have changed repeatedly. Currently 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 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.*, 1984). However, no phylogenetic studies have been comprehensively considered all 17 subfamilies (Musser and Carleton, 1993). Although the shared polytomous relationships across studies and markers suggest this pulse may be genuine, it is also possible that 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 further address this question. 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* reflect relationships previously identified. Paraphyly of *Clethrionomys* with respect to *Alticola* has previously been reported 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, submitted) 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 their 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, a lack of morphological synapomorphies that define clades within the genus (e.g. see Carleton, 1985), and molecular phylogenies (Conroy and Cook, submitted) 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 further characterize 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 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 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; 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; fossils [e.g., *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 at eight million years old (Repenning *et al.*, 1990). Other testable dates include the first appearance of modern genera (three to five million years ago) and the diversification among species of *Microtus* about two million years ago (Chaline and Graf, 1988).

To test these dates we averaged the divergence of third position TV's 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 3.6 (± 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 in Nikoletopoulos *et al.*, 1992) are also more ancient, suggesting 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 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.

**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 Mid-Miocene climate change, rather than Pleistocene glacial cycles, as previously suggested 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.

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**Table I.** Taxa used in phylogenetic analysis with cyt b. Complete data on all specimens are available from CJC.

| | Family | Subfamily | Arvicoline Tribe | species | Common Name | Reference Number |
|---|---|---|---|---|---|---|
| 1 | Muridae | Arvicolinae | Microtini | *Arvicola terrestris** | Water Vole | M13 |
| 2 | Muridae | Arvicolinae | Microtini | *Microtus agrestis** | Common Vole | AF3131 |
| 3 | Muridae | Arvicolinae | Microtini | *Microtus pennsylvanicus** | Meadow Vole | NK11205 |
| 4 | Muridae | Arvicolinae | Microtini | *Microtus montanus** | Montane Vole | NK55041 |
| 5 | Muridae | Arvicolinae | Microtini | *Microtus longicaudus** | Long-tailed Vole | AF2031 |
| 6 | Muridae | Arvicolinae | Clethrionomyini | *Alticola macrotis** | Lemming Vole | AF3791 |
| 7 | Muridae | Arvicolinae | Clethrionomyini | *Clethrionomys glareolus** | Bank Vole | AF3133 |
| 8 | Muridae | Arvicolinae | Clethrionomyini | *Clethrionomys rutilus* | Red-backed Vole | AF4853 |
| 9 | Muridae | Arvicolinae | Dicrostonichini | *Dicrostonyx groenlandicus** | Collared Lemming | AF2246 |
| 10 | Muridae | Arvicolinae | Dicrostonichini | *Dicrostonyx torquatus* | " " | AF5430 |
| 11 | Muridae | Arvicolinae | Ellobiini | *Ellobius tancrei** | Mole Vole | VF224 |
| 12 | Muridae | Arvicolinae | Ellobiini | *Ellobius fuscocapillus* | " " | VF226 |
| 13 | Muridae | Arvicolinae | Lemmini | *Lemmus trimucronatus** | Arctic Lemming | AF7421 |
| 14 | Muridae | Arvicolinae | Lemmini | *Myopus schisticolor** | Wood Lemming | AF1946 |
| 15 | Muridae | Arvicolinae | Lemmini | *Synaptomys borealis** | Bog Lemming | AF1196 |
| 16 | Muridae | Arvicolinae | Ondatrini | *Ondatra zibethicus** | Muskrat | AF7445 |
| 17 | Muridae | Arvicolinae | Phenacomyini | *Phenacomys intermedius** | 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** | House Mouse | Genbank V00711 |
| 23 | Muridae | Murinae | | *Rattus rattus** | Rat | Genbank X14848 |
| 24 | Muridae | Murinae | | *Acomys arensis* | Spiny Rat | Genbank X96996 |
| 25 | Muridae | Sigmodontinae | | *Bolomys amoenus* | ---------- | J Salazar |
| 26 | Muridae | Sigmodontinae | | *Calomys callosus* | Vesper Mice | J Salazar |
| 27 | Muridae | Sigmodontinae | | *Peromyscus keeni** | Deer Mouse | AF17750 |
| 28 | Sciuridae | Sciurinae | | *Sciurus aberti* | Abert's Squirrel | Genbank U10163 |
| 29 | Zapodidae | Zapodinae | | *Zapus trinotatus* | Jumping Mouse | AF18534 |
| 30 | Echimyidae | Echimyinae | | *Makalata didelphoides* | Tree Rat | Genbank U35413 |
| 31 | Echimyidae | Eumyopsinae | | *Proechimys amphichoricus* | Spiny Rat | Genbank L23363 |
| 32 | Echimyidae | Eumyopsinae | | *Mesomys hispidus* | Tree Rat | Genbank L23385 |

* Taxa include ND4 sequences.

**Table II.** Kishino and Hasegawa (1989) test of the ML tree against 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 | s.d. (diff) | T | p† |
|---|---|---|---|---|---|
| ML tree | 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 |

† Probability of finding a more extreme T-value under the null hypothesis of no difference between the two trees (two-tailed test).

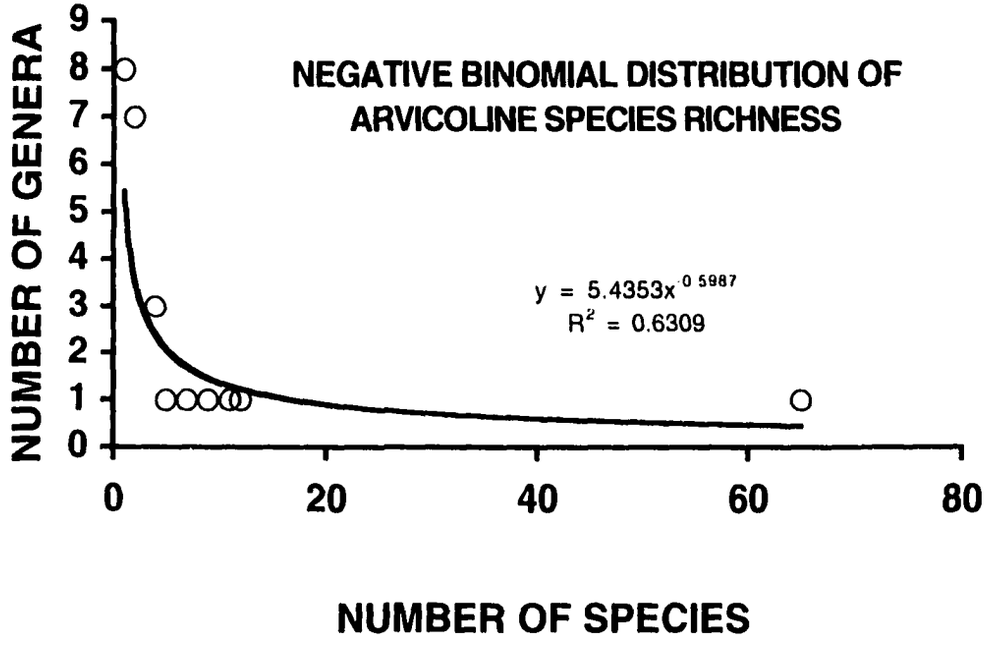


**Fig. 1.** The hollow curve distribution of species across the 25 genera of the Arvicolinae recognized by Musser and Carleton (1993).

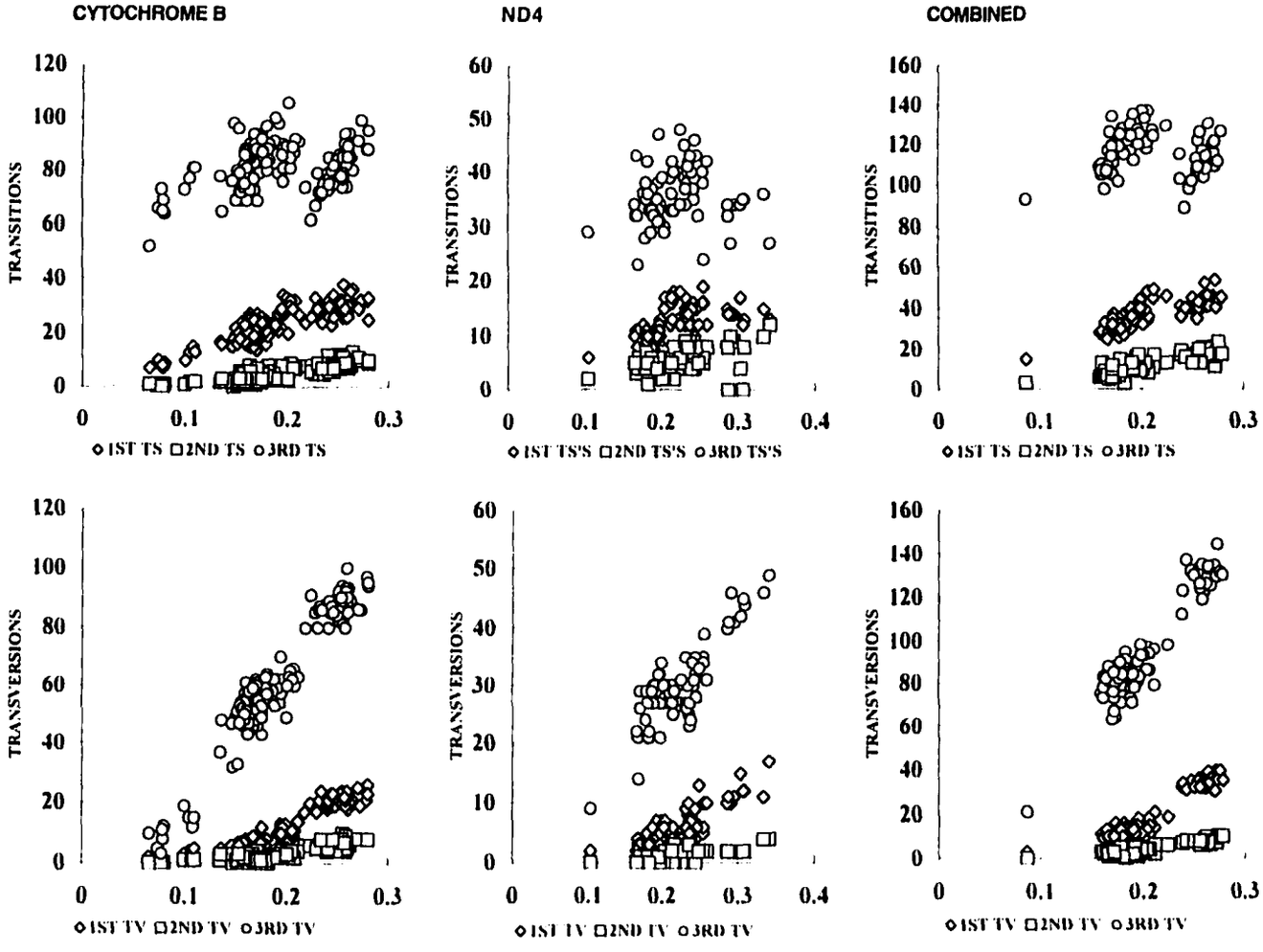


**Fig. 2.** Comparison of the number of pairwise TS (above) and TV (below) mutations (ordinate) plotted against maximum likelihood distance (abscissa) between arvicolines and Mus and Rattus.

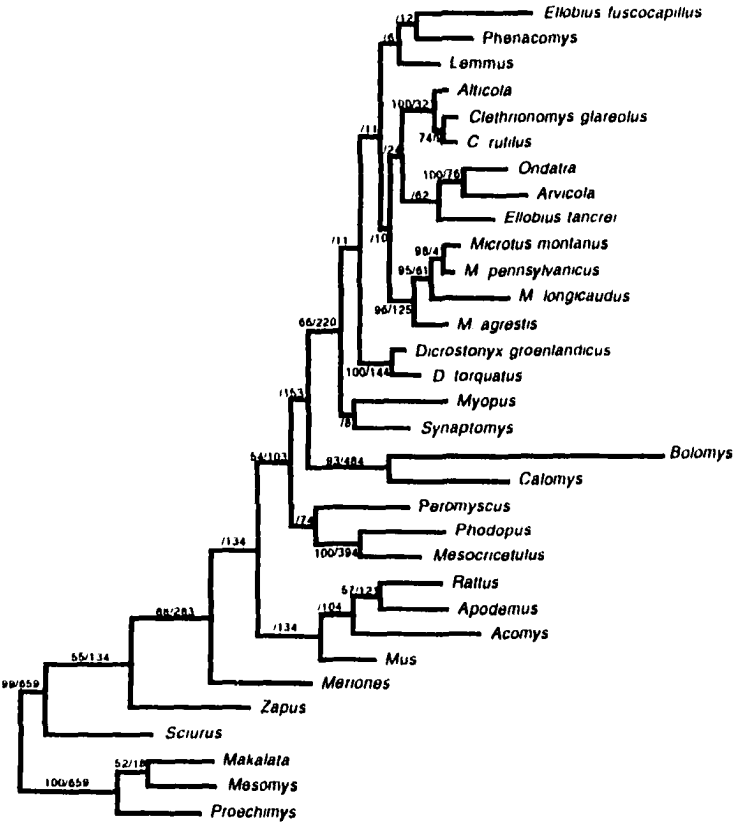


**Fig. 3 A.** Most parsimonious trees from heuristic search with 1:10 transition:transversion weighting and 4:13:1 codon weighting and 100 random additions of taxa. Tree length is 34,884, CI = 0.398, RI = 0.534, HI = 0.602. Values to the left of slashes are bootstrap percentages from 1,000 iterations, values to right are decay indices.

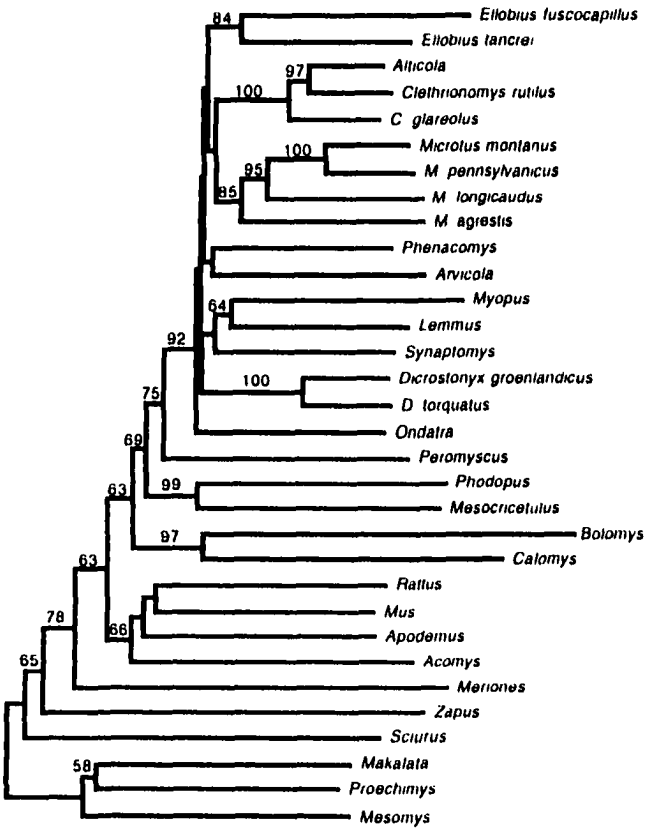


**Fig. 3 B.** NJ tree with Kimura two-parameter weighting and no rate heterogeneity based on complete cyt b sequences. Minimum evolution score was 3.22. Values adjacent to branches are bootstrap percentages from 1,000 iterations.

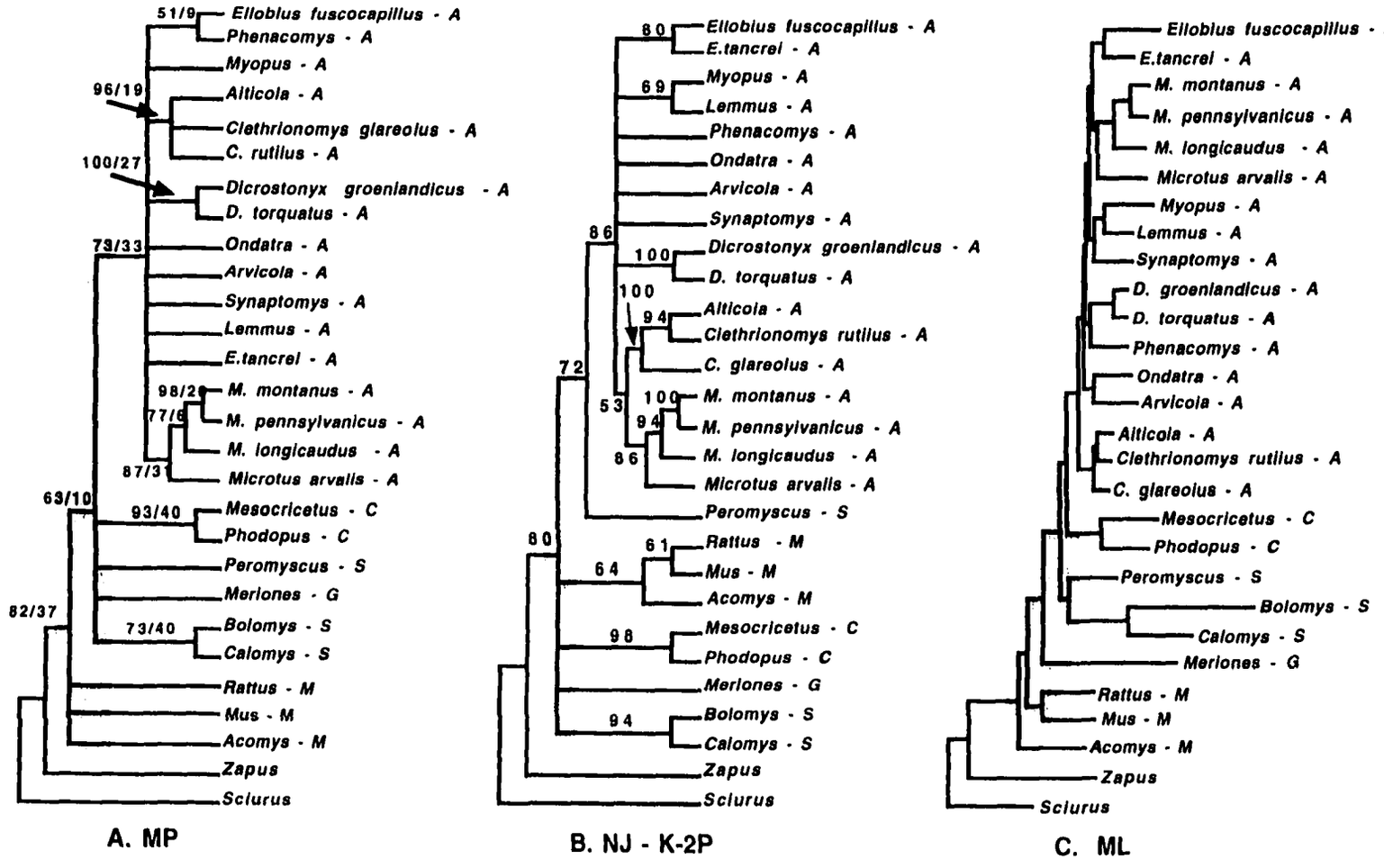


**Fig 4.** Phylogenetic analysis of cyt b sequences with *Sciurus aberti* (Sciuridae) as an outgroup to *Zapus trinotatus* (Dipodidae) and murid representatives (A) maximum parsimony, (B) neighbor-joining with Kimura two-parameter weighting, and (C) maximum-likelihood using F84 model. Values on branches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

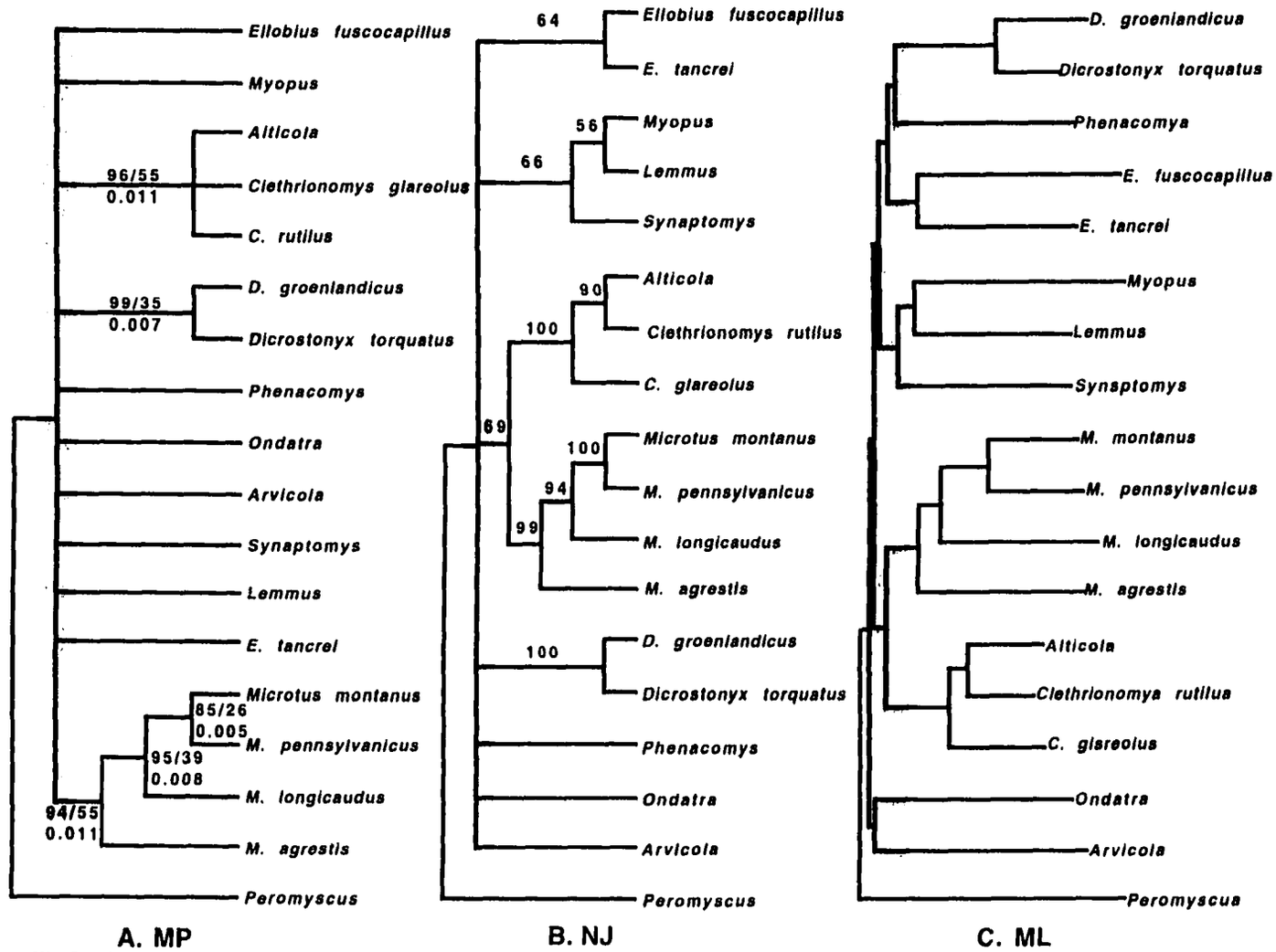


**Fig 5.** Phylogenetic analysis with *Peromyscus* as an outgroup to arvicoline representatives and including cyt b and ND4 sequences. (A) one of two maximum parsimony trees, (B) neighbor-joining with Kimura two-parameter weighting, and (C) maximum-likelihood using F84 model. Values on MP and NJ branches are bootstrap percentages from 250 iterations. The MP tree (4862 steps) had CI = 0.5197, HI = 0.4803, and RI = 0.3951. Gray shading indicates area of inferred pulse.

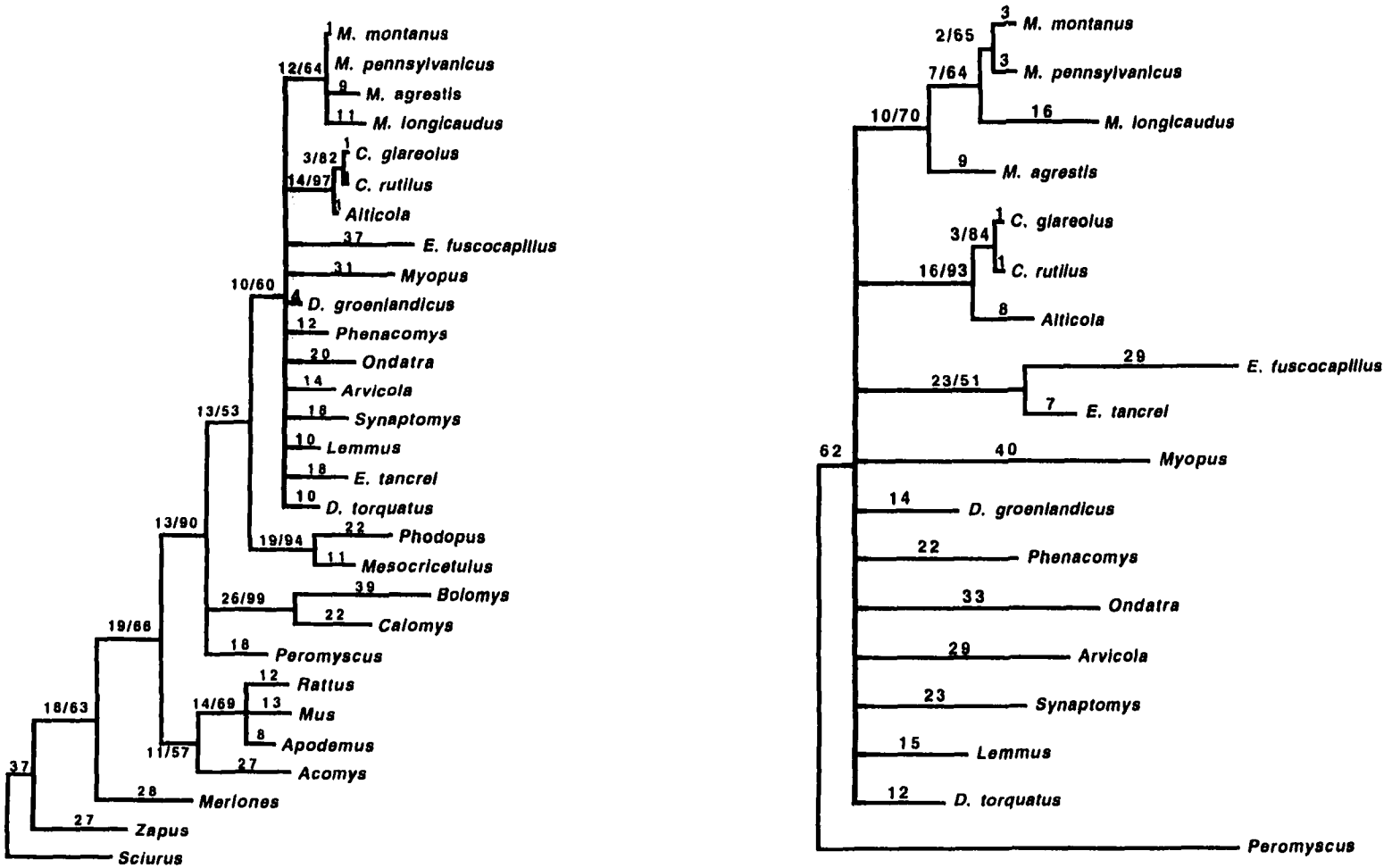


**Fig 6.** Maximum parsimony trees constructed only with amino acids. The tree on the left was constructed with only cyt b amino acids. The tree with *Peromyscus* as an outgroup also included ND4 amino acids as described for the nucleotide analyses. Branch lengths are indicated and where bootstraps were greater than 50 %, those values are shown after a slash (/).

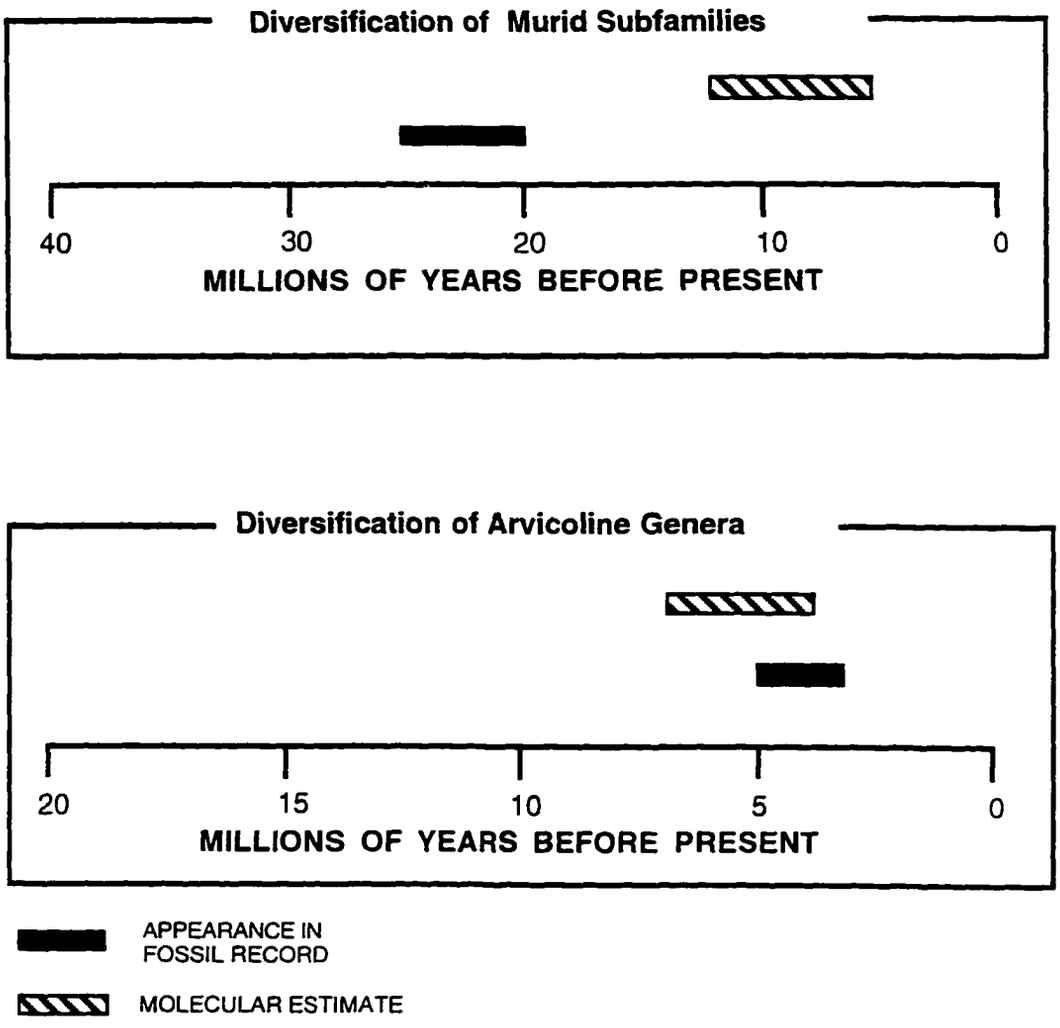


**Fig. 7.** Estimates of divergence from fossil and molecular data. Molecular estimates are based on change in third position transversions and assume a rate of 2.3 % per million years (Smith and Patton, 1993). Fossil estimates are from Chaline and Graf (1986) for arvicolines (three to five MY ago) and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

# Chapter 2

## Molecular Systematics of a Holarctic Rodent (Microtus: Muridae)[2]

ABSTRACT. -- The Bering Land Bridge was the itinerant connection that allowed the exchange of mammals between Asia and North America. Because some mammalian genera are widely distributed on both continents, recovery of their phylogenetic history may help reconstruct the sequence of intercontinental exchanges. The extant species of Microtus (Muridae: Rodentia) in North America are thought to be derived from a Eurasian ancestor. Their present distribution may be due to multiple invasions, or alternatively, a single invasion followed by subsequent speciation. However, an incomplete fossil record and an unstable taxonomic history suggest that some relationships have been difficult to recover. We sequenced mitochondrial cytochrome b gene sequences for 78 individuals representing 24 species of Microtus. Parsimony and likelihood methods were used to test competing phylogenetic and biogeographic hypotheses. One clade of taiga voles (M. pennsylvanicus, M. montanus, M. townsendii, and M. canicaudus), a clade of Asian species (M. kikuchii, M. fortis, M. montebelli, and M. middendorffi) with the Holarctic M. oeconomus, and several previously identified sister taxon pairs were supported. M. gregalis was genetically distant from M.

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abbreviatus and M. miurus, thus contradicting the monophyly of Stenocranius. Monophyly of the North American species was weakly supported because basal relationships were not robust, reflecting a single pulse of diversification about 1.3 million years ago. This pulse may obscure our ability to calibrate the timing of intercontinental invasions in this group.

## INTRODUCTION

Many species of terrestrial mammals are thought to have moved between North America and Asia during glacial periods of the Pleistocene via the Bering Land Bridge (Korth, 1994). These invasions may have initiated major continental radiations, the timing and extent of which have proven difficult to recover because the fossil record and evolutionary relationships of many of these taxa are poorly known. An assessment of the evolutionary relationships of a group may be crucial to our interpretation of the historical biogeography of a region. For example, Engel et al. (1998) suggested that the suspected radiation of the Sigmodontinae in South America needs to be re-evaluated because the group is apparently paraphyletic. Because molecular phylogenies provide opportunities to reconstruct the evolutionary history of taxa, they may be used to estimate the number and temporal order of invasions (Givnish, 1997).

We focus on the genus Microtus (Rodentia: Muridae), a Holarctic genus (Fig. 1) that could be important in interpreting the historical biogeography of the northern continents. Since the late Pliocene, Microtus diversified rapidly (Reig, 1989) into one of the more speciose mammalian genera (Musser and Carleton, 1993) with 65 species recognized in 14 subgenera. This rapid diversification may be partially responsible for the chaotic taxonomic history for the group (Anderson, 1985; Musser and Carleton, 1993). Evolutionary relationships among species remain unclear. Also, the limits of the genus are not clear. Numerous synonyms have been attributed to the group. However, at this time we include as Microtus a subset of those species classified by Musser and Carleton (1993) as Microtus or Volemys.

Species of Microtus are distributed in grassland, taiga, steppe and tundra ecosystems (Gromov and Polyakov, 1992; Hoffmann and Koeppl, 1985). Their fossil record indicates large fluctuations in distribution due to climate change (Graham et al., 1996; Repenning et al., 1990) with some species invading southerly regions of Eurasia and North America during cold phases. During subsequent warming trends, glacial relicts (e.g., on mountaintops) were isolated. These large fluctuations in distribution and apparent isolating events may be partially responsible for high species diversity in this genus.

Relationships among some Eurasian and North American species of Microtus have been explained by independent invasions across the Bering Land Bridge (Hoffmann

and Koeppl, 1985; Repenning et al., 1990). Rausch (1994) noted that movements of species across Beringia were not symmetric, with most species moving from Asia to North America. Asymmetric movement is suspected because western Beringia was directly connected to source populations further west in Eurasia during glacial maxima. However, eastern Beringia was isolated from southern areas of North America by the Laurentide and Cordilleran ice sheets. Because the fossil record and systematics of many Holarctic taxa remain largely unstudied, these hypotheses have not been thoroughly tested. Furthermore, although microtines were a common mammalian component of Beringia (Guthrie, 1982), the fossil record has not been investigated well enough to determine the diversity or persistence of species in this region.

Relationships among some species of Microtus have been proposed (e.g., as subgenera: Miller, 1896), but a phylogeny is crucial to examining how species of Microtus and other mammals invaded and diversified in North America. If the earliest Microtus originated in the Old World, a monophyletic origin for endemic North American species of Microtus would indicate either a single invasion (with a backward invasion of the Old World by M. oeconomus), or two invasions (resulting in the present New World group of endemics plus a later invasion by the Holarctic M. oeconomus). Sister relationships between particular North American and Asian/European clades would indicate more than two invasions. Phylogenies may aid in recovering the temporal

sequence of invasions. Taxa closely related to Eurasian sister taxa would indicate recent invasion, whereas deeper relationships may be the result of older invasions.

Alternatively, there is good reason to expect that basal relationships among species of Microtus may be difficult to recover. Because the paleontological record indicates a rapid appearance of many species of Microtus in North America about 500,000 years ago (Zakrzewski, 1985), some relationships may be polytomous. Previous systematic investigations of some species of Microtus were inconclusive or conflicted with other studies (Musser and Carleton, 1993). We address these issues by testing several phylogeographic hypotheses.

Phylogeographic Hypotheses. -- Karyotypes and molecular markers have provided an independent assessment of Microtus systematics (DeBry, 1992; Graf, 1982; Modi, 1987, 1996; Moore and Janecek, 1990; Nadler et al., 1978; Zagorodnyuk, 1990). However, the taxa and data in those studies often did not overlap. In this paper, DNA sequences for 24 species were used to assess the following biogeographic and systematic hypotheses described below: I) monophyly of North American Microtus, II) monophyly of Holarctic subgenus Stenocranius (Rausch, 1964), and III) monophyly of each of two groups of taiga-dwelling species of Microtus in North America (Hoffmann and Koeppl, 1985).

I. Interpretation of the fossil record of Pleistocene environments has led to a number of scenarios for the movement of particular species between Asia and North America. (e.g., Hoffmann and Koeppl, 1985; van der Meulen, 1978). If all endemic North American species diversified from a single invasion, they should be monophyletic. Alternatively, some endemic North America species may have sister species in Eurasia.

II. Phylogenetic relationships among species on separate continents may have been obscured by convergent morphological evolution. For example, the Holarctic subgenus Stenocranius (M. gregalis, M. miurus, and M. abbreviatus) is based on shared skull characteristics that ostensibly reflects a common origin. However, the high degree of morphological convergence in Arvicolinae (Courant et al., 1997), however, cautions that the morphology-based taxonomy of Microtus may not always reflect phylogenetic relationships.

III. Hoffmann (1981) and Hoffmann and Koeppl (1985) described a model of speciation wherein two purported clades of Microtus (Clade 1: M. pennsylvanicus, M. montanus, M. townsendii; Clade 2: M. xanthognathus, M. richardsoni, M. chrotorrhinus) expanded during interglacials, but contracted in three separate refugia (western coastal, western montane, eastern boreal) during glacial periods (Fig. 2). Speciation events that occurred over multiple glacial advances may produce a dichotomous phylogeny. An initial advance might lead to a single branching. A subsequent advance might then lead to branching within those daughters. However, rapid isolation during a single glacial period could result in polytomous branching if multiple daughter lineages were derived from a single ancestor at the same time.

# MATERIALS AND METHODS

Eight palearctic species, 15 nearctic species, and the Holarctic M. oeconomus were included to represent 10 of 14 subgenera of Microtus (Musser and Carleton, 1993 -- Table 1). Two species of Clethrionomys were used as outgroups (Conroy and Cook, submitted). DNA was extracted via a modified salt method (Medrano et al., 1990) from skin, liver, muscle, and/or heart tissue that was dried, frozen or preserved in ethanol. Symmetric PCR (Saiki et al., 1985) amplified the 1143 bp mitochondrial cytochrome b gene (cyt b) as described in Conroy and Cook (submitted). Sequence data were determined on an ABI 373a Stretch DNA sequencer using Prism® dye terminator technology. Sequences for two taxa were obtained from Genbank (Microtus arvalis, GenBank Accession #U54488; M. rossiaemeridionalis, GenBank Accession #U54474). MtDNA was sequenced for 78 individuals, including partial or complete cyt b sequences for multiple individuals for 21 of the 24 species. Species represented by multiple samples were all reciprocally monophyletic and apparently correctly identified. Due to computational limitations, phylogenetic analysis included only one representative per species (Table 1).

Saturation was examined by plotting maximum likelihood distance (DNADIST: Felsenstein, 1993) against transitions (TS) and transversions (TV) across each codon position (Fig. 3). Parsimony searches (MP) included a TS:TV bias of 2.6 based on

observed variation. That is, the average number of transitions between any two taxa was 2.6 times the average number of transversions between any two taxa. This was determined by summing pairwise transitions and transversions over all pairs. Trees were rooted with Clethrionomys glareolus and C. gapperi (Conroy and Cook, submitted) and bootstrapped 250 times using a heuristic search in PAUP* (test version 4.0d59, written by D. L. Swofford). A maximum-likelihood (ML) tree was estimated with PAUP* using an F84 (Felsenstein, 1984) + $\Gamma$ ($\alpha$ = 0.2159) model (with TS/TV ratio of 2.6). Skewness or $g_1$ statistics were generated from 1,000 random trees (PAUP*), with and without weighting at each position, and compared to values in Hillis and Huelsenbeck (1992) for statistical significance ($\alpha$ = 0.05). To evaluate the strength of alternate topologies, we usd likelihood ratios (Kishino and Hasegawa, 1989) to test the unconstrained ML tree against 1) ML trees constrained for particular phylogenetic hypotheses and 2) MP trees. To test the strength of relationships we 3) constrained the ML analysis to exclude two well-supported clades and tested those against the unconstrained ML tree.

To calibrate a rate of sequence evolution, we assumed that the deepest divergence among species of Microtus should correspond roughly to the initial diversification of the genus (approximately 2.1 million years ago; Repenning et al., 1990). To estimate the time of divergence, we used a distance based on the same maximum likelihood model used for estimating the ML tree. A molecular clock, the assumption of a linear relationship between molecular divergence and time, is often subject to error from excessive rate

heterogeneity. Therefore, we tested for rate heterogeneity among taxa by evaluating ML trees with and without a molecular clock constraint using a Chi-Square test (i.e., two times the log-likelihood difference with n [= number of taxa] minus two degrees of freedom; Felsenstein, 1988). To evaluate individual taxa, we used the Wu and Li (1985) relative rate test, as implemented by algorithms in Muse and Weir (1992), with software (K2WuLi) distributed by L. Jermiin.

## RESULTS

Composition and Variation. -- Of the 1143 base pairs, 459 (40 %) were variable and 361 of those were phylogenetically informative (Table 2) across the individuals used in the phylogenetic analysis. Similar to other studies of mammalian cyt b evolution (Irwin et al., 1991; Ma et al., 1993), most polymorphic sites were in third positions (340, 74 %) followed by first positions (94, 20 %), and second positions (25, 5 %). Base pair composition differed across codon position and between nucleotides (Table 4). Guanine nucleotides were underrepresented at second and third positions (12.3 % and 3.7 %, respectively), thymine nucleotides were overrepresented in second positions (41.7 %) and adenine nucleotides were overrepresented in third positions (40.9 %).

Interspecific Kimura (1980) 2-P distance (Table 5) ranged from 1.5 % (M. abbreviatus and M. miurus) to 18.0 % (M. oregoni and M. gregalis). Expected differences

in variation among codon and substitution type were seen in saturation curves (Fig. 3). None displayed saturation. G1 statistics (Table 2) indicated that the data have phylogenetic signal (Hillis and Huelsenbeck, 1992).

Phylogenetic Results. -- ML and MP supported monophyly of the subset of North American species of Microtus sampled in this study (Fig. 4). Several sister relationships (e.g., M. fortis and M. middendorffi, M. arvalis and M. rossiaemeridionalis, M. abbreviatus and M. miurus, M. canicaudus and M. townsendii, M. pennsylvanicus and M. montanus, and M. pinetorum and M. richardsoni) were consistent across methods. The clade including M. oeconomus, M. middendorffi, M. montebelli, M. kikuchii and M. fortis (hereafter the "Asian clade") and the clade including M. pennsylvanicus, M. montanus, M. townsendii, and M. canicaudus (hereafter the "M. pennsylvanicus clade") were present in both methods. No analyses supported monophyly of the subgenus Stenocranius or monophyly of the second clade of taiga voles (M. xanthognathus, M. chrotorrhinus, and M. richardsoni) as proposed by Hoffmann and Koeppl (1985). Though weakly supported, North American species were monophyletic in both methods. They formed a sister clade to European species, and Asian species were basal to them. Bootstrap support was weak for basal nodes and only one alternate topology was rejected by the likelihood ratio test.

The M. pennsylvanicus clade (subgenus Mynomes, Musser and Carleton, 1993) was identified previously by karyotypes (Modi, 1987), skeletal morphology (Hooper

and Hart, 1962), nuclear DNA (Modi, 1996) and allozymes (Moore and Janecek, 1990). M. oregoni and M. longicaudus were basal to the M. pennsylvanicus clade in the ML tree. M. canicaudus was not sister to M. montanus, as has been previously suggested (Musser and Carleton, 1993), but instead was sister to M. townsendii. Modi (1986) also noted significant chromosomal differences between these species. DeBry (1992) investigated the monophyly of the M. pennsylvanicus clade with mtDNA RFLP data and did not find it to be monophyletic, but also could not reject their monophyly with a likelihood ratio test. Although DeBry (1992) suggested that mtDNA sequences might provide more synapomorphies to make this test more sensitive (phylogenetically informative sites: RFLP, 53; cyt b sequence, 361), we were also unable to reject a ML tree constrained against this clade.

The Asian clade has not been recognized previously, though Zagorudnyuk (1990) placed M. fortis in the M. middendorffi species group of subgenus Alexandromys. By retaining M. kikuchii within Microtus, we depart from the taxonomy of Musser and Carleton (1993) and Zagorudnyuk (1990) who placed M. kikuchii and three other species from southeastern Asia (Volemys clarkei, V. millicens, and V. musseri) in a separate genus, Volemys. The position of M. kikuchii suggests the need for further sampling of Asian species including the three additional species of Volemys. Zagorudnyuk (1990) also suggested that M. oeconomus and M. montebelli may be sister taxa (Fig. 4). M. oeconomus and M. montebelli share an ancestral form of X-Y chromosome pairing, the

lack of which is shared by most species of Microtus (Borodin et al., 1997). Chromosomal pairing needs to be investigated in other members of the Asian clade. M. gregalis, and in North American species to resolve this hypothesis.

Of the 346 relative rate tests, 32 indicated unequal rates of evolution ($|Z| > 1.96$). These departures from equal rates involved nearly all taxa and rate heterogeneity was not greater than expectations (Chi-Square = 2.68) under a molecular clock (Felsenstein, 1988). Constraining the oldest interspecific divergence to 2.1 million years ago (Repenning et al., 1990) yielded a rate of 7.5 million years per unit of likelihood distance. A plot of pairwise differences (Fig. 5) with a unimodal distribution suggested a single pulse of diversification among species about 1.3 million years ago. This pulse corresponds to the early Pleistocene appearance of several lineages in North America (Hoffmann and Koeppl, 1985; Repenning, 1980).

## DISCUSSION

Our primary goal was to use molecular characters and a relatively large taxonomic sample to test taxonomic hypotheses in this diverse group. The monophyly of the M. pennsylvanicus and Asian clades, and several sister taxon pairs (e.g., M. arvalis and M. rossiaemeridionalis, M. fortis and M. middendorffi, M. abbreviatus and M. miurus,) were well supported (Fig. 4). However, in addition to resolving some clades, our data also indicated weak relationships across many internal branches in the parsimony analysis

(Fig. 4A). The likelihood ratio tests (Table 3) indicated that only one of the alternative topologies we tested was significantly less likely than that depicted in the maximum likelihood tree.

We suggest three possible explanations for this failure to reject alternate topologies. The first is that rapid diversification may have led to short internodal branches. Altering the topology across these short branches does not significantly change the likelihood. The second is that as likelihood models are made complex to more realistically model DNA evolution, the sampling variance increases and the ability to reject alternative trees decreases. A third possibility is that the data lack phylogenetic signal and all trees are equally likely or unlikely. However, we can probably reject the last explanation because g1 statistics are significant and saturation is not apparent. Because both MP and ML displayed equivalent topologies, the weakly supported relationships most likely reflect the rapid diversification, especially notable at the base of the phylogeny.

Pulses of diversification apparently have been repeated throughout murid evolution (Conroy and Cook, submitted). This pulse of speciation in Microtus may correspond to an environmental change, such as the period of global warming (Chaline et al., 1993) about 1.3 million years ago. Our calibration, based on the minimum age of fossils of Microtus, corresponds to the Late Villafranchian interglacial epoch in Europe (Kurtén, 1968) and the beginning of the Kansan glaciation in North America (Zakrzewski,

1985). Though a single and severe climatic phenomenon may have been important to speciation in Microtus, fine scale variability on a millennial scale may also be a potential cause of increased speciation or extinction rates (Roy et al., 1996). Climatic oscillations shifted from 41,000 to 100,000 year cycles at about 1.2 million years ago (Imbrie, et al. 1993). However, the effects of this shift on mammalian evolution are unstudied. This calibration of the apparent pulse of speciation in Microtus should be further tested. For instance, it may be appropriate to subtract genetic variation in the putative ancestor before estimating interspecific differences (i.e., net divergence; Avise and Walker, 1998; Edwards, 1997). Intraspecific variation of the cytochrome b gene in Microtus is being investigated elsewhere (CJC and JAC).

It is apparent that many relationships across these species remain to be tested with the inclusion of more taxa and independent characters. The historical implications of the relationships supported by our data are summarized in the following sections.

North American Monophyly. -- During Pleistocene glacial maxima (Kansan, Nebraskan, Illinoian, and Wisconsinan), ocean levels dropped sufficiently to expose the Bering Land Bridge and unite Beringia (Hopkins et al., 1982). The biogeography, ecology, and systematics of many North American mammals hinges on the nature of Beringia's vegetational composition and climate during and since the Pleistocene (Guthrie, 1990; Kontrimavichus, 1986). Much controversy exists over these questions (Colinvaux, 1996; Elias et al., 1996), but establishing the chronology of mammal invasion between Asia and

North America could help to reconstruct the environments through which they passed and could help to reconstruct the tempo at which North American species evolved.

Microtus has a long association with Beringia with an origin approximately 2.1 million years ago when it is thought to have first invaded North America from Asia after the Blancan V glaciation in Laurentia (Repenning et al., 1990). Because the Palearctic has an older fossil record (Gromov and Polyakov, 1992), a Eurasian ancestor appears more probable. The placement of Palearctic species basal to the North American taxa (Fig. 4) is consistent with a Eurasian origin for the genus.

From paleontological and zoogeographical data, Hoffmann and Koeppl (1985) suggested that distinct lineages of Microtus invaded North America across the Bering Land Bridge in the early, middle, and late Pleistocene (until about 13,000 years ago). Some of the earliest species (e.g., Microtus deceitensis, M. paroperarius) are extinct and their relationships to extant species are unclear. Three hypothesized survivors of the earliest invasion are M. californicus and M. umbrosus (Martin, 1974) and M. guatemalensis (Repenning, 1980). Survivors of Middle Pleistocene invaders are thought to be M. quasiater and M. oaxacensis of the Mexican cloud forest, M. pinetorum of the eastern North American forest, and M. ochrogaster of the Great Plains. During the late Pleistocene most other North American species appeared in the fossil record, except for M. canicaudus, M. oregoni, M. townsendii, and a few insular allospecies (Hoffmann and

Koeppl, 1985). Two possible recent arrivals are M. miurus (Hoffmann and Koeppl, 1985) and M. oeconomus (Lance and Cook, 1998).

Our data suggest a different history of invasion into North America than that previously inferred. Unfortunately, weak basal relationships limit our ability to discriminate multiple invasions. Monophyly of the endemic North American species of Microtus we sampled indicates only two invasions (these plus M. oeconomus) and potentially refutes proposed taxonomic affinities (e.g., M. longicaudus a member of Eurasian Chilotus: Anderson, 1985; M. richardsoni within European Arvicola: Bailey, 1900; Hooper and Hart, 1962; Miller, 1896; Nadler et al., 1978; and M. pinetorum within Eurasian Pitymys: Gromov and Polyakov, 1992). Monophyly of North American species also was supported by Graf's (1982) allozymic data, although sampling of taxa was less extensive in that study relative to this.

Because our data only address the history of extant species, there may have been other invasions of North America whose descendants have since gone extinct. For example, M. paroperarius and M. deceitensis, now extinct, were present in North America in the early Pleistocene (Repenning et al., 1990). They share the four-triangle m1 with M. oeconomus (Zakrzewski, 1985), a holarctic species we found to be distantly related to North American species of Microtus and a late Pleistocene invader of North America (Lance and Cook, 1998). It is more probable that most extant species of Microtus in North America evolved from a later invader in the middle Pleistocene. Due to

the apparent monophyly of North American species and an early pulse of diversification. only two invasions may have occurred: the first resulting in species restricted to North America and the second in M. oeconomus (Lance and Cook, 1998). Our estimate of this group's phylogeny should improve with the inclusion of other species that were likely to have been early invaders: M. quasiater, M. oaxacensis, M. umbrosus, and M. guatemalensis.

Albeit weakly supported, the sister relationship between the North American species and European species (M. agrestis, M. arvalis, M. rossiaemeridionalis) may suggest that some Asian species were isolated in a separate refugium while a corridor existed between Beringia and Eurasia. Guthrie (1990) described a "mammoth steppe," or high latitude steppe grassland belt that extended from Europe to eastern Beringia during glacial periods. Though M. oeconomus and M. middendorffi are widely distributed throughout Asia, M. kikuchii, M. montebelli (now both island endemics), and M. fortis are distributed south of this corridor and may have been isolated from it during glacial advances. More thorough sampling of east Asian species should provide a test of this hypothesis.

Monophyly of subgenus Stenocranius. -- Stenocranius was diagnosed originally by the long and narrow skull and short tail of the Asian M. gregalis (Kaschenko, 1901). North American M. miurus (Rausch, 1964) and M. abbreviatus of Hall (Miller, 1899) and St. Matthew (Rausch and Rausch, 1968) islands (Bering Sea) were later included in the clade indicating a trans-Beringian distribution for the subgenus. An invasion of North America during the Illinoian Age ( ≈ 300,000 years ago, Rausch, 1964; Zakrzewski, 1985)

by a Stenocranius ancestor was hypothesized to explain their Holarctic distribution. Subsequently, M. abbreviatus was isolated on Hall and St. Matthew islands at the end of the Wisconsin glaciation (Hoffmann and Koeppl, 1985; Rausch and Rausch, 1968). Its similar morphology and karyotype to M. miurus suggested to Rausch and Rausch (1968) that the two were closely related. However, monophyly of Stenocranius has been questioned on the basis of differences in behavior, dental morphology (Gromov and Polyakov, 1992) and karyotypes (Fedyk, 1970). For example, M. abbreviatus and M. middendorffi (subgenus Alexandromys) were hypothesized to be sister taxa based on similar karyotypic and morphologic characteristics (Lyapunova and Krivosheev, 1969; Matthey and Zimmermann, 1961).

It appears that M. miurus originated in North America (Fig. 4) and is morphologically convergent with M. gregalis. M. abbreviatus was a close sister to M. miurus based on cyt b sequences (Table 5). In addition, chromosomal similarity supports their conspecific status. Morphological and chromosomal similarities between M. middendorffi and M. abbreviatus (Matthey and Zimmermann, 1961; Vorontsov and Lyapunova, 1986) appear to be convergent (Fig. 4 A-C). Thus, our data support the interpretation of Stenocranius as "pseudoamphiberingian" (Vorontsov and Lyapunova, 1986). The basal position of M. gregalis agrees with an early Pleistocene origin from the extinct M. gregaloides (Chaline, 1990; Gromov and Polyakov, 1992). M. miurus and M. abbreviatus were consistently sister to M. xanthognathus and part of the North American clade.

Taiga Vole Speciation. -- Pleistocene glaciations have been implicated as an important factor in speciation in birds and mammals (Rand, 1948, 1954). Ecosystems

expanded, contracted, and fragmented along the fringes of ice sheets and along elevational gradients at lower latitudes (Hewitt, 1996). Hoffmann and Koeppl (1985) attributed the speciation of two taiga adapted vole clades in North America to allopatry during Pleistocene glacial phases (Fig. 2). They suggested that ancestors of these clades were widespread during interglacials and then became isolated in refugia during glacial advances (Rand, 1948, 1954; Hoffmann, 1981). For the M. pennsylvanicus clade, refugia were hypothesized for the eastern boreal (M. pennsylvanicus), the western montane (M. montanus), and Pacific coastal (M. canicaudus, M. townsendii) areas. This clade (T1 in Fig. 4) was well supported in our analyses. M. canicaudus has been considered a peripheral isolate of M. montanus, but our data suggest it is sister to M. townsendii, suggesting a different series of speciation events.

The other taiga voles were suggested to have arisen in the eastern boreal (M. chrotorrhinus and M. xanthognathus) and western montane (M. richardsoni) refugia. However, these species were not monophyletic (Table 3, T2 in Fig. 4). The position of M. chrotorrhinus was poorly defined. M. xanthognathus was sister to M. miurus and M. abbreviatus, while M. richardsoni was sister to M. pinetorum. The latter relationship was unexpected and has never been suggested. M. pinetorum is often considered to be closely related to members of the subgenus Pitymys of Europe (Gromov and Polyakov, 1992) and Mexico (Musser and Carleton, 1993). Gromov and Polyakov (1992:275) explained this disjunct distribution as "a result of the complex Pleistocene history of

alpine-forest biomes in the New World." Although M. richardsoni has been considered highly divergent, other studies (Jannett, 1992; 1997; Matthey, 1957; Zakrzewski, 1985; Conroy and Cook, submitted) support its inclusion within Microtus. Adding more taxa (e.g., European and other North American members of the subgenus Pitymys) will help address the problem of long-branch attraction, although ML is generally robust to its effect (Gaut and Lewis, 1995).

Our data suggest that all taiga specialized species are not phylogenetically related. A tree constrained to monophyly for the six taiga species was rejected by the Kishino-Hasegawa test. Two other species that occur in taiga, M. longicaudus and M. oregoni, have been considered distinctive due to differences in karyotypes (Modi, 1987), gonosomal mosaicism in M. oregoni (Ohno et al., 1963), large B-chromosome complement in M. longicaudus (Judd and Cross, 1980), and genic comparisons (Moore and Janecek, 1990).

The Pleistocene glacial refugia model of speciation (Rand, 1948, 1954) has been criticized as a primary mechanism of speciation during the last glaciation (Klicka and Zink, 1997). This was because avian examples appear more divergent than expected based on a rate of sequence evolution of 2% per site per million years (Shields and Wilson, 1987). Pairwise differences among species of passerines range from the Pliocene to the late Pleistocene, suggesting that diversification in this group was not centered around a single point in time, particularly the latest Pleistocene. A single pulse of

speciation among species of Microtus implies a different history than that experienced by the diverse North American passerines, which evolved over a much longer period. Refinements to the molecular dating approach are possible (e.g., Avise and Walker, 1998; Rambaut and Bromham, 1998), but establishing a date of origin for Microtus from fossils remains problematic.

The work presented here is a step toward recovering the history of faunal interchange among the northern continents. The rapid appearance of species of Microtus during the Pleistocene may be responsible for the difficulty in resolving basal phylogenetic relationships, yet numerous lower level relationships were well supported. Some of these relationships based on these mitochondrial data differ from those proposed based on morphology. These should be tested with other unlinked markers and against other taxa with similar distribution and diversity (e.g., other boreal mammals, birds, and plants).

These data do not support more than two invasions of North America. An invasion by the ancestor of most species occurred in the early Pleistocene, followed by M. oeconomus in the late Pleistocene. More paleontological research in conjunction with molecular studies of the four remaining North American and 36 Old World species not included here, as well as other closely related genera, such as: Volemys, Blanfordimys, Chionomys, Lasiopodomys, and Proedromys would further clarify intercontinental relationships.

Another important step to enhance understanding of speciation within Microtus will be investigations of intraspecific and interspecific variation. Peripheral isolates models, for example, have testable phylogenetic predictions at the species level (Frey, 1993). The model suggested by Hoffmann and Koeppl (1985) for the divergence in the M. pennsylvanicus clade is consistent with our data, but other aspects of their hypothesis were not. Phylogeographic research (Avise et al., 1987) is a logical extension to bridge the gap between speciation and population genetics in Microtus and should provide insight into rates of divergence during and since the Pleistocene (Avise and Walker, 1998).

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Table 1.—Species of Microtus (Musser and Carleton, 1993), including Volemys, examined in the study. X in sequence indicates full cyt b (1143 bp). n = number of specimens examined with at least partial cyt b. ? in subgenus indicates taxonomy is unclear.

| Subgenus | species | n | Subgenus | Species | n |
|---|---|---|---|---|---|
| Agricola | agrestis | 2 | Mynomes | Oregoni | 4 |
| Alexandromys | fortis | 1 | Mynomes | pennsylvanicus | 2 |
| Alexandromys | middendorffi | 2 | Mynomes? | californicus | 4 |
| Aulacomys | chrotorrhinus | 4 | Pallasiinus | montebelli | 4 |
| Aulacomys | richardsoni | 3 | Pallasiinus | oeconomus | 4 |
| Aulacomys | xanthognathus | 6 | Pedomys | ochrogaster | 7 |
| Aulacomys | longicaudus | 5 | Pitymys | pinetorum | 3 |
| Microtus | arvalis | 2* | Stenocranius | abbreviatus | 4 |
| Microtus | rossiaemeridionalis | 2* | Stenocranius | gregalis | 3 |
| Microtus? | mexicanus | 2 | Stenocranius | miurus | 2 |
| Mynomes | canicaudus | 4 | Volemys | kikuchii | 3 |
| Mynomes | montanus | 3 | Mynomes | townsendii | 2 |

* = obtained from Genbank

Table 2. — Sequence variation and $g_1$ statistics from 1,000 random trees (PAUP*). All are significant at $p < 0.01$). Significance from Table 2 in Hillis and Huelsenbeck (1992). All searches were run three times to verify results (data not shown). Values from first search presented.

| | First Position | Second Position | Third Position |
|---|---|---|---|
| # base pairs | 381 | 381 | 381 |
| # variable sites | 94 | 25 | 340 |
| # parsimony informative variable sites | 65 | 11 | 285 |
| g1 statistic with no weights | -0.28 | -2.13 | -0.39 |
| g1 statistic with TS/TV = 2.6 | -0.30 | -1.83 | -0.71 |

Table 3. — Results of Kishino and Hasegawa (1989) test of tree topologies. See text for description of tree construction. One topology was significantly different from the ML tree.

| | Tree | -lnL | Diff-lnL | s.d.(diff) | T | P* |
|---|---|---|---|---|---|---|
| 1 | ML Tree with no constraints | 8976.26 | (best) | - | - | - |
| 2 | MP Tree #1 | 8990.22 | 13.96 | 10.78 | 1.29 | 0.196 |
| 3 | MP Tree #2 | 8987.03 | 10.78 | 10.50 | 1.03 | 0.305 |
| 4 | Stenocranius monophyly enforced | 8995.19 | 18.93 | 13.71 | 1.38 | 0.168 |
| 5 | Taiga vole clade #1 enforced | 8976.26 | 0.00 | 0.00 | 0.00 | 1.000 |
| 6 | Taiga vole clade #2 enforced | 8989.96 | 13.70 | 9.36 | 1.46 | 0.144 |
| 7 | North American Monophyly enforced | 8976.26 | 0.00 | 0.00 | 0.00 | 1.000 |
| 8 | All Taiga voles forced monophyly | 9000.41 | 24.16 | 10.81 | 2.23 | 0.026** |
| 9 | Asian Clade rejected | 8980.72 | 4.46 | 10.64 | 0.42 | 0.68 |
| 10 | Pennsylvanicus clade rejected | 8982.02 | 5.76 | 8.41 | 0.68 | 0.49 |

** Significant at $P < 0.05$

Table 4. — Percent nucleotide base composition, by codon position and by nucleotide, for complete cytochrome b gene sequences averaged across 24 species of Microtus[a].

| Nucleotide | Overall | Position | | |
|---|---|---|---|---|
| | | 1st | 2nd | 3rd |
| G | 13.0 | 22.9 | 12.3 | 3.7 |
| A | 30.7 | 30.3 | 20.9 | 40.9 |
| T | 27.2 | 23.1 | 41.7 | 16.8 |
| C | 29.1 | 23.6 | 25.0 | 38.7 |

[a]G, guanine; A, adenine; T, thymine; C, cytosine.

Table 5. — Kimura pairwise distances (Kimura, 1980) for 24 species of Microtus and 2 outgroup species of Clethrionomys. Values are percentages times 100.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. Clethrionomys glareolus | | | | | | | | | | | | |
| 2. C. gapperi | 7.2 | | | | | | | | | | | |
| 3. Microtus abbreviatus | 15.9 | 16.2 | | | | | | | | | | |
| 4. M. agrestis | 15.0 | 16.2 | 13.9 | | | | | | | | | |
| 5. M. arvalis | 15.3 | 16.8 | 13.8 | 14.3 | | | | | | | | |
| 6. M. californicus | 15.3 | 16.2 | 14.1 | 13.1 | 15.1 | | | | | | | |
| 7. M. canicaudus | 16.3 | 16.7 | 12.4 | 13.7 | 12.8 | 13.7 | | | | | | |
| 8. M. chrotorrhinus | 14.1 | 14.3 | 13.6 | 13.4 | 13.1 | 12.7 | 12.3 | | | | | |
| 9. M. fortis | 14.7 | 14.7 | 14.3 | 13.5 | 14.0 | 13.2 | 13.6 | 12.3 | | | | |
| 10. M. gregalis | 16.8 | 17.8 | 17.6 | 17.7 | 17.1 | 15.8 | 16.2 | 16.8 | 15.5 | | | |
| 11. M. kikuchii | 14.2 | 14.7 | 13.3 | 13.9 | 14.3 | 11.8 | 12.5 | 12.9 | 12.3 | 14.8 | | |
| 12. M. longicaudus | 17.1 | 17.7 | 14.4 | 15.6 | 14.1 | 15.3 | 12.3 | 13.0 | 14.9 | 17.5 | 14.4 | |
| 13. M. mexicanus | 15.3 | 16.5 | 13.5 | 13.6 | 13.4 | 12.0 | 11.9 | 12.2 | 14.5 | 15.8 | 13.4 | 14.5 |
| 14. M. middendorffi | 14.7 | 15.3 | 13.8 | 13.4 | 13.3 | 13.4 | 14.1 | 11.9 | 9.1 | 15.3 | 10.9 | 14.7 |
| 15. M. miurus | 15.0 | 16.0 | 1.5 | 13.8 | 13.0 | 13.8 | 12.7 | 13.0 | 13.6 | 16.9 | 13.2 | 14.0 |
| 16. M. montanus | 15.2 | 16.5 | 14.5 | 14.6 | 14.0 | 13.1 | 9.3 | 12.7 | 13.9 | 16.6 | 12.9 | 12.3 |
| 17. M. montebelli | 14.0 | 14.4 | 14.7 | 12.7 | 13.3 | 14.0 | 13.0 | 12.5 | 12.0 | 15.0 | 10.3 | 15.7 |
| 18. M. ochrogaster | 14.8 | 15.2 | 13.9 | 15.5 | 14.0 | 12.5 | 13.7 | 12.7 | 15.0 | 14.8 | 13.6 | 15.0 |
| 19. M. oeconomus | 14.7 | 15.3 | 13.5 | 13.3 | 12.9 | 13.6 | 12.8 | 11.8 | 10.4 | 14.6 | 9.7 | 13.7 |
| 20. M. oregoni | 18.4 | 20.2 | 14.6 | 16.3 | 15.2 | 14.2 | 12.4 | 14.2 | 16.9 | 18.0 | 14.5 | 14.7 |
| 21. M. pennsylvanicus | 16.3 | 17.4 | 13.8 | 15.0 | 14.6 | 13.1 | 10.2 | 13.7 | 15.4 | 16.9 | 13.8 | 12.6 |
| 22. M. pinetorum | 14.4 | 16.2 | 13.4 | 15.9 | 14.0 | 13.3 | 13.7 | 13.0 | 14.1 | 15.5 | 13.3 | 14.4 |
| 23. M. richardsoni | 15.2 | 16.1 | 14.4 | 15.3 | 14.7 | 13.1 | 12.8 | 12.5 | 13.8 | 16.7 | 13.9 | 13.4 |
| 24. M. rossiaemeridionalis | 16.4 | 18.5 | 14.8 | 14.9 | 6.5 | 15.3 | 14.4 | 13.9 | 14.2 | 17.5 | 14.4 | 14.8 |
| 25. M. townsendii | 16.3 | 16.8 | 12.7 | 12.7 | 13.7 | 13.0 | 5.3 | 12.4 | 14.1 | 16.1 | 11.7 | 12.3 |
| 26. M. xanthognathus | 15.9 | 15.9 | 12.7 | 14.6 | 13.6 | 13.2 | 12.7 | 13.1 | 14.5 | 16.5 | 13.6 | 13.4 |

Table 5. cont.

| | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 14. M. middendorffi | 14.5 | | | | | | | | | | | | |
| 15. M. miurus | 12.8 | 12.9 | | | | | | | | | | | |
| 16. M. montanus | 12.3 | 14.7 | 14.2 | | | | | | | | | | |
| 17. M. montebelli | 14.4 | 10.9 | 13.8 | 14.0 | | | | | | | | | |
| 18. M. ochrogaster | 13.9 | 15.0 | 14.4 | 13.6 | 14.5 | | | | | | | | |
| 19. M. oeconomus | 13.5 | 9.3 | 13.0 | 13.9 | 9.5 | 14.5 | | | | | | | |
| 20. M. oregoni | 14.5 | 16.4 | 13.8 | 13.1 | 14.8 | 15.2 | 16.3 | | | | | | |
| 21. M. pennsylvanicus | 13.7 | 16.2 | 14.2 | 7.6 | 14.4 | 14.6 | 14.8 | 14.5 | | | | | |
| 22. M. pinetorum | 13.0 | 14.3 | 12.8 | 13.6 | 13.6 | 13.6 | 13.9 | 14.7 | 14.5 | | | | |
| 23. M. richardsoni | 12.4 | 13.8 | 13.9 | 11.4 | 14.2 | 13.6 | 13.3 | 14.0 | 12.9 | 12.3 | | | |
| 24. M. rossiaemeridionalis | 13.9 | 14.7 | 14.2 | 15.0 | 13.9 | 14.5 | 13.4 | 15.3 | 14.7 | 14.9 | 14.7 | | |
| 25. M. townsendii | 11.6 | 14.0 | 12.6 | 8.3 | 12.7 | 13.7 | 12.8 | 11.7 | 9.4 | 13.8 | 13.0 | 14.4 | |
| 26. M. xanthognathus | 13.5 | 14.6 | 12.0 | 12.8 | 14.1 | 13.6 | 13.4 | 14.5 | 13.3 | 14.6 | 14.0 | 15.7 | 11.7 |

# APPENDIX

Specimens included in the phylogenetic analysis were obtained from the following collections: Museum of Southwestern Biology, University of New Mexico (NK), University of Alaska Museum (UAM or AF), Burke Museum (HEH and SAR), Museum of Vertebrate Zoology (MVZ), University of Michigan Museum of Zoology (UMMZ), and Rick Jannett (FJ). Quad refers to USGS 1:250,000 quadrangle.

Clethrionomys gapperi: Washington, Kittitas County (NK 3221); Clethrionomys glareolus: Finland, Lieksa (AF3133); Microtus abbreviatus: Alaska, St. Matthew Island (UAM 7762, AF21237, AF21238, AF21239); Microtus agrestis: Finland, Lieksa (AF3131, AF3304); Microtus californicus: California, Contra Costa County (MVZ3941), San Bernadino County (AF15889, AF15890, AF15891); Microtus canicaudus: Oregon, Benton County (AF18618, AF18619, AF18723, AF18724); Microtus chrotorrhinus: Minnesota, Cook County (AF17691, AF17692, AF17693, FJ47595); Microtus fortis: Korea (MVZ1524); Microtus gregalis: Russia, Yamal Peninsula (AF14463, AF14464, AF14465); Microtus kikuchii: Taiwan (MVZ1243, MVZ1245, MVZ1373); Microtus longicaudus: Alaska, Yakutat Quad (AF2031), Washington, Kittitas County (NK3135), Oregon, Lincoln County (AF18526), Arizona, Apache County (NK1924), Montana, Carbon County (AF10901); Microtus mexicanus: New Mexico, Union County (NK9222), Mexico, Coahuila State (NK9501); Microtus middendorffi: Russia, Yakutia

Republic (SAR6117, SAR6118); Microtus miurus: Alaska, Philip Smith Mountains Quad (AF5101), Healy Quad (AF1846); Microtus montanus: Utah, Salt Lake County (NK55041), White Mountains (NK3446), California, Mono County (NK5897): Microtus montebelli: Japan, Honshu Island (NK6066, NK6078, NK6084, NK6117): Microtus ochrogaster: Minnesota, Clay County (NK1946, NK7945), Montana, Carbon County (AF5275), New Mexico, Mora County (NK11180, NK11181), Arkansas, Lonoke County (NK3331, NK3332): Microtus oeconomus: Alaska, Montague Island (AF545), Russia, Kuril Islands, Rassua Island (HEH040), Shimishur Island (HEH024), Ketoi Island (HEH065); Microtus oregoni: Washington, Clallam County (NK3205), Oregon, Lane County (AF24989), Tillamook County (AF24992), Douglas County (AF24993): Microtus pennsylvanicus: Alaska, Mitkof Island (AF2511), New Mexico, San Juan County (NK11205); Microtus pinetorum: Arkansas, Pulaski County (NK2734), Saline County (NK9815), Massachusetts, Franklin County (NK9145): Microtus richardsoni: Oregon, Linn County (NK2786), Montana, Glacier County (UMMZ57934), Wyoming, Teton County (UMMZ67979); Microtus townsendii: Oregon, Tillamook County (AF18520, AF18523); Microtus xanthognathus: Alaska, Hughes Quad (AF3401, AF7953), Beaver Quad (AF3817), Nulato Quad (AF5372), Ruby Quad (AF3101), Tanacross Quad (AF10290).

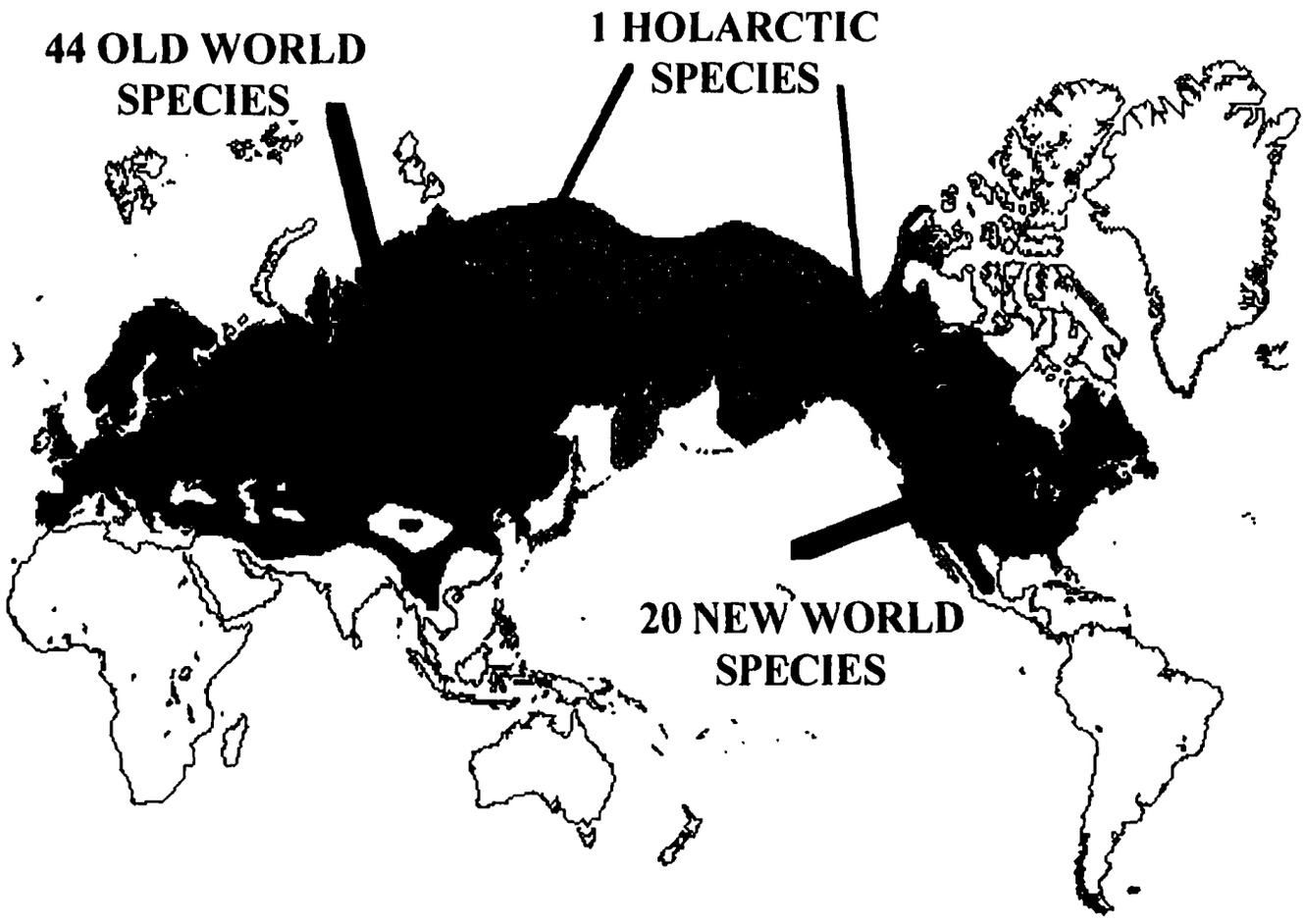


Fig. 1. — Current distribution of Microtus (black) and postulated extent of Beringia (Hopkins, et al., 1982) at peak glaciation (grey). Species diversity follows Musser and Carleton (1993) including Volemys. Distribution of Microtus follows Gromov and Polyakov (1977).

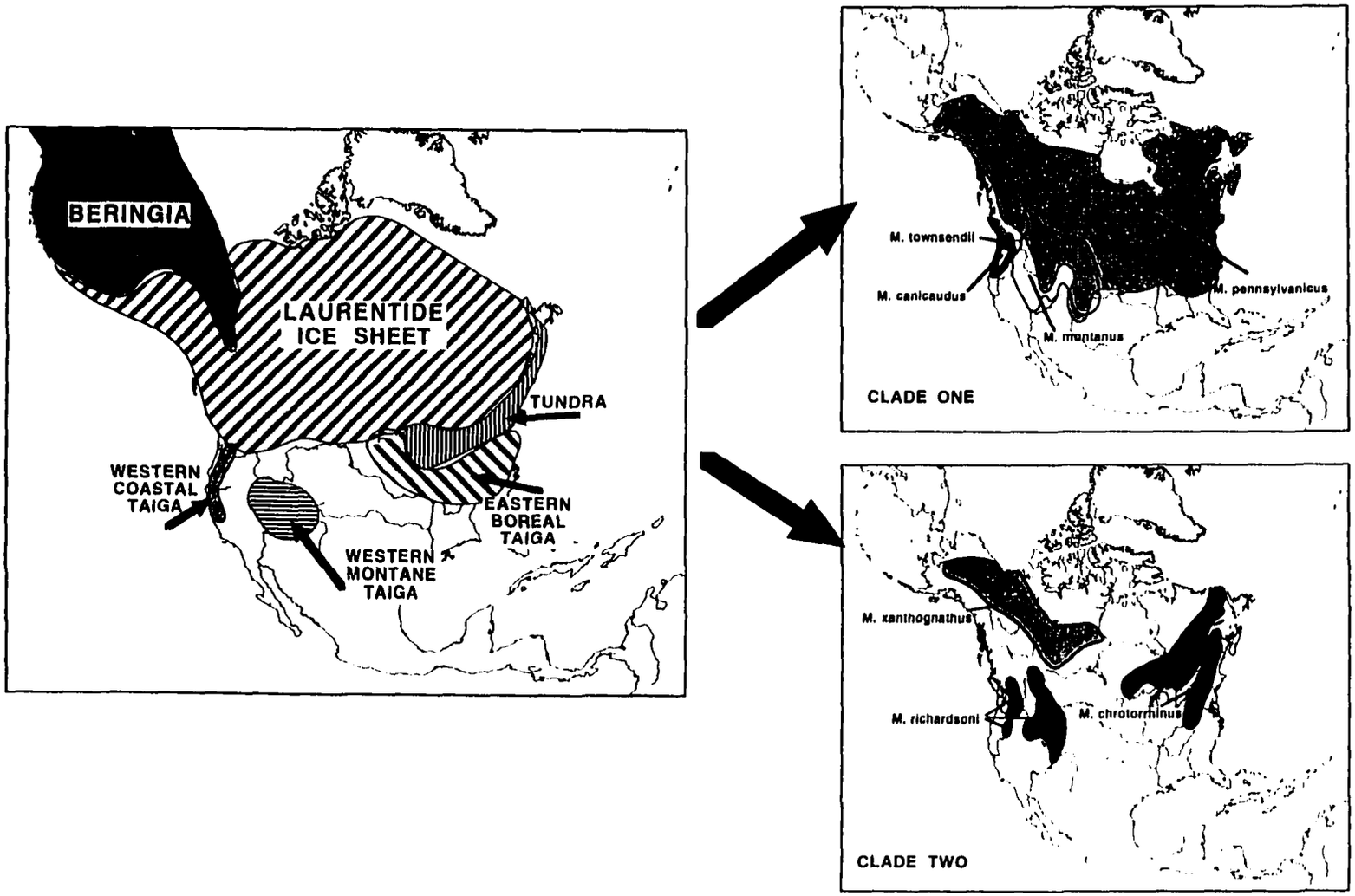


Fig. 2. — Model of taiga biome expansion and contraction through Pleistocene glaciations. Glacial refugia modified from Hoffmann (1981) and distribution of species modified from Hoffmann and Koeppl (1985).

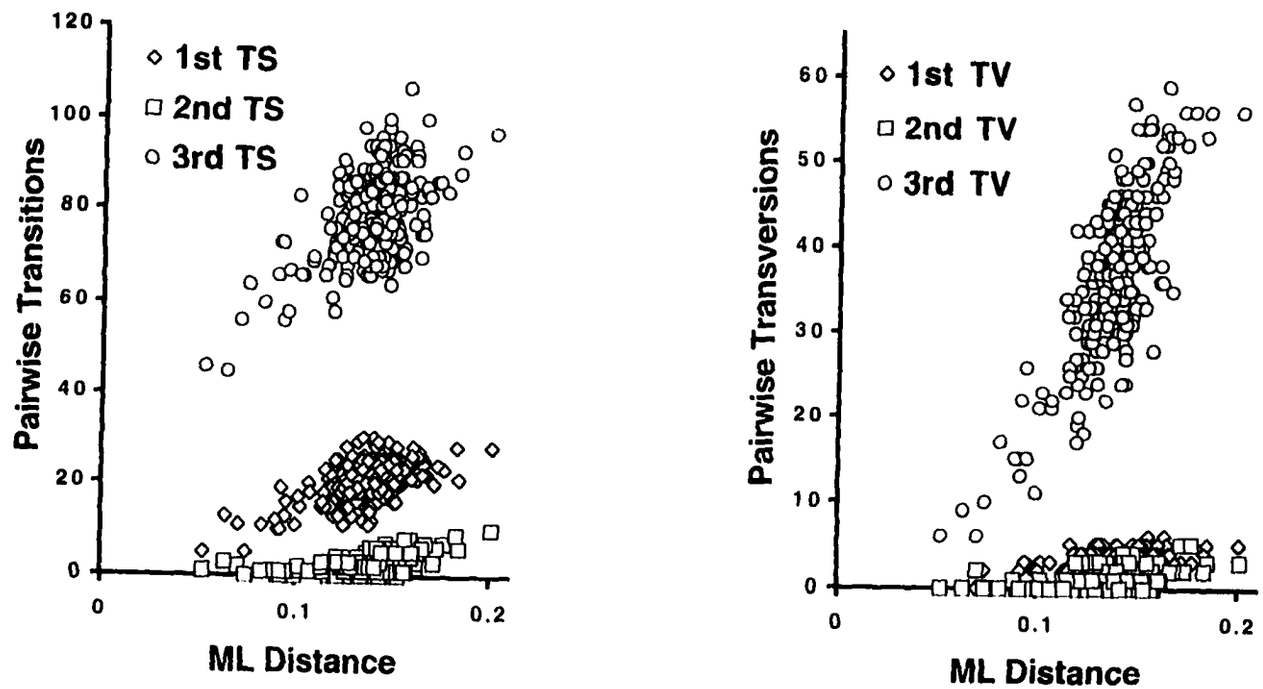


Fig. 3. - Pairwise numbers of transitions and transversions for first, second, and third amino acid positions plotted against maximum likelihood distance (F84) between species of *Microtus*.

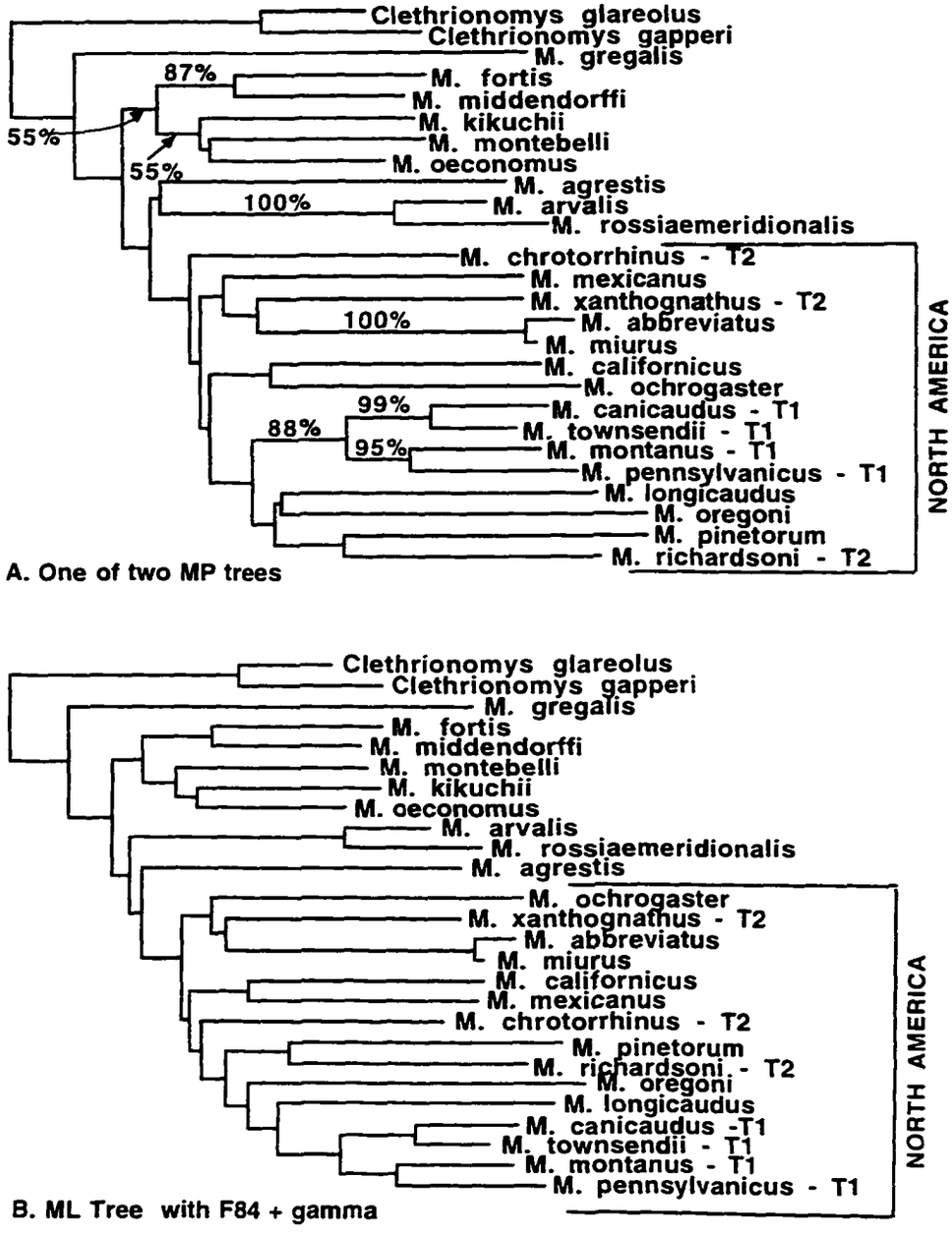


Fig. 4. — A. One of two maximum parsimony trees. B. Maximum-likelihood tree (F84). T1 and T2 following taxon indicate first and second taiga vole clades, respectively.

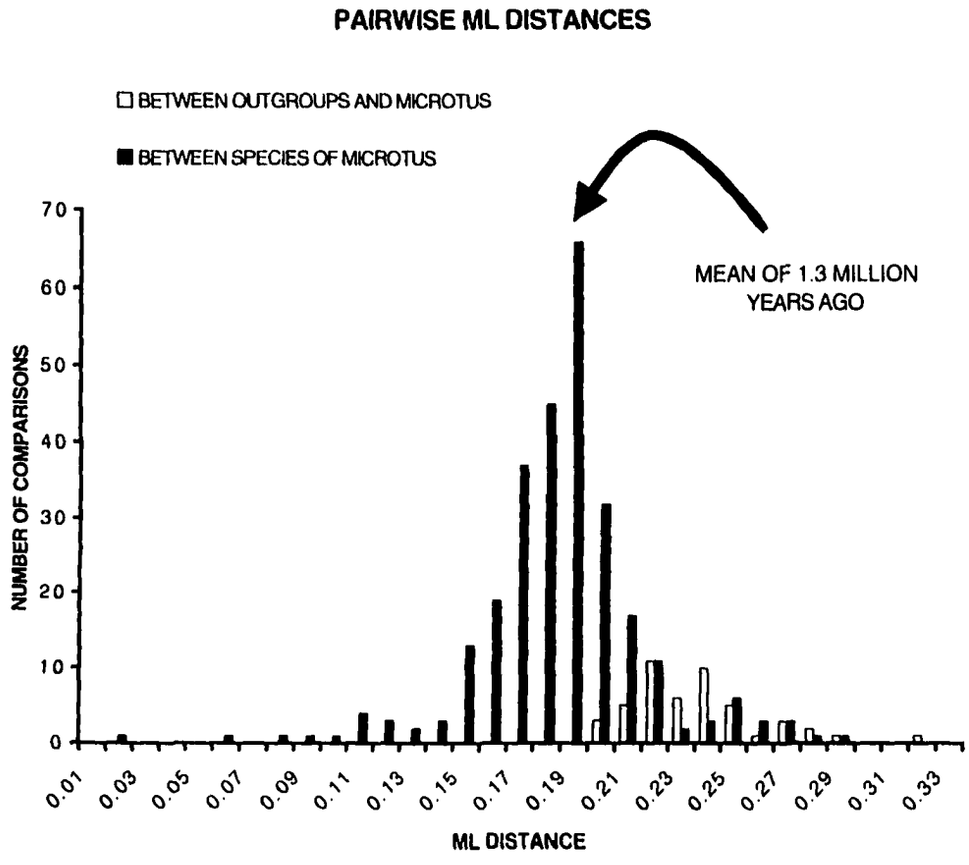


Fig. 5. - Pairwise distances between species of Microtus and between all species of *Microtus* and both species of *Clethrionomys*. The X axis is the same ML distance used in construction of the ML tree (see methods).

# Chapter 3

## Phylogeography of a Post-Glacial Invader: Microtus longicaudus (Muridae: Rodentia)[3]

Abstract:

The molecular phylogeography of Microtus longicaudus was investigated with DNA sequences of the mitochondrial cytochrome b gene. We used phylogenetic and pairwise distance methods to reconstruct the history of the species with a particular emphasis on the Pacific Northwest. Our data are consistent with post-glacial expansion following the receding Laurentide and Cordilleran ice sheets. Genetic variation across the species appears to be related to vicariant events during the Pleistocene followed by expansion. The largest break (> 6 % uncorrected percent sequence divergence) exists between populations found southeast of the Colorado River (eastern Arizona, Colorado, Wyoming, and New Mexico) and all other western populations. Other well-supported subclades were composed of samples from 1) the islands and north coast of southeast Alaska, 2) eastern Alaska, British Columbia, Washington, and Oregon, and 3) northern California, Idaho, and Montana. Within subclades divergence was low. Our results suggest that the close relationships among haplotypes within northern subclades are due

[3] Conroy, C. J., and J. A. Cook. In prep. Phylogeography of a Post-Glacial Invader: *Microtus longicaudus* (Muridae: Rodentia). Molecular Evolution.

to recent invasion whereas among subclade divergence is due to earlier, possibly mid-Pleistocene isolation events.

**Introduction**

Co-distributed organisms often respond to large environmental changes in predictable ways (Avise 1994, Hewitt 1993). The retreat of the Laurentide and Cordilleran ice sheets in North America at the end of the Pleistocene (ca. 10,000 years ago) provides an opportunity to assess the response of recolonizing communities of organisms. Graham *et al.* (1996) concluded that, based on the fossil record, vertebrate species apparently responded to deglaciation independently. Genetic markers can reveal geographic structure within and between species that is not necessarily exhibited morphologically (i.e. cryptic variation, Baker *et al.* 1995). Often these phylogeographic patterns are shared across species (e.g. Avise 1992, Bermingham & Moritz 1998). Molecular markers thus provide an alternative perspective to morphology to test geographic structuring within and among species.

Glacial retreat in North America led to range expansion northward by many taxa. Generalized predictions for genetic and phylogeographic structuring for these recolonizing populations include factors such as 1) population expansion, 2) vicariance, 3) differing forms of dispersal, and 4) refugial isolation (Hewitt 1993, 1996, Ibrahim *et al.* 1996, Marjoram & Donnelly 1991, Slatkin & Hudson 1991). For example, populations inhabiting recently deglaciated regions should exhibit patterns of lineage branching such as star phylogenies and genetic diversity typical of range expansion such as smooth mismatch distributions in pairwise comparisons of DNA sequences (e.g. see Fedorov *et*

*al.* 1996; Jaarola & Tegelstrom 1996; Merilä *et al.* 1997; Slatkin & Hudson 1991). Within a particular species, variation between clades is predicted if there has been expansion from separate refugia. Species may not share phylogeographic patterns if they expanded from geographically disjunct refugia in post-glacial periods. Conversely, patterns across species may be coincident if they expanded from shared refugia and responded to similar geographic barriers. Widely divergent haplotypes within an area might indicate secondary contact. Populations that have persisted in or near glacial refugia should have deeper among clade branch lengths than expanding populations (Bernatchez & Wilson 1998). With good comparative data at higher taxonomic levels, we can begin to assess the relative timing of phylogeographic events (Avise & Walker 1998).

Extant populations that occur in previously glaciated regions of North America are the descendents of lineages that invaded during the Holocene. These are characterized generally by reduced levels of genetic variation when compared to more southern conspecific populations (Hayes & Harrison 1992, Merilä *et al.* 1997, Sage & Wolff 1986, Soltis *et al.* 1997). However, levels of genetic variation in populations in northwestern North America may be confounded by the possibility of admixture among populations expanding from multiple refugia south of the ice sheets (Soltis *et al.* 1997), or refugia farther north along the Pacific Coast (Heaton *et al.* 1996), or Beringia (MacDonald & Cook 1996).

*Microtus longicaudus*

M. longicaudus is an herbivorous rodent that occurs in western montane taiga from New Mexico and Arizona north into British Columbia. Yukon Territory and Alaska (see review in Smolen & Keller 1987). It occurs in isolated mesic habitats on western and southwestern mountains, but has a more continuous distribution at higher latitudes and along the North Pacific Coast. Many mammals that expanded northward following glacial retreat apparently also were isolated in the mountains of the Great Basin and the southern Sierras (Lomolino *et al.* 1989, Patterson 1995).

Because patterns seen in mammals may not indicate broader community responses to environmental change, examining additional taxa such as plants could be informative of broader responses. For instance, Lamb *et al.* (1997) interpreted phylogeographic patterns in Abert's squirrel, Sciurus aberti, within the context of the distribution of ponderosa pine (Pinus ponderosa). The distribution of M. longicaudus is largely shared with P. contorta. P. contorta has responded rapidly to climate change (Anderson 1996, Critchfield 1985) and may be a general indicator of movement of boreal-forest associated species. Because the pollen of P. contorta is preserved in many late Holocene lakes throughout western North America and is easily identified, it is possible to track the movement of P. contorta forest over time.

Much of the data that bears on interglacial refugial isolation and expansion has been derived from the fossil record (Graham *et al.* 1996). However, those data are

frequently limited in the temporal scope of questions they might address. Because molecular approaches provide an independent view of intraspecific divergence and relationships, they can be used to address questions related to Pleistocene differentiation and recolonization.

Few boreal taxa have been studied over large geographic areas to investigate both refugial and expansion responses to Holocene warming (Wooding & Ward 1997). Although the Pleistocene fossil record has been interpreted to generally suggest that most taxa exhibit independent responses to climatic change (Graham *et al.* 1996), the growing body of molecular data at the intraspecific level provides a more detailed perspective on the geography of plant and animal movement in the Holocene. For instance, phylogeographic studies (see review in Molecular Ecology, Volume 7) can be quite useful for identifying Pleistocene refugia, post-glacial invasions, and variation within species previously undetected by morphological studies.

We test the genetic predictions of post-glacial range expansion by examining DNA sequence variation across populations of long-tailed voles (Microtus longicaudus, Muridae). We focus on populations along the North Pacific Coast and place them within the context of variation across the entire range of the species.

## Materials and methods

### *Sampling*

Specimens were obtained from throughout the range of M. longicaudus with a particular focus on localities north of 54° north latitude and along the North Pacific Coast (Fig. 1, Appendix). This region has a complex biogeographic history (Conroy *et al.* in press, Klein 1965, MacDonald & Cook 1996, Scudder and Gessler 1989) and has been suggested to support paleoendemic populations (Heaton *et al.* 1996). Because many northern populations descended from periglacial lineages within the last 10,000 years ago, we investigated genetic variation within and between phylogenetically defined lineages (e.g. Bonatto & Salzano 1997, Redd *et al.* 1995, Shields *et al.* 1992).

### *Molecular Methods*

We examined variation in the mitochondrial cytochrome b gene (cyt b) in 111 specimens of M. longicaudus. Of those, we sequenced the complete cyt b gene (1143 bases) from 72 and a portion of the gene (409 bases) from 39 specimens using methods described in Conroy & Cook (in press). PAUP* (test version 4.0d64, written by D. L. Swofford) was used for genetic and phylogeographic analyses. Variable sites were compared within and among species. The synonymous to replacement ratio was examined both between and within species with a G test (MacDonald & Kreitman 1991). We rooted parsimony trees with closely related species M. pennsylvanicus and M.

montanus (Conroy & Cook submitted). Neighbor-joining trees (Saitou & Nei 1987) were based on all specimens and Kimura two-parameter distances (Kimura 1980). This distance method corrects for multiple substitutions, but assumes equal base frequencies. However, trees constructed with more complex models (e.g. Tamura-Nei, Tamura & Nei 1993) were not different in branching order among the major subclades. Only individuals with complete cyt b sequences were used in bootstrap resampling (500 replicas) to identify well-supported clades. Patterns of variation in cyt b within M. longicaudus were compared with 24 other species of Microtus (Conroy & Cook submitted).

Clades with at least five individuals and bootstrap support greater than 50% were used for analysis of within-clade diversity. Gene diversity was calculated for all samples and also examined within clades using Arlequin (Excoffier *et al.* 1997). Pairwise mismatches were calculated, plotted, and tested against a sudden expansion model for expanding populations (Rogers 1995, Watterson 1975).

We estimated the relative time of divergence of mitochondrial lineages using a maximum likelihood distance based on interspecific comparisons (Conroy & Cook submitted). We assumed that the deepest phylogenetic splits within the genus Microtus should approximate the oldest branching events in the group. Therefore, we set the genetic distance between M. gregalis and M. oregoni, the deepest split, to 2.2 million years divergence, based on the fossil record (Repenning *et al.*, 1990). Chi-square tests of rate heterogeneity indicated that species of Microtus were evolving in a clocklike manner

(Conroy & Cook submitted). We estimated the average pairwise distances within clades under the HKY85 (Hasegawa *et al.* 1985) + Γ model ($\alpha = 0.2159$, ti/tv ratio = 2.6) with PAUP*, and then subtracted these distances from between clade differences for a net divergence time (Avise & Walker 1998, Edwards 1997). We also tested for rate heterogeneity among the complete cyt b sequences within Microtus longicaudus by evaluating ML trees with and without a molecular clock constraint (Felsenstein 1988).

**Results**

Variation across third (178 variable sites, 78 % of all variable sites), first (39, 17 %) and second (12, 5 %) positions of codons was distributed as suggested for genuine sequences of mammalian cyt b. Base composition (A: 31 %, C: 27 %, G: 13 %, T: 29 %) is similar to other mammals (Irwin *et al.* 1991, Lessa & Cook 1998) and other species of Microtus (Conroy & Cook submitted). The distribution of forty-five variable amino acid sites along the gene was consistent with structural and functional models of variation (e.g. Irwin *et al.* 1991). Comparisons of within and between species variation suggest selection on cyt b is not apparent (McDonald & Kreitman 1991). That is, the ratio of replacement to synonomous sites was the same between as within species.

Parsimony and distance analysis retrieved topologically equivalent trees that differed only at nodes with bootstrap values below 50 % (Fig. 2). Four primary clades were identified. A clade of haplotypes from Colorado, Wyoming, eastern Arizona, and New Mexico was highly divergent from all other haplotypes. These are at the eastern and

southeastern edges of the Great Basin (hereafter Southern Rockies clade). A second clade included samples from northern California, Idaho and Montana (hereafter Central clade). Samples from central Washington, coastal Oregon, British Columbia, the east coast of southeast Alaska, and interior Alaska were monophyletic (hereafter Northwest clade). Finally, samples from southwestern Yukon Territory, southeast Alaska islands, the Pacific coast from Haines, Alaska, west to Yakutat, Alaska, and southern interior Alaska formed another clade (hereafter Island clade). The Island and Northwest clades apparently contact in the vicinity of Haines, Alaska. Samples from the north rim of the Grand Canyon in Arizona were not strongly monophyletic and were intermediate to the Central, Northwest, and Island clades.

The pairwise distance histogram of all samples (Fig. 3) indicated variation both within and among populations or clades (Marjoram & Donnelly 1991). Mean pairwise numbers of differences within M. longicaudus was 29.2 ± 12.9 (= 1 SD). Pairwise analysis suggested the Central clade and Southern Rockies clade were significantly different from expectations under Rogers (1995) Sudden Expansion Model. However, pairwise differences within the Island (12.3 ± 5.7) and Northwest (11.2 ± 5.3) clades were indistinguishable from an expansion model. Mean pairwise difference was lowest in the Central clade (10.1 ± 5.2). The Southern Rockies clade exhibited much deeper divergence (28.1 ± 13.8).

The test of rate heterogeneity indicated that some lineages may be evolving at different rates under the Kimura two-parameter model. This weakens the inferences we can make by applying a molecular clock assumption to these data. However, we present this analysis as a heuristic tool for exploring the relationship between genetic divergence and time since last common ancestor within this species. An estimate of the net divergence time for M. longicaudus from other Microtus suggested a divergence beginning $0.918 \pm 0.018$ (= 1 SD) millions of years ago (MYA) under the assumption of a molecular clock (Table 2, Fig. 4). The Island and Northwest clades were estimated to have diverged $0.091 \pm 0.024$ MYA and these clades diverged from the Central clade about $0.246 \pm 0.029$ MYA. These clades in turn diverged from the Southern Rockies clade approximately $0.342 \pm 0.067$ MYA.

**Discussion**

Climatic change can impact organismal evolution at many temporal and geographic scales. Cycles of fluctuating climate since the Pliocene, for example, may be partially responsible for elevated mammalian species richness in western North America (Mönkkönen & Viro 1997). More recent events in the late Pleistocene, however, may only have effects below the species level (Avise & Walker 1988). For instance, late Pleistocene climatic cycles may have resulted in distinctive genetic lineages within M. longicaudus that were undetected previously with morphological characters. This

variation can be used to test the predictions of post-glacial expansion on the distribution of genetic variation and test the geographic limits of genetic lineages. However, these results should be tested with other morphological and genetic markers and by examining additional specimens of M. longicaudus, particularly from southern populations. This might allow identification of morphological characters that correlate with these results.

*History of Invasions*

Pairwise analysis of DNA sequences has been used to recover the history of population movement, particularly in humans (Harpending 1994, Rogers 1995, Rogers & Jorde 1995, Shields et al. 1993:555). Distribution of pairwise mismatches for example, may indicate whether populations are expanding geographically or, alternatively, are older and have had a relatively constant size (i.e. not substantially bottlenecked). A bimodal distribution pattern is expected from gene trees with a single major bifurcation (Slatkin & Hudson 1991), such as that seen between the Southern Rockies clade and all others. The pattern of pairwise mismatches within the southern clades departs from expectations of Rogers (1995) model of sudden expansion; this suggests they may be older. These clades are contrasted with the two northern clades which have rapidly expanded their ranges northward into deglaciated areas. The phylogeny also supports this interpretation with well-supported branches for suspected older populations, but star-like topology for the expanding clades (Slatkin & Hudson 1991).

The distribution of pairwise differences in the northern clades (i.e. relatively small differences and a single peak) may indicate a recent bottleneck (Marjoram & Donnelly 1994, Merilä *et al.* 1997). Serial bottlenecking may be a common theme among animals invading recently deglaciated regions (Merilä *et al.* 1997, Sage & Wolff 1986) and is the likely case for M. longicaudus. The relatively low DNA divergence within the Island and Northwest clades indicates rapid population level radiation and is consistent with other high latitude arvicoline rodents (Myopus schisticolor, Fedorov *et al.* 1996; Microtus agrestis, Jaarola & Tegelstrom 1996). Soltis *et al.* (1997) documented a reduction in lineages among post-glacial populations of plants in the Pacific Northwest. This pattern may be contrasted with southern M. longicaudus which exhibited larger genetic differences over equivalent geographic distances; a situation consistent with longer isolation (e.g. on mountain tops). Restricted gene flow due to isolation on mountains has been suggested for other southwestern mammals (e.g. mountain sheep, Ramey 1995).

Our sampling scheme, which emphasized sampling in the northern latitudes, also may have contributed to the levels of variation seen in the northern clades (Fig. 3). Average pairwise differences were less within the two northern clades than within more southern clades. However, more extensive sampling of populations, particularly those at lower latitudes, will be necessary to test the significance of this sampling artifact. Further sampling may also allow tests of forms of dispersal over extended time periods (e.g. normal versus leptokurtic, Ibrahim *et al.* 1996).

*Post-Glacial Invasion Pathways*

Most of the northern half of the current range M. longicaudus was likely glaciated until 15,000 years before the present. Since then, this vole has reinvaded these areas, possibly through several different pathways. These possibilities for post-glacial colonization include 1) northward expansion along the North Pacific Coast from refugia south of the Laurentide and Cordilleran ice sheets, 2) expansion from coastal refugia along the North Pacific Coast, 3) northward expansion east of the coast range, or 4) expansion southward from the Beringian refugium (Fig. 5B). The low genetic variation within the northern clades over large geographic areas suggests this expansion was rapid.

A northward post-glacial coastal invasion might have occurred as early as 13,500 to 10,400 years before present when there was a rapid retreat of ice along the coast of British Columbia and southeast Alaska exposing large areas of land (Josenhans *et al.* 1995, Mann & Hamilton 1995). These exposed low-relief areas may have been productive habitat for early succession generalists (Heusser 1960) such as M. longicaudus. Old-growth associated species, such as the northern flying squirrel, Glaucomys sabrinus, probably arrived much later (Demboski *et al.* in press). The absence of M. longicaudus from the Queen Charlotte and Vancouver islands, which are isolated by deep-water channels, is consistent with a mainland coastal expansion of the Island clade from a southern refugium. The absence of the Island clade along the mainland in southeastern

Alaska, other than the northern coast, is inconsistent with a post-glacial coastal northward invasion.

An alternative to a post-glacial colonization of southeast Alaska by M. longicaudus is persistence in a refugium in southeast Alaska during the latest glaciation as has been suggested for Ursus arctos (Heaton *et al.* 1996) and some tree species (Hansen & Engstrom 1996). Fossil evidence of long-term occupation of voles in southeast Alaska is lacking. However, presence on numerous islands of the Alexander Archipelago, presence in southern interior Alaska, and divergence of the Island clade from other northwest populations is consistent with isolation in a refugium. Pinus contorta, which is often co-distributed with M. longicaudus, has occurred in southeast Alaska throughout the Holocene, and possibly through glacial advances (Hansen & Engstrom 1996). This suggests that other boreal taxa also may have been present through these glacial advances in or near southeast Alaska. Although our data are consistent with a refugium in the area, precisely locating a Northwest Coast refugium is problematic because of a lack of information for mid-glacial sea levels and environments in the region. Thorough geographic sampling is necessary to establish the boundaries of potential refugia. Preliminary data from coastal British Columbia (175 bases, light strand only from Goose Lake, Haney Experimental Forest [n = 1]; Surf Inlet, Belmont [n = 1]; Kynoch Inlet [n = 2]; Garibaldi Provincial Park [n = 1], and Goose Island [n = 2]) indicates the Northwest clade is continuously distributed on the coast from Oregon to Haines, Alaska. This

suggests the Island clade is restricted to the islands in southeastern Alaska and mainland areas north of 58° North latitude.

The glacial history of southeastern Alaska may have significantly impacted the distribution of genetic lineages in the region. The mainland south of Juneau, Alaska (Fig. 1), was probably the area most recently deglaciated by the receding Cordilleran ice sheet which probably melted from the edges towards the middle. Numerous glaciers and ice fields still occur in the Coast Mountains of southeastern Alaska and British Columbia. Absence of the Island clade from this area is consistent with an eastern expansion from the outer edge of the Alexander Archipelago that was halted by mainland glaciers. The presence of the Island clade in southcentral Alaska could have been facilitated by invasion along exposed beaches and morraines during lowered sea levels. Other taxa such as Pinus contorta were probably prevented from expanding west of Yakutat by the Malaspina and Bering glaciers (Heusser 1960). The range of M. longicaudus, however, barely extends into interior Alaska and Yukon Territory. We suggest that these populations were established relatively recently.

The presence of M. longicaudus on at least 25 islands (MacDonald & Cook 1996) suggests that movement may have been more common when sea levels were depressed and distances between islands were reduced. However, absence of Microtus from the nearby Queen Charlotte islands argues against a refugium for voles in that archipelago as has been suggested for other taxa (Byun *et al.* 1996, Foster 1965, Scudder & Gessler

1989). An abundance of closely related haplotypes distributed across the Alexander Archipelago is consistent with a past genetic connection of these populations. The lack of geographical structure among those lineages within southeast Alaska, however, is consistent with an initial colonization by a bottlenecked population followed by isolation and subsequent divergence on different islands. The paucity of shared identical haplotypes between localities suggests modern gene flow is relatively rare.

Invasion routes between eastern Beringia (interior Alaska and Yukon Territory) and southern regions in North America were not available until 12-15,000 years ago when the Laurentide and Cordilleran ice sheets had melted sufficiently (Mandryk 1996). This corridor has implications for the peopling of North America and expansion of populations from various other refugia (Fladmark 1978, Rogers *et al.* 1990, Rogers *et al.* 1991, Rogers *et al.* 1992). The presence of the relatively undifferentiated Northwest clade of long-tailed voles (Oregon to interior Alaska) is consistent with a rapid post-glacial invasion northward along an interior route (Fig. 5).

Although this interior route was used by mammals that moved both north and south, it seems unlikely that M. longicaudus expanded south from Beringia after glacial retreat. M. longicaudus lacks a Beringian fossil record (Morlan 1989) and interior Alaskan populations are closely related to populations in Washington and Oregon. Relatively few North American mammal taxa were present in both Beringia and southern refugia; most were south of the ice sheets and dispersed northward (Hoffmann 1981).

However, no fossils of M. longicaudus have been documented in Oregon or Washington. Thus, it is possible that M. longicaudus expanded from Beringia and the more southern populations of this lineage were the result of recent colonization. Larger sample sizes and a faster evolving molecular marker will be necessary to discriminate the relative age of these populations. For instance, application of isolation by distance models could be informative of the direction of invasion.

Coastal versus mainland or interior taxonomic divisions occur within a number of taxa (mammals, MacDonald and Cook 1996; birds, Passerella iliaca, Zink 1994; trees, Pinus contorta, Critchfield 1985). We found that the Northwest and Island clades overlap in the vicinity of Haines, Alaska (Fig. 1). This might be a region of overlap for other interior-coast pairs. However, it is not clear when these M. longicaudus clades may have come into contact. Coastal and interior forms of Pinus contorta are found in the region, but the fossil record suggests they did not move through White Pass, which has always been alpine tundra at higher elevations (Spear & Cwynar 1997). Introgression between the coastal and interior forms of Pinus contorta is not very extensive (Wheeler & Guries 1982).

*Southern Refugia*

Findley & Anderson (1956) suggested that a number of sister taxa meet across the Wyoming Basin and the Green River, a tributary of the Colorado River. Our preliminary

data also suggest a phylogeographic break in M. longicaudus across this region. This deep division in long tailed voles has not been identified previously either on the basis of subspecific taxonomy (Hall 1981) or chromosomal variation (Judd & Cross 1980). Other taxa that appear to be morphologically undifferentiated across this region should be examined for cryptic genetic differences.

The similarity of many mammal communities throughout the southern Rocky Mountains suggests these taxa dispersed between areas through the Pleistocene (Davis *et al.* 1988, Luikart & Allendorf 1996). However, it is also possible that this region supported distinct Pleistocene refugia for mammals of low vagility. For example, other small mammals also exhibit large phylogeographic breaks in this region (e.g. Onychomys leucogaster , Riddle *et al.* 1993; Sciurus aberti , Lamb *et al.* 1997). Extensive genetic divergence among southern haplotypes in M. longicaudus (and other taxa) could be due to rare invasion events during cooler periods in the Pleistocene (Lomolino *et al.* 1989, Roy *et al.* 1996) followed by isolation during interglacials. Early phylogeographic events occurred in M. longicaudus approximately 0.34 MYA, which corresponds to the Kansan glaciation (Winograd *et al.* 1997). Though Pleistocene climatic oscillations may not have been as important to mammalian and ornithological speciation as previously thought (Riddle 1995, Klicka & Zink 1997), this period may have been key to many phylogeographic breaks (Avise & Walker 1998). Our divergence estimates, which placed the major phylogeographic break within M. longicaudus within the mid-Pleistocene is

consistent with findings for other taxa. Our estimate, based on net divergence, significantly reduced estimates of between clade divergence (Avise & Walker 1998, Edwards 1997).

Fossils can provide temporal information regarding the history of populations (Graham *et al.* 1996). However, fossils of Microtus longicaudus are scarce and limited both temporally and geographically (Graham *et al.* 1996, Zakrzewski 1985). The shallow age of fossils for this species (early Wisconsinan) limits inferences to the last glacial episode. Unambiguous M. longicaudus fossils are largely distributed within the contemporaneous distribution and are restricted to the eastern side of the distribution of the species (Nevada, Idaho, Montana, Utah, Colorado, Wyoming, New Mexico, Nevada, and Alberta). Whether this depicts the late Pleistocene distribution of Microtus longicaudus or is due to a bias in preservation is unknown. There are many other fossils in western North America that may be M. longicaudus, but morphological similarity to M. montanus and M. pennsylvanicus inhibits positive identification to species level (Zakrzewski 1985). The molecular characters used in this study have provided an alternative, but testable, perspective to the limited direct evidence available from fossils.

**Conclusion**

M. longicaudus apparently responded to climate change in two ways during the mid to late Pleistocene. An early separation across the Colorado River led to the

accumulation of significant amounts of genetic variation equivalent to recent species level divergence. The retreat of glaciers in the early Holocene led to a rapid but spatially organized northward invasion. Consequently, specimens examined over large geographic areas in the north (e.g. British Columbia to interior Alaska) are very closely related genetically, but show geographic differentiation that suggests multiple paths of expansion. The timing and geographical origins for the Island clade might support the presence of a glacial refugium in or near southeast Alaska.

Many processes affect the distribution of genetic variation across populations. Similar phylogeographic patterns across species indicate the possibility of common processes or history. Though many species appear to respond to their environments independently and not share geographic patterns (Graham *et al.* 1996, Zink 1994, Bernatchez & Wilson 1998, Taberlet *et al.* 1998), more empirical data are necessary to test this observation. Conclusions for M. longicaudus should be tested against other phylogeographic studies of mammals, plants, and birds common to boreal habitats of western North America. Unfortunately, few boreal taxa of North America have been investigated with molecular methods at both northern and southern extremes. Future phylogeographic studies should test the spatial distribution of genetic variation in other mammals that occur on recently deglaciated areas in Alaska and Canada, many of which have conspecific populations isolated in mountains of the southern Rocky and Sierra mountains. Investigations of lineage differentiation in other taxa, particularly plants and

invertebrates, would provide a robust test of the response of organisms to climatic change. An appreciation for the genetic response of organisms to widespread northward invasion may be useful for predicting future responses to global climatic change as well.

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**Table 1** Descriptive statistics of genetic variation by major clade.

| | SOUTHERN ROCKIES | NORTHWEST | ISLAND | CENTRAL | TOTAL |
|---|---|---|---|---|---|
| No. of samples | 8 | 25 | 27 | 8 | 71 |
| Observed transitions | 58 | 55 | 96 | 30 | 211 |
| Observed transversions | 7 | 11 | 8 | 1 | 33 |
| transition/transversion ratio | 8.29 | 5 | 12 | 30 | 6.39 |
| Substitutions | 65 | 66 | 104 | 31 | 244 |
| Polymorphic sites | 64 | 63 | 104 | 31 | 229 |
| Observed transitions | 57 | 55 | 96 | 30 | 208 |
| Observed substitutions | 64 | 63 | 104 | 31 | 229 |
| C | 34.57% | 35.94% | 36.40% | 26.21% | 37.66% |
| T | 29.49% | 26.79% | 26.10% | 37.90% | 26.30% |
| A | 21.68% | 20.13% | 22.86% | 20.16% | 27.20% |
| G | 14.26% | 17.14% | 14.64% | 15.73% | 8.83% |
| Mean pairwise differences | 28.12 ± 13.80 | 11.21 ± 5.26 | 12.26 ± 5.71 | 10.07 ± 5.16 | 29.2 ± 12.9 |
| Nucleotide diversity | 0.44 ± 0.25 | 0.18 ± 0.09 | 0.12 ± 0.06 | 0.32 ± 0.19 | 0.09 ± 0.05 |
| Mismatch observed mean | 28.11 | 11.21 | 12.26 | 10.07 | 29.2 |
| Mismatch observed variance | 255.06 | 22.61 | 16.43 | 15.11 | 264.46 |

**Table 2** Molecular clock estimates of divergence between clades in millions of years (Mya). Net divergence is the average gross estimate minus the average pairwise distance within clades. Net divergence between *M. longicaudus* and other species is the average distance between *M. longicaudus* and sister taxa minus the average distance within *M. longicaudus* based on all pairwise comparisons.

| Between | And | Gross (Mya) | Net (Mya) |
|---|---|---|---|
| *M. longicaudus* | Other species of *Microtus* | 1.13 | 0.918 |
| SOUTHERN ROCKIES | All other subclades | 0.440 | 0.342 |
| CENTRAL | NORTHWEST + ISLANDS | 0.320 | 0.246 |
| NORTHWEST | ISLANDS | 0.169 | 0.091 |
| NORTHERN HALF OF NORTHWEST CLADE | SOUTHERN HALF OF NORTHWEST CLADE | 0.102 | 0.058 |

Appendix: Specimens obtained from the following collections or individuals: Museum of Southwestern Biology, University of New Mexico (NK), University of Alaska Museum (UAM or AF), Lee Simons, University of California at Davis (LHS), Bruce Hayward (BJH), Jack Sullivan, University of Idaho (JMS), and Cowan Vertebrate Museum, University of British Columbia (CVM). Quad refers to USGS 1:250,000 quadrangle.

*Clethrionomys gapperi*, Washington, Clallam County (NK3221);

*Microtus montanus*, Utah, Salt Lake County (NK55041);

*M. pennsylvanicus*, New Mexico, San Juan County (NK11205);

*M. longicaudus*:

**Alaska.**

Circle Quad, Big Windy Hot Springs (AF15867, AF15868);

Big Delta Quad, Goodpaster R. (UAM1894);

Bradfield Canal Quad, mouth of Unuk R. (AF4366, AF4426);

Craig Quad, Prince of Wales Island, Dunbar Inlet (AF10405); Anguilla I. (AF12411); Orr I. (AF12434); Tuxecan I. (AF12487); Prince of Wales I., near El Capitan (AF14456); Prince of Wales I., Polk Inlet (AF2156); Coronation I., Egg Harbor (AF3982, AF4485, AF4486, AF5170, AF5171, AF5172, AF5173); Prince of Wales I., 19 km E of Craig (AF4503); Suemez I., Refugio Bay (AF4517); Cleveland Peninsula, Union Bay (AF4717, AF4718); Marble I. (AF4832); Warren I., Warren Cove (AF8345, AF8347);

Dixon Entrance Quad, Forrester I. (AF16751, AF16752); Dall I., Essowah Lakes (AF4687);

Juneau Quad, Chichagof I., Game Creek (AF10376); Lynn Canal, Excursion Inlet, W side (AF17242); Lynn Canal, Excursion Inlet, E side (AF17254); Chichagof I., 11 mi

SE of Hoonah (AF1809); Glacier Bay, Bartlett Cove, 10 km NW Gustavus Airport (AF3752); Chichagof I., Game Creek (AF6519); Chichagof I., Otter Lake (AF8619, AF8662);

Ketchikan Quad, Revillagigedo I., Ella Cr. (AF4333); Revillagigedo I., Behm Canal, Portage Cove (AF4773);

McCarthy Quad, near Kennecott (UAM3553, AF3553);

Petersburg Quad, Etolin I., Anita Bay (AF14451, AF14452, AF14453, AF14454, AF2583); Mitkof I. (AF2440); Kupreanof I., (AF2960, AF4843); mouth of the Chickamin R. (AF4902, AF4910); Revillagigedo I., Orchard Lake (AF4986); Thomas Bay (AF5269, AF5270);

Port Alexander Quad, Kuiu I. (AF3725);

Prince Rupert Quad, Pearce Canal, Hidden Inlet, Gwent Cove (AF8389);

Sitka Quad, Chichagof I., Salt Lake Bay (AF10195);

Skagway Quad, White Pass, (AF12501, AF12502); Taiya River tidal flats (AF12516, AF12517); Haines Hwy., 3.9 km WNW Haines (AF12535); 17 km W, 20 km N Klukwan, Kelsall R drainage (AF8014, AF8015, AF8034, AF8038, AF8090); 10 km E, 9 km S Klukwan (AF8075); Klehini R, 5 km W Klukwan (AF8116);

Taku River Quad, Crescent Lake (AF8299, AF8308, AF8317); Fish Creek (AF8464, AF8467);

Yakutat Quad, Cannon Beach near Yakutat (AF2031);

**Arizona**: Apache County (NK1924); Coconino County (NK8521, NK8524, NK8525);

**California**: Siskiyou County (LHS558, LHS567, LHS569, LHS577, LHS616, LHS641);

**Colorado**: Chaffee County (BJH9871, BJH9873, BJH9874);

**Idaho:** Latah County (JMS 138);

**Montana**: Carbon County (AF10901);

**New Mexico**: Sandoval County (NK1719); Cibola County (NK9766);

**Oregon**: Lincoln County (AF18526), Lane County (AF18528);

**Washington**: Kittitas County (NK3135);

**Wyoming**: Carbon County (AF23201);

**British Columbia**: Salmon River (AF12713); Stikine River (AF12847, 12860); near Atlin (AF12909); Sicamous Creek (AF14020, AF18740, AF24886, AF24988, AF24990); Opax Mountain (AF14909); Goose Lake (CVM10610); Surf Inlet (CVM5059); Kynoch Inlet (CVM2754, CVM2755); Garibaldi Provincial Park (CVM3607); Goose Island (CVM2734, CVM2739);

**Yukon Territory**: near Haines Junction (AF10424, AF10426).

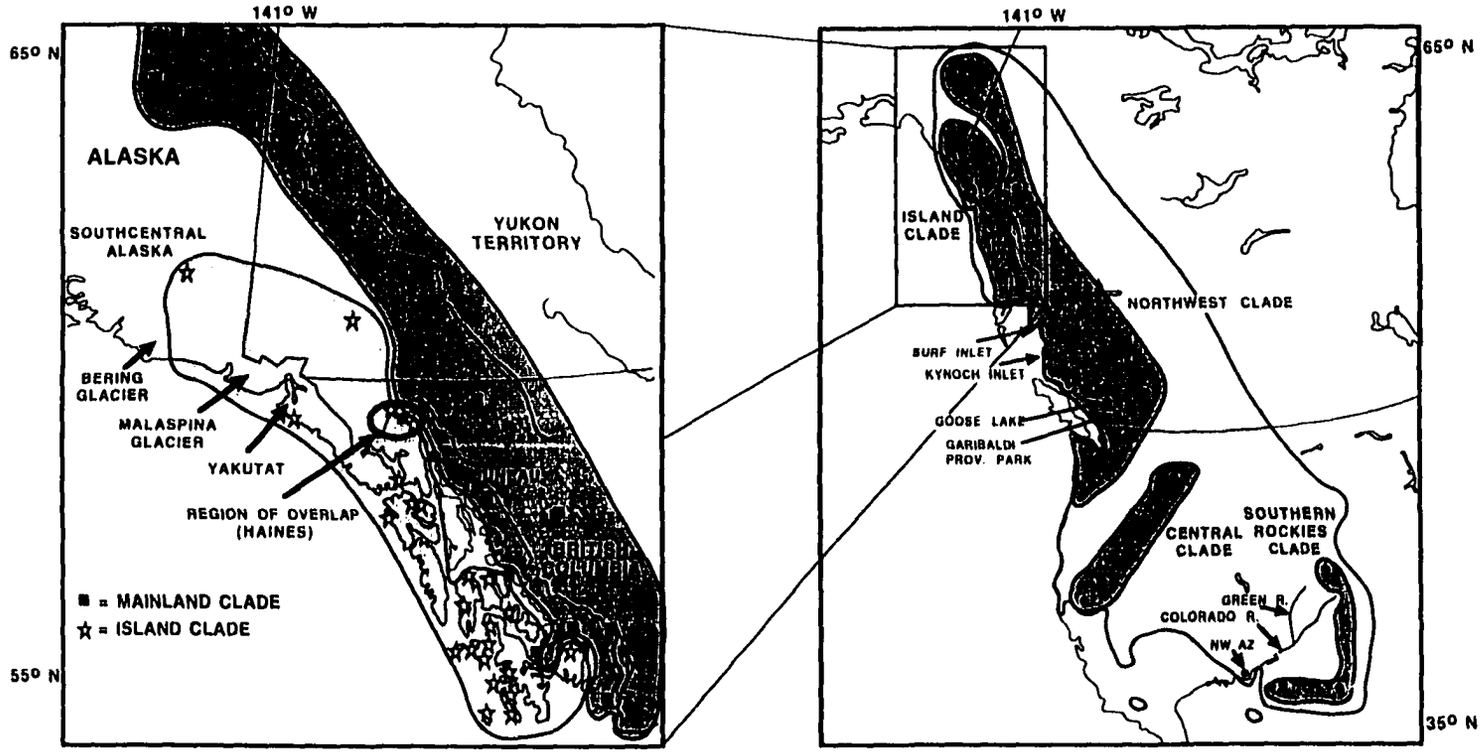


**Fig. 1.** Map with distribution of sampled localities and general distribution of clades within M. longicaudus. Lines enclosing clades should not be construed as the actual geographic limit.

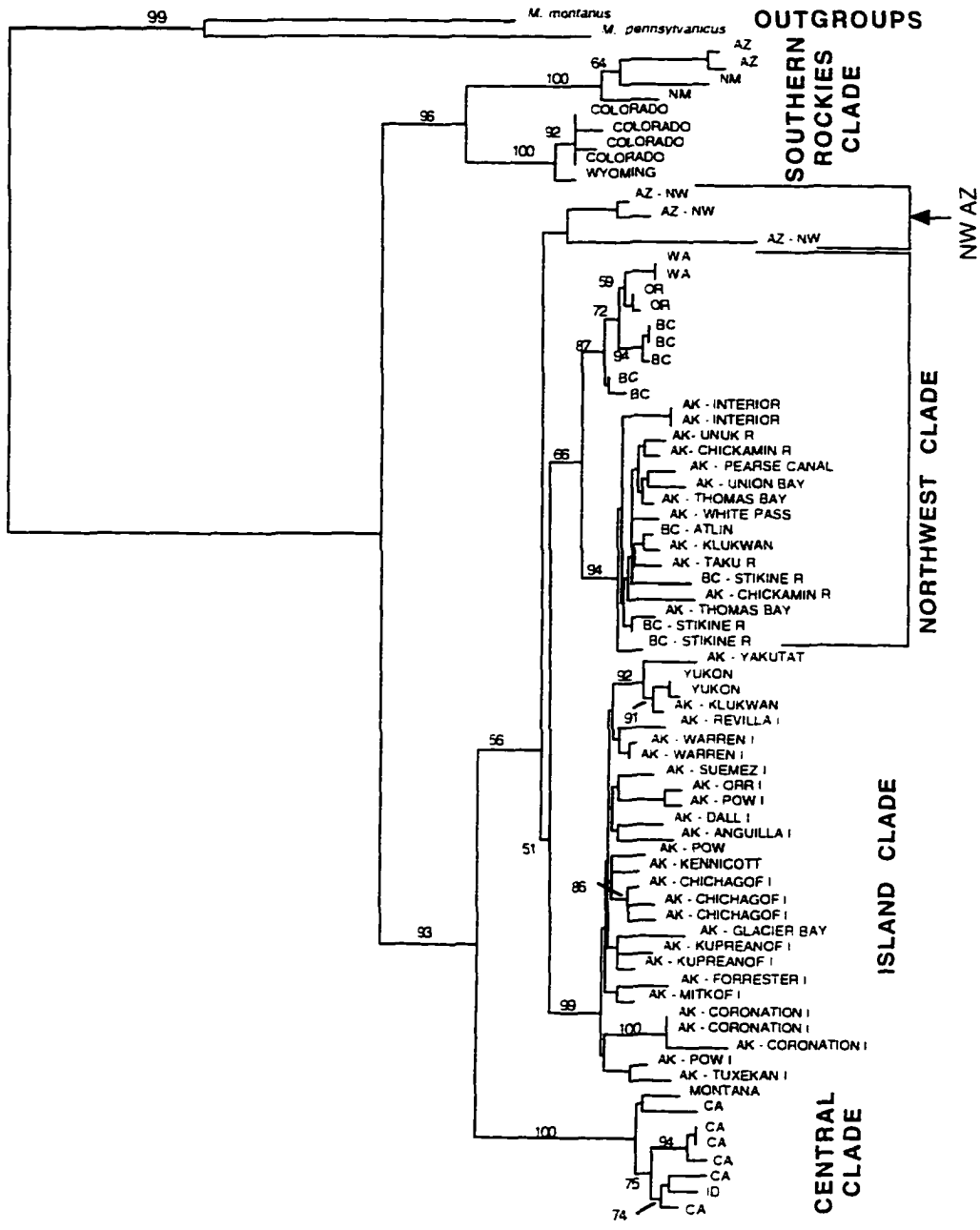


**Fig. 2** Neighbor-joining phylogram based on Kimura (1980) two-parameter distance from complete cyt b sequences. Values above or adjacent to branches indicate bootstrap percentages from 5.000 iterations.

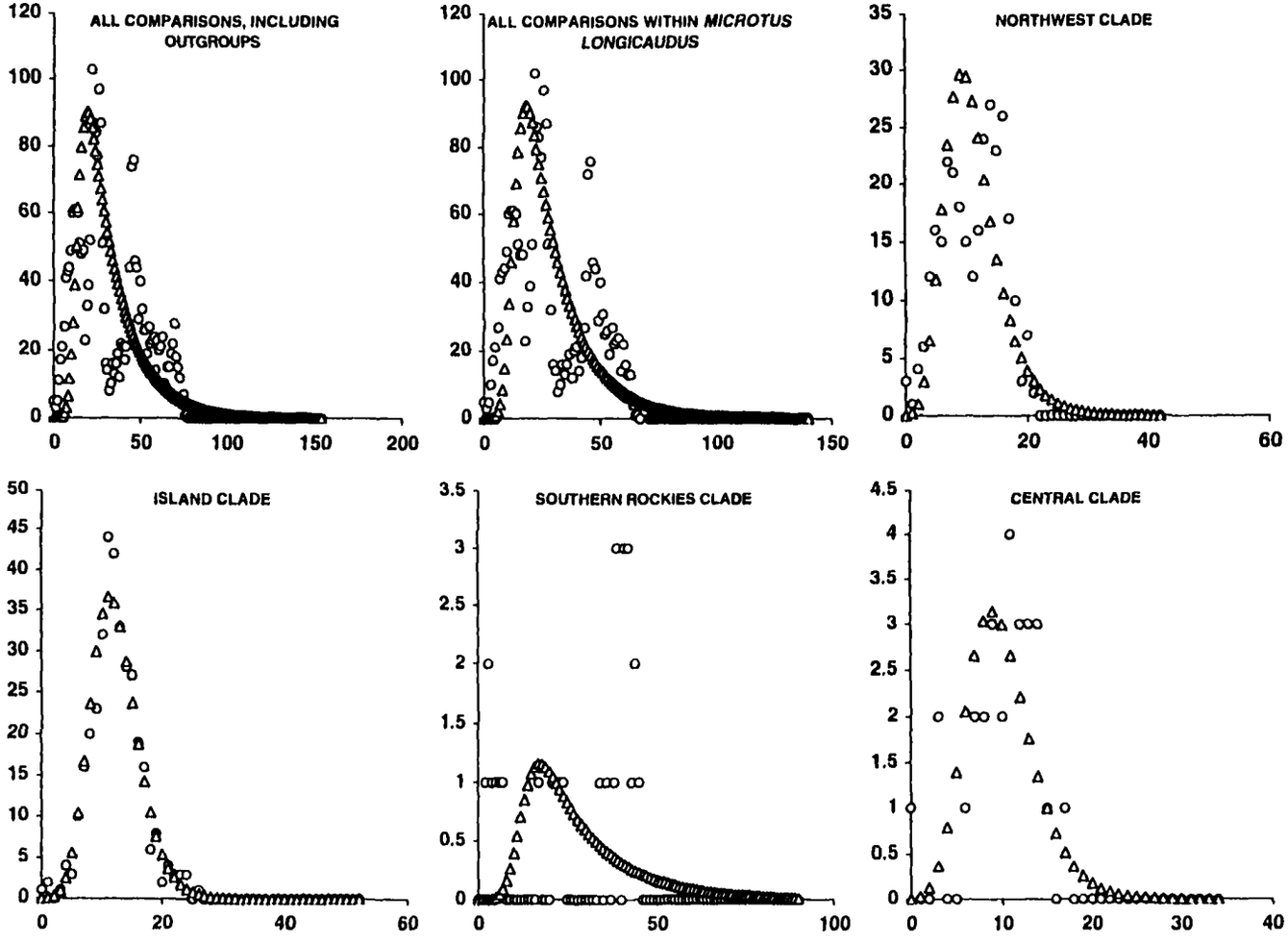


**Fig. 3** Charts of pairwise analysis. The Y axis is the number of nucleotide differences between any pair of sequences and the X axis is the number of times that amount of difference was found in that set of sequences. Circles indicate observed differences and triangles indicate predictions under Roger's (1995) expansion model.

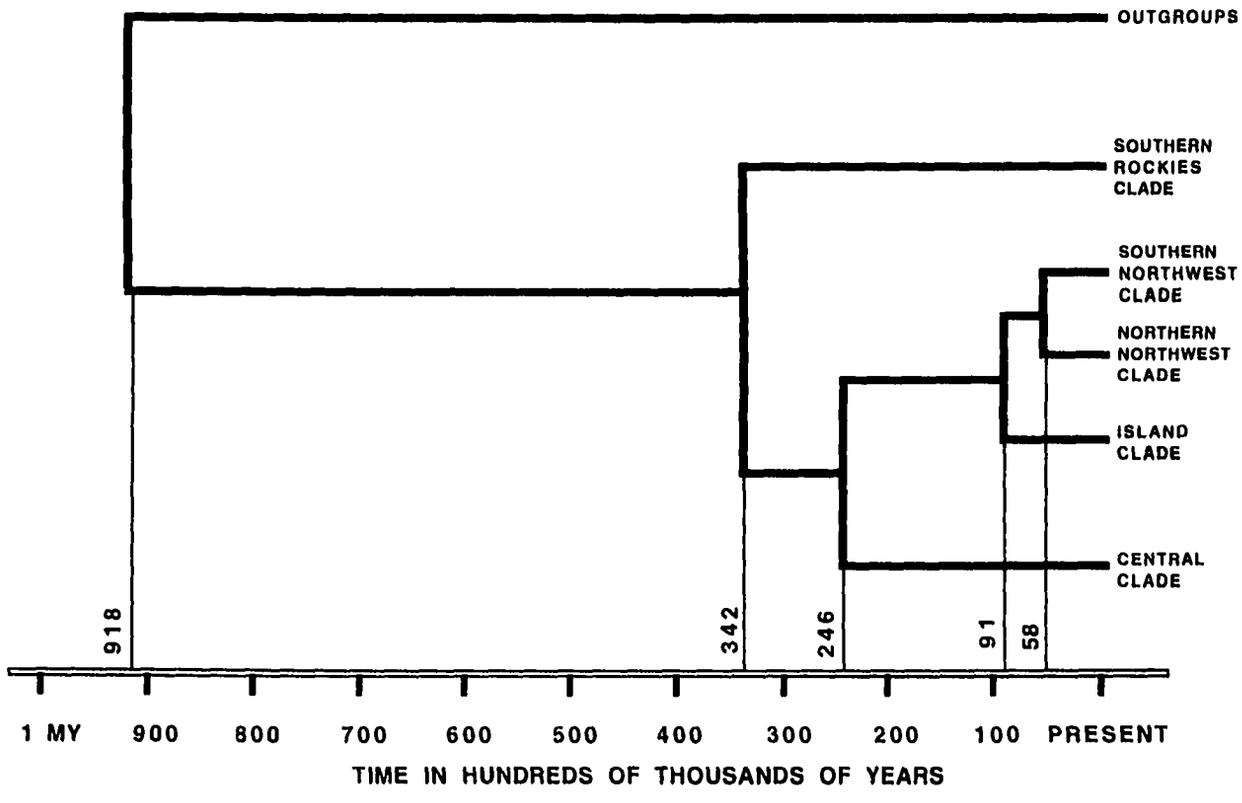


**Fig. 4** Timescale of net divergence between clades. Date is calibrated at 2.2 million years between *Microtus gregalis* and *M. oregoni*, using an HKY85 + G distance model. Average within clade diversity was subtracted from average distance between two clades. See Table 2 for gross divergence.

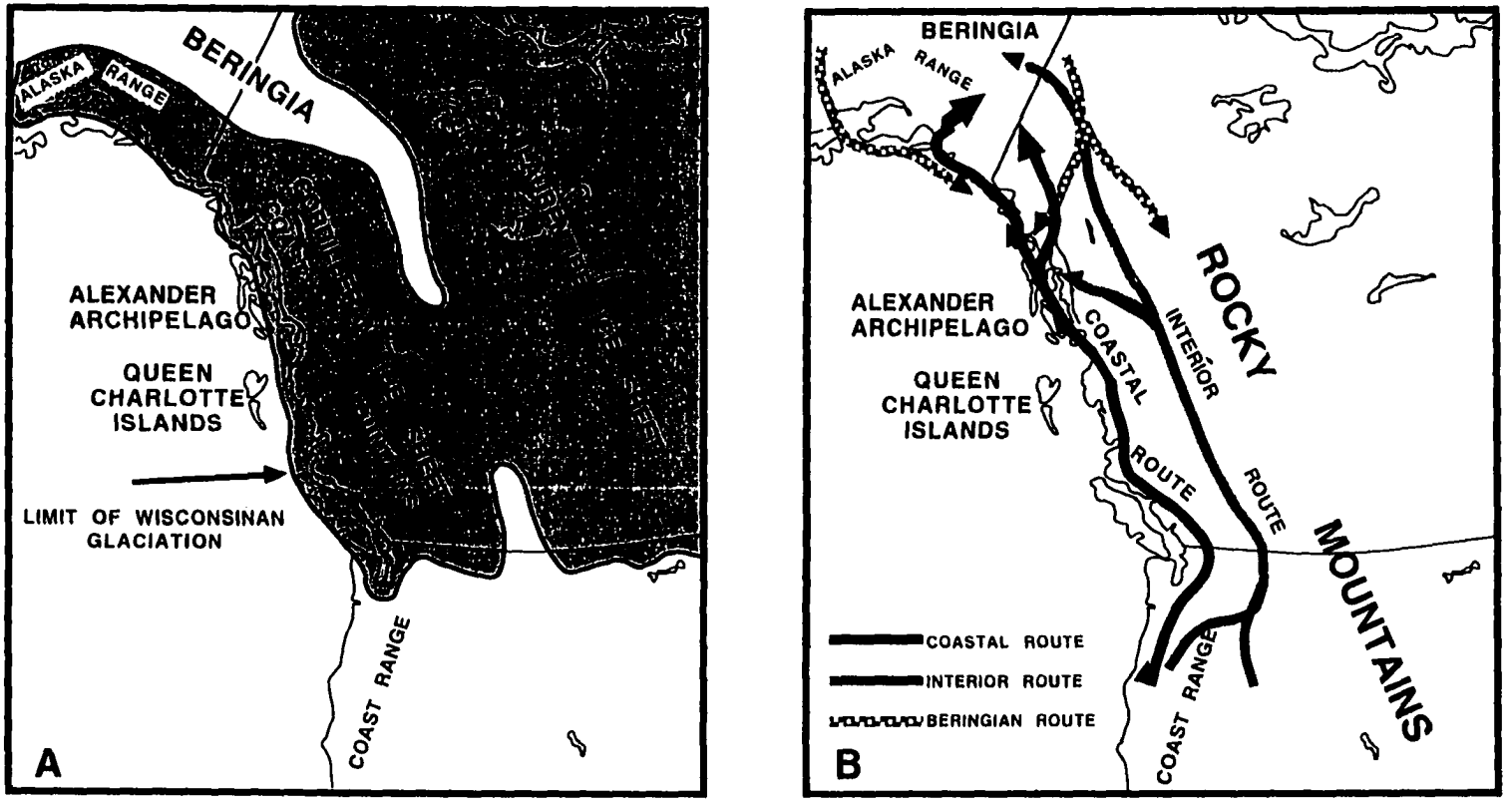


Fig. 5 A) Limits of peak Wisconsinan glaciation. The limits of the ice sheets have not yet been clearly determined, nor has the timing of deglaciation in many areas (Mann and Hamilton 1995, Mandryk 1996). The ocean depth around southeastern Alaska during peak glaciation and during deglaciation is unknown, but exposed surfaces may have supported refugia or permitted early post-glacial invasion. B) possible routes of post-glacial recolonization from the south along the coast, or through the interior, or southward from Beringia

# Chapter 4

## The Phylogenetic Position of Southern Relictual Species of *Microtus*[4].

Abstract:

The most southern species of *Microtus* (*M. guatemalensis*, *M. oaxacensis*, *M. quasiater*, and *M. umbrosus*) may be relics, isolated in the mountains of Mexico and Guatemala by the warming phase at the end of the Pleistocene. To investigate their biogeographic history, we used parsimony and likelihood analyses of complete mitochondrial cytochrome b gene sequences of 28 species of *Microtus*, including several Eurasian species, holarctic *M. oeconomus*, and all extant North American species. North American *Microtus* were monophyletic under the maximum-likelihood criterion, but paraphyletic under parsimony. Likelihood ratio tests and bootstrapping indicated a rapid basal radiation in this group with apparently short intervals between cladogenic events. However, many sister taxon relationships (e.g. *M. quasiater* and *M. pinetorum*) were robust to bootstrapping or were consistent between methods. We found that *M. quasiater* was sister to *M. pinetorum*, as previously predicted from morphology, and these taxa were sister to a clade of *M. oaxacensis* and *M. guatemalensis*. The

[4] Conroy, C. J., J. A. Cook, Y. Hortelano, and F. Cervantes. In prep. The phylogenetic position of southern relictual species of *Microtus*. Canadian Journal of Zoology.

phylogenetic position of *M. umbrosus*, however, was unclear. Monophyly of the southern relics was rejected by a likelihood ratio test. The paraphyly of the southern relics suggests multiple invasions. Conservation strategies for these mountain top relics should incorporate phylogenetic data for other co-distributed taxa since the relationships among other taxa may not be apparent from current taxonomy.

## INTRODUCTION

The mountains of Mexico and Guatemala host a highly endemic flora and fauna (Ramamoorthy et al. 1993) including a diverse set of organisms associated with mesic environments that apparently invaded the region during cooler periods and then became isolated at higher elevations as conditions became drier and warmer. This invasion and isolation cycle may have occurred numerous times during the Pleistocene and has led to a complex biogeographic history (Sullivan et al. 1997).

The genus *Microtus* is primarily holarctic in distribution and reaches its southern limit in Central America. At higher latitudes, up to five species (e.g. *M. longicaudus*, *M. miurus*, *M. oeconomus*, *M. pennsylvanicus*, and *M. xanthognathus* in Yukon Territory) may be found in close proximity. However, species tend to be more allopatrically distributed at southern latitudes. For example, *M. guatemalensis*, *M. oaxacensis*, *M. quasiater*, and *M. umbrosus* are endemic to separate mountains in the cloud and pine forests of Mexico and Guatemala (Fig. 1). *Microtus quasiater*, the Jalapin vole, is found

in the more southern parts of the Sierra Madre Oriental in Central Mexico. *Microtus oaxacensis*, the Tarabundi vole, is isolated in the Sierra de Juarez of Oaxaca, but ranges in elevation from 1,600 (Sanchez et al. 1996) to 2,499 meters (Jones and Genoways 1967). *Microtus umbrosus*, the Zempoaltepec vole, is restricted to approximately 80 $km^2$ at elevations ranging from 1,829 to 3,000 meters (Frey and Cervantes 1997) around Mt. Zempoaltepec (type locality 8,200 feet) in the mountains of Oaxaca. *M. guatemalensis*, the most southern of the relics, occurs from the mountains of central Chiapas south to central Guatemala. These species may be the result of peripheral isolation of ancestors that were more widely distributed during cool periods of the early to middle Pleistocene (Hoffmann and Koeppl 1985).

In contrast to the four highly restricted species, *M. mexicanus* is widespread in Mexico and occurs in limited sympatry or parapatry with *M. oaxacensis*, *M. quasiater*, and *M. umbrosus* with a disjunct range extending from New Mexico and Arizona to southern Mexico (Hall 1981). Its fossil record is limited to the late Wisconsinan and is restricted to San Josecito in northeastern Mexico and localities farther north (Zakrzewski 1985). Phylogenetic relationships between *M. mexicanus* and other species of *Microtus* are unclear, but there is little indication that this species shares a common ancestor with other Meso-American species. Thus, its presence in Mexico probably reflects an independent invasion.

We expand the investigation of holarctic *Microtus* phylogeny reported earlier (Conroy and Cook, submitted) by testing the monophyly of four low latitude relics, *M. guatemalensis*, *M. oaxacensis*, *M. quasiater*, and *M. umbrosus*. While monophyly of these possibly relictual survivors would indicate a single early invasion, paraphyly would suggest multiple invasions. Timing of invasions might be ascertained through examination of relative depth of divergence and branching order among clades. Although correlating depth of genetic divergence with absolute time of isolation is problematic, we propose to compare branch lengths among species pairs within this genus to estimate relative times of divergence for the southern relics relative to other "temporally calibrated" divergence points within the genus. Finally, we propose to evaluate whether these species were likely to have been isolated simultaneously by a single climatic event, in which case we would expect a polytomy or very short internodal branch lengths among species (Zink and Blackwell 1998).

## MATERIALS AND METHODS

DNA was extracted from ethanol-preserved tissues of these four southern *Microtus* (Table 1) with methods described in Conroy and Cook (in press). The cytochrome b gene (hereafter cyt b) was amplified in three sections and sequenced in both directions (Perkin-Elmer Prism® dye terminator kit [Fst-RR, 402119]) on an ABI 373 automated sequencer. We included cyt b sequences from 24 species of *Microtus* and two

species of *Clethrionomys* (Conroy and Cook in press, submitted). In the phylogenetic analysis we represented each species with a single individual, but we examined intraspecific variation where possible by including multiple representatives for 24 of the 28 species (data available from authors). We used unweighted parsimony (MP) and maximum likelihood (ML) with the software PAUP* (Swofford 1998). We estimated parameters of simple to more complex likelihood models (JC [Jukes and Cantor 1969], HKY85 [Hasegawa et al. 1985], and GTR [Yang 1994a], and the latter two with gamma distributed among-site rate variation [Yang 1994b]) and tested among them with likelihood ratio tests that are chi-square distributed. As tests of node strength, we bootstrapped the parsimony analysis 1,000 times and used 1,000 random addition sequences to locate multiple tree islands. We tested several alternative phylogenetic topologies against the ML tree (Kishino and Hasegawa 1989): 1) monophyly of the four relictual Meso-American species, and 2) the four shortest maximum parsimony trees. Besides testing relationships among these new taxa, several tests were conducted to ascertain the effects of adding these four southern species to more general hypotheses concerning the systematics of *Microtus* previously conducted (Conroy and Cook submitted). These included: 1) monophyly of subgenus *Stenocranius* (Rausch 1964), 2) monophyly of the "*M. pennsylvanicus*" clade of taiga voles (*M. pennsylvanicus, M. montanus, M. townsendii, M. canicaudus*, Hoffmann and Koeppl 1985), 3) monophyly of

a second taiga vole clade (*M. richardsoni, M. xanthognathus, M. chrotorrhinus*), and 4) monophyly of all North American taiga voles (Hoffmann and Koeppl 1985).

## RESULTS

Base pair composition of the cyt b gene was similar to other *Microtus* (Conroy and Cook submitted) as well as most other mammals (Irwin et al. 1991). Of the 1143 base pairs, 472 were variable across 28 species of *Microtus* and two species of *Clethrionomys*. When outgroups were excluded, 460 sites were variable. Of these, 100 were in the first position, 23 in the second position, and 337 in the third position of codons. Of the 381 amino acids, 76 (20 %) were variable across species of *Microtus* and the replacement pattern was consistent with structural models (e.g. Irwin et al. 1991). There were 351 parsimony informative nucleotide sites and $g_1$ statistics ($g_1$ = -0.325) indicated phylogenetic signal in the data.

Maximum parsimony searches recovered four equally parsimonious trees (Fig. 2), each including a basal clade of *M. ochrogaster*, a North American species, and *M. gregalis*, an Asian species. A clade of *M. oeconomus, M. middendorffi, M. montebelli, M. kikuchii* and *M. fortis* (hereafter the "Asian clade"), and the *M. pennsylvanicus* clade were present in the four trees. The branch leading to a sister relationship between *M. pinetorum* and *M. quasiater* was in all trees, had high bootstrap support (99 %) and relatively high decay values (12). This relationship has been predicted based on

morphology. *M. oaxacensis* and *M. guatemalensis* were sister taxa in three of four trees, but the branch leading to this pair had weak bootstrap support (< 50 %) and a decay index of zero. Other clades were found in three or four of these shortest trees, but bootstrap support was generally low across basal relationships.

The HKY85 + Γ likelihood model (transition/transversion ratio = 3.4, $\alpha = 0.213$) was chosen since more complex models (e.g. GTR + Γ) were not significantly more likely but produced the same topology (not shown). As in other studies (e.g. Sullivan et al. 1997), the addition of the gamma-distributed rate parameter contributed significantly to model likelihood. This model produced one tree (Fig. 3) in which *Microtus gregalis* was basal, followed by the Asian clade. North American endemic species formed a monophyletic clade and the European species formed a sister clade to North American species. The Meso-American endemics were not basal within the clade of North American species. Three Meso-American species (*Microtus guatemalensis*, *M. oaxacensis*, and *M. quasiater*) displayed the same branching pattern as in the parsimony trees, while *M. umbrosus* was sister to *M. chrotorrhinus*. Other relationships were similar to previous analyses (Conroy and Cook submitted). For example, the *M. pennsylvanicus* and Asian clades were consistently supported and *M. mexicanus* was sister to *M. californicus*. In likelihood ratio tests (Table 2), few of the alternate topologies could be rejected. However, one of the MP trees and two trees obtained from a ML search constrained to monophyly of the four southern species were rejected.

Relative depth of divergence was estimated with pairwise likelihood differences between taxa estimated under the same model used for the ML phylogeny. *M. abbreviatus* and *M. miurus*, which were probably split at the end of the Pleistocene when rising sea levels in the Bering Strait isolated *M. abbreviatus* on islands, differ by 0.015. Another late Pleistocene split may be *M. canicaudus* and *M. townsendii* which differ by 0.058. Another pair considered closely related include *M. arvalis* and *M. rossiaemeridionalis* (0.072), while two widespread sister taxa, *M. montanus* and *M. pennsylvanicus*, differ by 0.086. Divergence between the southern species (*M. quasiater* and *M. pinetorum* [0.094], *M. guatemalensis* and *M. oaxacensis* [0.113], and *M. chrotorrhinus* and *M. umbrosus* [0.137]) is deeper than any of the preceding examples.

## DISCUSSION

Previous investigations into the history of microtines suggested an evolutionary history closely tied to fluctuating boreal ecosystems (Hoffmann and Koeppl 1985). The biogeographic history of species of *Microtus* in Mexico and Guatemala has been enigmatic. The elevational distribution suggests expansion and contraction of cool, moist forests may have resulted in isolation in relatively small areas. The paucity of synapomorphic characters, but apparent abundance of autapomorphic characters has led to their characterization as ancient, highly divergent species. This paper reconsiders the biogeographic and evolutionary history of the southern relics in light of molecular

characters examined within a wider taxonomic sampling (28 species) for the genus. These southern species lack a fossil record, which might provide minimum estimates for their age.

**Biogeography**

The four southern species of *Microtus* are in two clades: three species are closely related to *M. pinetorum*, while the fourth is a sister taxon to *M. chrotorrhinus*. Our data do not support the hypothesis that species of *Microtus* restricted to the mountains of Mexico and Guatemala may be relics of an invasion of North America prior to other invasions leading to other species at higher latitudes (Hoffmann and Koeppl 1985). These species were not basal in the North American clade, nor were they monophyletic. However, interspecific distances suggest they may have speciated earlier than some other more northern pairs, particularly late Pleistocene peripheral isolates (e.g. *M. abbreviatus* and *M. miurus*). The split between North American *Clethrionomys gapperi* and Eurasian *C. glareolus*, 0.079, occurred about the time their common ancestor invaded North America. This suggests *Microtus* may have been present and fragmented into isolated populations in Mexico and Guatemala prior to the invasion of North America by *Clethrionomys*, which may have occurred during the early Pleistocene (Repenning et al. 1990). Although the rapid basal radiation in *Microtus* obscures relationships, a topology

constrained for monophyly of these southern species was significantly worse than the ML topology. This supports two invasions by *Microtus* into the southern latitudes.

Sullivan et al. (1997) found that the Isthmus of Tehuantepec was a strong geographic barrier for the *Peromyscus aztecus* group in Mexico and Guatemala. They summarized divergence in other taxa (see Ramamoorthy et al. 1993) and recommended that their phylogeography be tested with co-distributed taxa. No species of *Microtus* is distributed across the Isthmus of Tehuantepec. However, *M. guatemalensis*, which is south of the barrier, is sister to *M. oaxacensis*, found north of the isthmus. Their common ancestor may have been distributed across this barrier with northern and southern populations subsequently diverging. This difference in the depth of divergence around the Isthmus of Tehuantepec (e.g., intraspecific in *Peromyscus*, interspecific in *Microtus*) suggests that although *Microtus* and *Peromyscus* are widely sympatric, they apparently responded to Pleistocene climatic fluctuations differently. The Isthmus of Tehuantepec may have isolated populations of *Microtus* much earlier than populations of *Peromyscus*. Although few studies have addressed the significance of the Isthmus of Tehuantepec as a barrier, the great diversity in mammal species in the region indicates there is ample material to test these hypotheses.

**Systematics**

*Microtus quasiater* is a member of the subgenus *Pitymys* (Musser and Carleton 1993) and shares dental morphology with extinct *Microtus* (*Pitymys*) *meadensis*, a widespread species of mid-Pleistocene North America and Mexico (Repenning 1983). Morphological characters and DNA sequences are congruent in placing *M. quasiater* as sister to *M. pinetorum* (Musser and Carleton 1993). *M. quasiater previously* was considered sister to *M. ochrogaster* (Moore and Janecek 1990) in an allozyme study, but only nine of 21 North American species (Musser and Carleton 1993) and no Palearctic species were examined.

The evolutionary relationships of *M. guatemalensis* and *M. oaxacensis* have not been addressed in detail, although they are thought to be relatively divergent from one another (Musser and Carleton 1993). *Microtus guatemalensis* is in the monotypic subgenus *Herpetomys*, but may have affinities with *Pitymys* (Martin 1987). There is little evidence to suggest *Herpetomys* should be a distinct genus, as indicated by its original description, or that it is related to *Phenacomys*, as suggested by Hinton (1926). The relationship between *M. oaxacensis* and other species is obscure (Musser and Carleton 1993), but it too has been considered a part of an early pitymyine invasion (Hoffmann and Koeppl 1985, Martin 1974). Though no details were given, Jones and Genoways (1967:320) noted that *M. oaxacensis* "resembles *quasiater* in external features." A widespread ancestor (e.g. *M. meadensis*) may have given rise to the clade consisting of *M.*

*pinetorum* and *M. guatemalensis*, *M. quasiater*, and *M. oaxacensis*, prior to peripheral isolation in the eastern deciduous forests and southern cloud forests (Hoffmann and Koeppl 1985). The branching order suggests isolation occurred first between an ancestor of *M. pinetorum-M. quasiater* and an ancestor of *M. guatemalensis-M. oaxacensis*. A later cladogenic event split each of these pairs. The latter pair may have been split after invasion across the Isthmus of Tehuantepec, while the former pair may have diverged following an interglacial-aged episode of range retraction.

The sister relationship between *M. umbrosus* and *M. chrotorrhinus* was unexpected because they are not similar morphologically. *Microtus umbrosus* is the sole member of the subgenus *Orthriomys* (Musser and Carleton 1993) and has been considered a relic from an early invasion from Asia during the mid-Pleistocene by the extinct *Phaiomys* (Martin 1987). Though previously considered closely related to *M. xanthognathus* (Hall and Kelson 1959), *M. chrotorrhinus* was later differentiated based on chromosomal complement (Rausch and Rausch 1974). The lack of similarity between *M. chrotorrhinus* and *M. umbrosus* and the "pitymyine" species suggests an independent invasion of the southern latitudes by a common ancestor of *M. umbrosus* and *M. chrotorrhinus*.

We included 28 of approximately 65 species of *Microtus* (Musser and Carleton 1993), but the addition of more taxa may lead to more accurate phylogenies (Hillis 1996). The addition of the four taxa in this study did not significantly alter the ML topology

previously obtained with only 24 species of *Microtus* (Conroy and Cook submitted). The North American species and the Asian and *M. pennsylvanicus* clades remained monophyletic. Several constraints previously tested (Conroy and Cook submitted) were consistent with the ML tree (e.g. North American monophyly, *M. pennsylvanicus* group monophyly), and are not reported. Also, *M. richardsoni* became basal to a clade of *M. californicus* and *M. mexicanus*. The expanded analysis suggested two differences based on likelihood ratio tests. Without the Meso-American species, we rejected the topology ($p = 0.026$) which constrained all North American taiga voles as monophyletic (sensu Hoffmann and Koeppl 1985). However, with the inclusion of Meso-American species, we did not reject this hypothesis ($p = 0.136$). In the expanded analysis we rejected one of the four equally parsimonious trees ($p = 0.013$). That topology indicated *M. chrotorrhinus* was basal among all species of *Microtus* sampled and that *M. gregalis* (Russia) was within a clade of North American species. It is unclear whether this new arrangement will be stable with the addition of taxa and other characters.

Morphological material for interspecific comparison is abundant, but the phylogenetic utility of morphological characters such as tooth pattern has been criticized because they are too variable within and between species (Guthrie 1965, Zakrzewski 1985). Only *M. quasiater* has been included in an allozyme study (Moore and Janecek 1990) and DNA data have not previously been used. Despite the availability of standard karyotypes for these and other species of *Microtus*, chromosomes might not be

phylogenetically informative since the rates of chromosomal evolution varies greatly between species (Cervantes et al. 1997. Modi 1987). Indeed. our phylogeny suggests a complicated series of events are needed to explain chromosomal rearrangements in *Microtus* (Fig. 2). Species with low fundamental numbers are not sister to each other. For example. *M. oaxacensis* (2N = 30) is sister to *M. guatemalensis* (2N = 52). and *M. canicaudus* (2N = 24) is sister to *M. townsendii* (2N = 50). This supports Cervantes et al.'s (1997) contention that chromosomal evolution may be independent of phylogenetic history in *Microtus*.

**Conservation**

Mexico has one of the richest mammalian faunas on Earth partially because it shares both elements of neotropical and nearctic biomes (Fa and Morales 1993). Conservation efforts for this rich fauna are complicated by the diversity of the fauna and the variety of threats (Ceballos and Navarro L. 1991). Protection of the mountains of Oaxaca, a region of high mammalian diversity (Arita et al. 1997), would impact the three relics, *M. oaxacensis*, *M. umbrosus*, and *M. quasiater*. *M. guatemalensis* is found in Chiapas and south into Guatemala. Whether conservation criteria focus on rarity. diversity, or levels of endemism these southern relics and their habitats warrant conservation concern.

Molecular systematic studies of other endemic taxa should be considered in planning conservation efforts in this region (Baker et al. 1995). Our analysis suggests that temporal scales may be a crucial component to interpreting the significance of biogeographic barriers. Barriers apparent for recently diverged taxa may be less apparent for taxa with deeper histories. Detection of patterns at different temporal scales could help resolve shared histories of taxa in the region (Avise 1994) and be used to manage for historical associations of flora and fauna.

## CONCLUSIONS

Species of *Microtus* in Mexico and Guatemala are not monophyletic but instead are the result of at least two invasions of this region during the Pleistocene. One invasion may have been by the ancestors of *M. oaxacensis*, *M. quasiater*, and *M. guatemalensis*. Another invasion might have occurred by the ancestor of *M. umbrosus*. A third invasion might have led to the distribution of *M. mexicanus*. A lack of fossils inhibits dating cladogenesis among these species, however, depth of divergence relative to other splits within *Microtus* suggest mid-Pleistocene divergence. Morphological similarity between some species (e.g. *M. pinetorum* and *M. quasiater*) and formerly widespread (mid to late Pleistocene) taxa that are now extinct (e.g. *M. meadensis*) suggest isolation by range retraction is a viable hypothesis. Morphological studies support the shared history of

several of the pitymyine species. The sister relationship between *M. umbrosus* and *M. chrotorrhinus* was not predicted and suggests it should be tested further. Phylogenetic analysis of other organisms of the region should be used to identify regions of endemism and significant biogeographic barriers.

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Table 1. Specimens of Meso-American species of *Microtus* examined in this study (in addition to those reported in Conroy and Cook [submitted]).

| Species | Collection location | UNAM Museum Catalog Number (collector number) |
|---|---|---|
| *M. umbrosus* | Mexico: Oaxaca Cerro Zempoaltepetl, 5 Km N Sta Ma. Yacochi. Mpio. Tlahuitoltepec, 2450 m. | 34890 (JMV 1460). 34894 (JMV 1466). |
| *M. guatemalensis* | Mexico: Chiapas: Cerro Tzontehuitz, 13 Km NE San Cristobal de las Casas, Mpio Chamula, 2880 m. | 35262 (JMV 1590). |
| *M. oaxacensis* | Mexico: Oaxaca: 11 km SW La Esperanza, Mpio. Santiago Comaltepec, 2000 m.; Oaxaca: 11 Km SE La Esperanza, Mpio. Santiago Comaltepec, 1000 m. | 27415 (JMV 277). 33815 (JMV 1390). |
| *M. quasiater* | Mexico: Veracruz: 5 Km. W Naolinco, Mpio. Naolinco, 1650 m. | 35282 (YHM 295). 35274 (YHM 279). |

Table 2. Kishino-Hasegawa likelihood ratio tests of tree topologies. The "*Stenocranius*" constraint forced *M. miurus*, *M. gregalis*, and *M. abbreviatus* to be monophylyetic. The "Second taiga vole" constraint enforced *M. xanthognathus*, *M. chrotorrhinus*, and *M. richardsoni* monophyly. The "All taiga voles" constraint enforced the "Second taiga vole" species with *M. pennsylvanicus*, *M. montanus*, *M. townsendii*, and *M. canicaudus* monophyly. The "Meso-Americans" constraint enforced *M. guatemalensis*, *M. oaxacensis*, *M. quasiater*, and *M. umbrosus* monophyly. Two trees were obtained from the ML search under this last constraint. The parsimony trees were derived from unweighted heuristic searches with ten random-addition replicates.

| Tree | Constraint | -lnL | Diff- lnL | s.d. (diff) | T | P* |
|---|---|---|---|---|---|---|
| 1 | None | 9868.811 | (best) | | | |
| 2 | *Stenocranius* Monophyletic | 9891.258 | 22.447 | 16.723 | 1.342 | 0.180 |
| 3 | Second taiga vole clade | 9879.176 | 10.365 | 9.080 | 1.142 | 0.254 |
| 4 | All Taiga voles Monophyletic | 9897.044 | 28.233 | 18.904 | 1.494 | 0.136 |
| 5 | Meso-Americans monophyletic I | 9910.346 | 41.535 | 11.613 | 3.577 | 0.0004* |
| 6 | Meso-Americans monophyletic II | 9910.346 | 41.535 | 11.613 | 3.577 | 0.0004* |
| 7 | Parsimony Tree 1 | 9908.109 | 39.298 | 22.586 | 1.740 | 0.082 |
| 8 | Parsimony Tree 2 | 9908.743 | 39.932 | 20.492 | 1.949 | 0.052 |
| 9 | Parsimony Tree 3 | 9908.493 | 39.682 | 20.581 | 1.928 | 0.054 |
| 10 | Parsimony Tree 4 | 9919.839 | 51.027 | 20.528 | 2.486 | 0.013* |

* Significant at $P < 0.05$

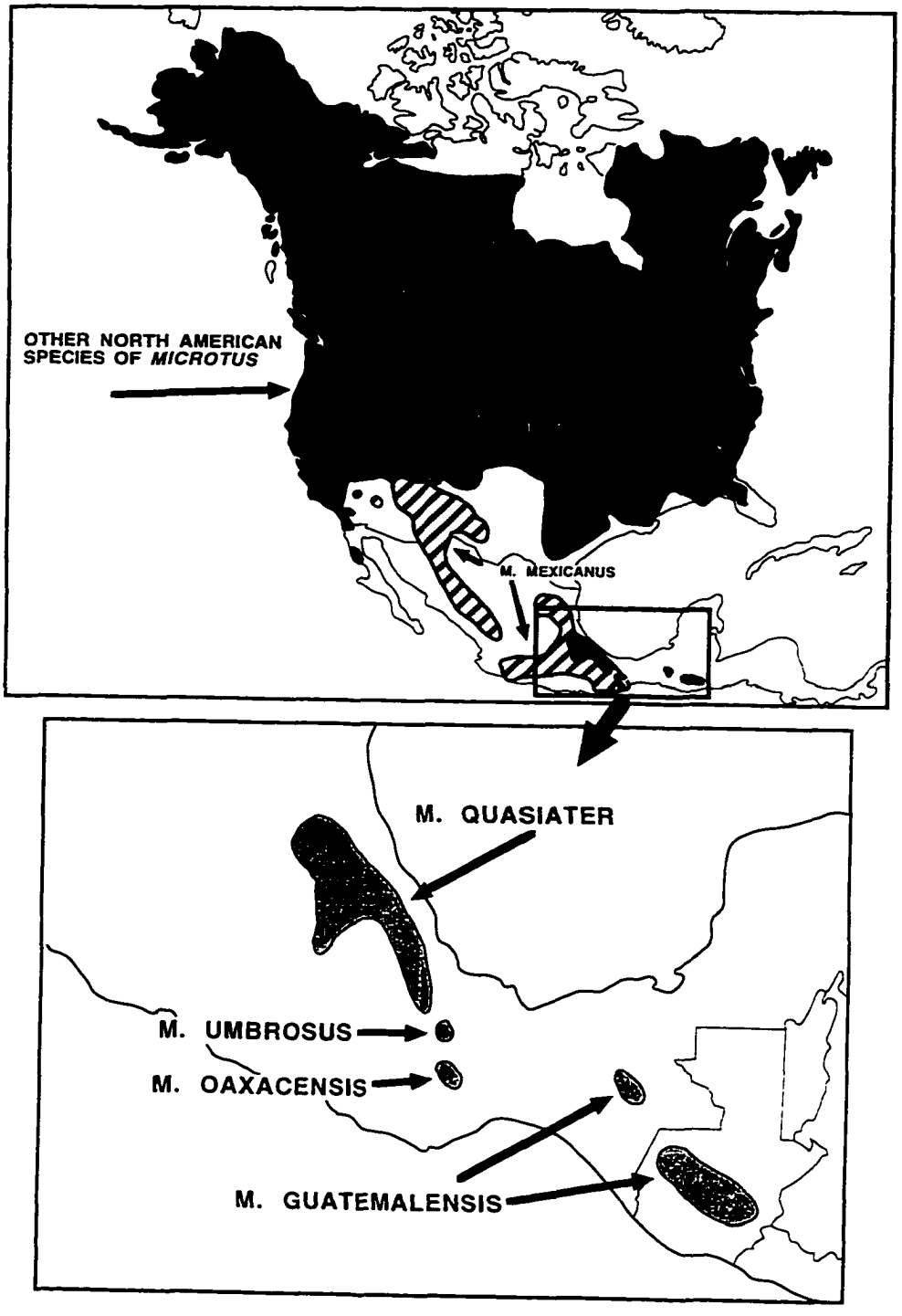


Fig. 1. Map of the distribution of species of *Microtus* in North America and into Guatemala (redrawn from Hoffmann and Koeppl 1985).

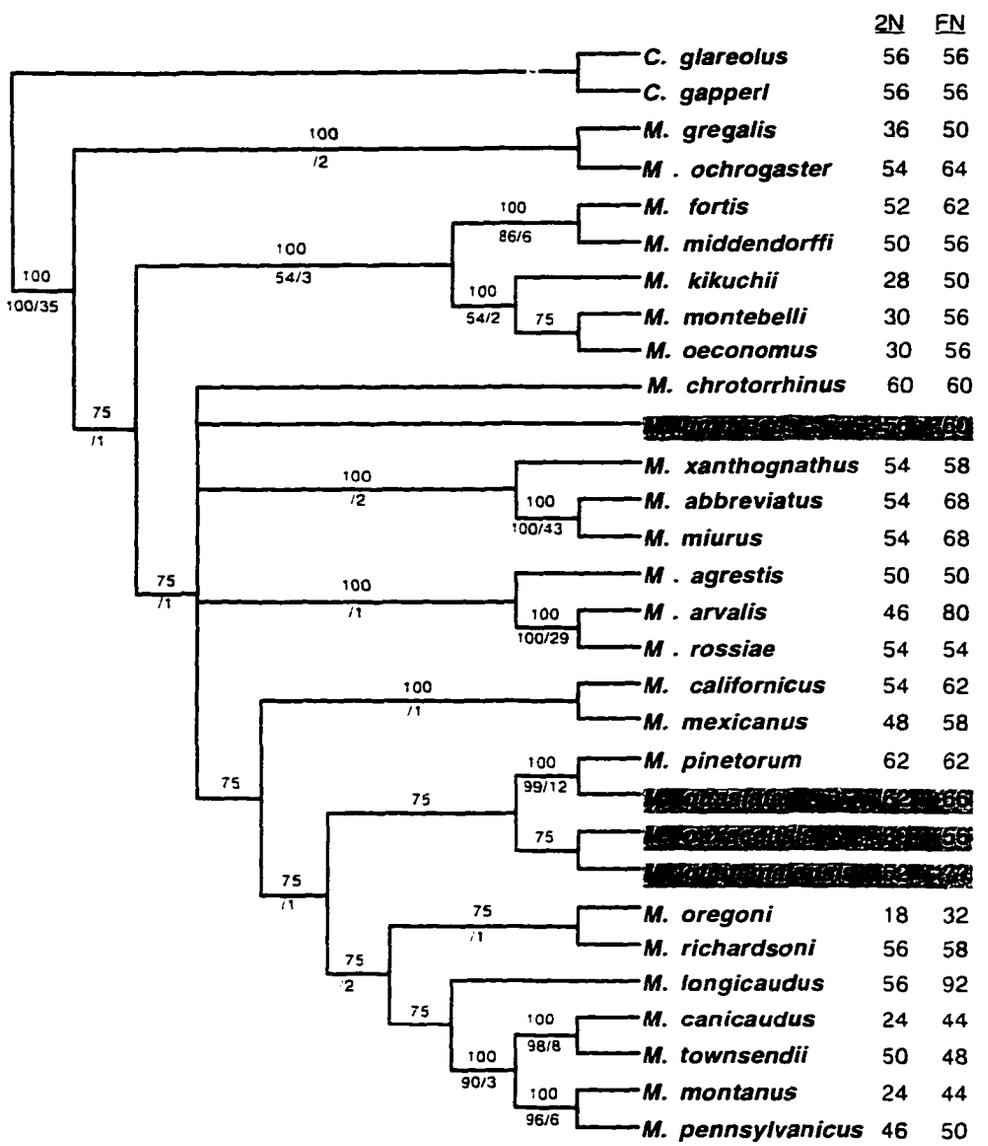


Fig. 2. Summary of MP searches. Values above branch are the percentage of equally parsimonious trees that share that relationship (e.g., 75 = three of four trees). Values below branchs to the left of slash are the percent of 1,000 bootstraps, simple addition of taxa: right of slash indicates decay index calculated with 10 random-addition replicates for each search. Diploid (2N) and fundamental (FN) numbers are from Cervantes et al. (1997), Matthey (1957), Modi (1985, 1986), and Zagorodnyuk (1990).

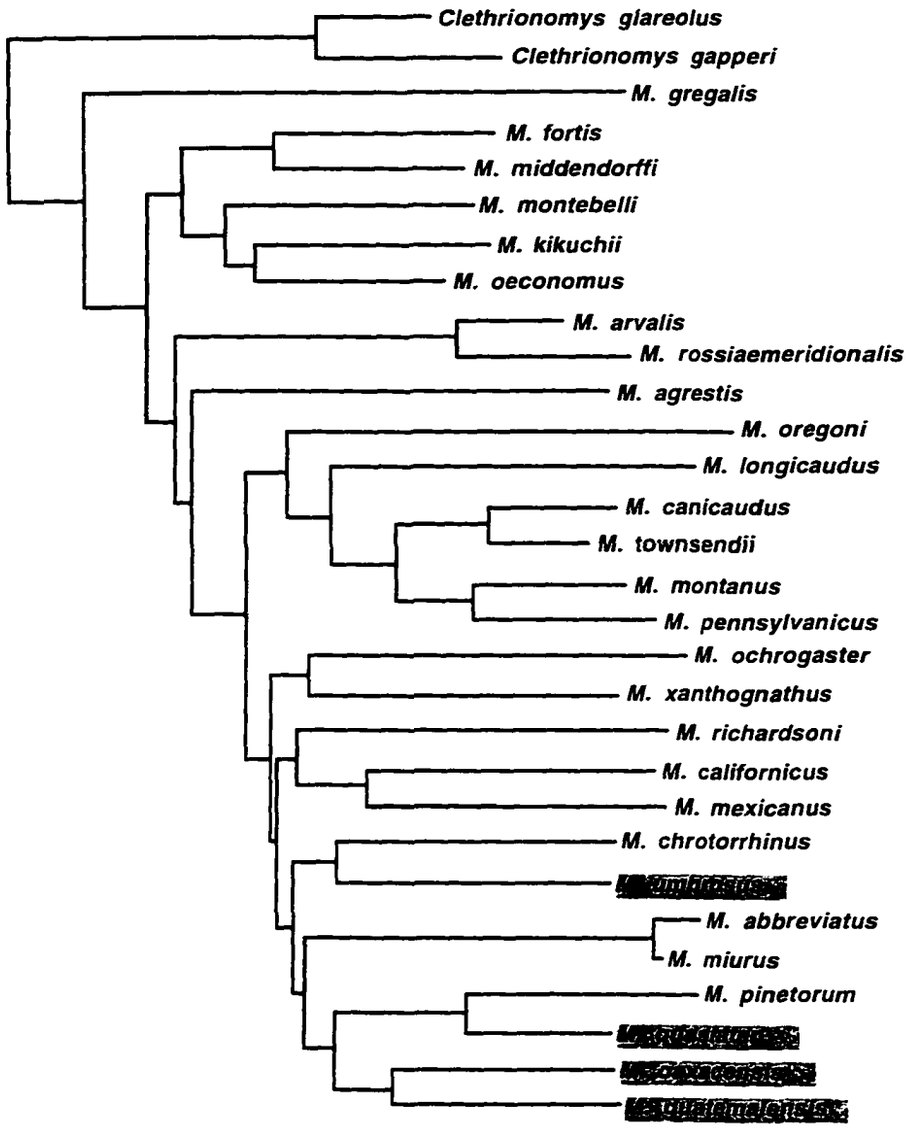


Fig. 3. Maximum likelihood phylogenetic tree based on the HKY85 + Γ model (see text for parameter values).

# XI. CONCLUSIONS:

These molecular genetic studies, summarized in the preceding chapters, supported radiations within the arvicolines at the levels of genera (Chapter 1) and species within *Microtus* (Chapters 1, 2, 4; Chaline and Graf, 1988). A pulse among murid subfamilies was supported, but taxonomic and character sampling for this level remains limited. Other studies also support a rapid radiation at this level (Catzeflis et al., 1992; Engel et al., 1998; Robinson et al., 1997). Processes responsible for these pulses of diversification may include an intrinsic factor in the group's genetic background (e.g. rapid chromosomal evolution), or some extrinsic factor, such as rapid climate response (Vrba, 1992). With a more precise estimate of the relationship between molecular divergence and time, it may be possible to precisely correlate the timing of cladogenic events with environmental events, such as glacial or interglacial peaks. Unfortunately, the field of molecular phylogenetics is far from resolved on the use of molecular clocks other than as speculative tools (Johns and Avise, 1998).

Contrary to the polytomies at some higher levels, I found support for several previously postulated species groups within *Microtus* (Chapters 2, 4). This evolutionary framework provides a basis for investigating the evolution of a variety of characteristics within this group. For instance, behavior or ecology among species of *Microtus* now may be cast in a phylogenetic perspective. That is, the similarity among taxa in behavior or ecological attributes made be in part due to their evolutionary similarity.

The work presented in Chapter 2 is a step toward recovering the history of faunal interchange between the northern continents. Contrary to previous hypotheses of multiple invasions, these data support no more than two invasions of North America from Eurasia. More paleontological research in conjunction with molecular studies of the 36 Old World species not included here, as well as *Volemys*, *Blanfordimys*, *Chionomys*, *Lasiopodomys*, and *Proedromys*, would further clarify intercontinental relationships.

The results of Chapter 3 suggested major differentation in the southeastern portion of the range of *M. longicaudus* and widespread expansion in recently deglaciated regions in the north. The major split within *Microtus longicaudus* may represent incipient species and should be tested with other characters. One of my primary goals was to investigate the status of *M. coronarius*, the Coronation Island vole, from a mitochondrial DNA perspective (Chapter 3). Samples of *M. coronarius* (Coronation [n = 6], Warren [n = 2], and Forrester [n = 2] islands) were considered in perspective with larger sample sizes in the surrounding regions. Our larger sample of long-tailed voles from southeast Alaska can be referred to the subspecies *M. longicaudus littoralis* and *M. l. vellerosus*. The border between these morphologically defined taxa was described as the coast range (Osgood, 1900; Swarth, 1922, 1933) with some morphological overlap in the Haines and Juneau area. However, as discussed in Chapter 3, the cyt b data suggested that mainland populations south and east of Haines are distinct from nearby island populations.

Populations of *M. coronarius* originally were elevated to species due to color and size differences (Swarth, 1911). However, the status of *M. coronarius* has been called into question (e.g., Musser and Carleton, 1993). These mtDNA data do not distinguish individuals on Coronation, Warren, or Forrester islands. However, mtDNA may conflict with other markers (Avise, 1994) and further investigation of nuclear markers, karyology, and skeletal morphology is needed to critically evaluate the status of this taxon (Conroy and Cook, in press). Neutral nuclear alleles take approximately four times as long to fixate through drift as neutral mtDNA haplotypes. A genetically unique population may have persisted in a Northwest Coast glacial refugium. Gene flow, due to post-glacial colonization, may have led to a loss of unique mitochondrial markers in *M. coronarius*. It is also possible that *M. coronarius* is an artifact of excessive species level splitting (Anderson, 1985). This is the first systematic reanalysis of this taxon since its description. Although samples from Coronation, Warren, and Forrester islands were not distinct, samples that coincide with the subspecies *M. longicaudus littoralis* (the Island clade) appear divergent from other clades in the Pacific Northwest and are consistent with genetic isolation in a glacial refugium. These clades are not nearly as divergent as other sister species in the genus *Microtus*, but these data illustrate the effectiveness of placing previous systematic hypotheses (e.g., subspecies of *M. longicaudus*) within a phylogenetic framework.

Despite the information obtained in these chapters, these phylogenetic hypotheses remain a framework to be tested with more paleontological data and further taxon and character sampling. Recent work in molecular systematics (e.g., Hillis, 1996) suggests that intensive taxonomic sampling can improve the accurate recovery of branching topologies. Therefore, these phylogenetic and phylogeographic studies will be more accurate reconstructions of history when the sampling of species and populations is enhanced. Other markers must be considered to obtain the most robust perspective on these groups because gene trees are not necessarily species trees.

An area of molecular phylogenetics that requires more attention is the application of molecular clocks (Chapters 1 and 2). Molecular phylogenetics will benefit from the development of techniques to more reliably interpret molecular data within a temporal framework. Therefore, critical estimates of cladogenic events among arvicoline lineages will require better dates from the fossil record and better methods of correcting DNA distances.

This thesis challenges several well-recognized systematic relationships including monophyly of the Sigmodontinae, the validity of genera *Volemys* and *Alticola* and the taxonomic status of *Microtus coronarius*. However, several unexpected sister pairs (e.g., *M. umbrosus* and *M. chrotorrhinus*, *M. canicaudus* and *M. townsendii*) were also recovered. Some of these relationships conflict with previous analyses and therefore warrant additional testing with wider sampling of taxa and independent markers. Studies

based on DNA sequences can be readily expanded with the addition of taxa or other characters. However, this directly leads to the challenge of computational limits imposed by current computer software. Progress in uncovering arvicoline evolution will thus rely on 1) an expanded fossil record, 2) sampling more taxa, 3) development of independent loci, and 4) methods to address the computational limits to phylogenetic inference.

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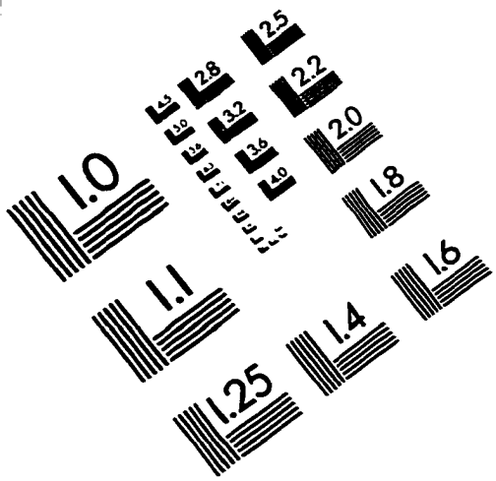


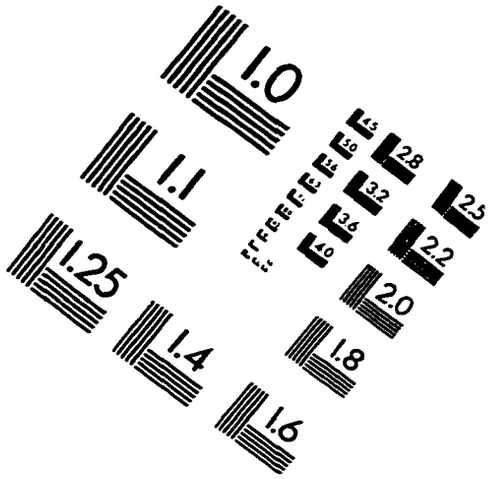


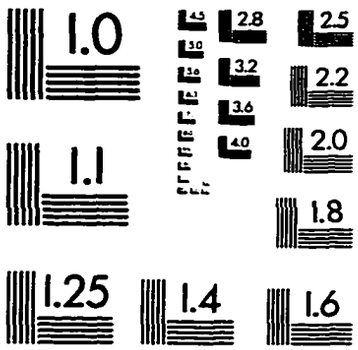


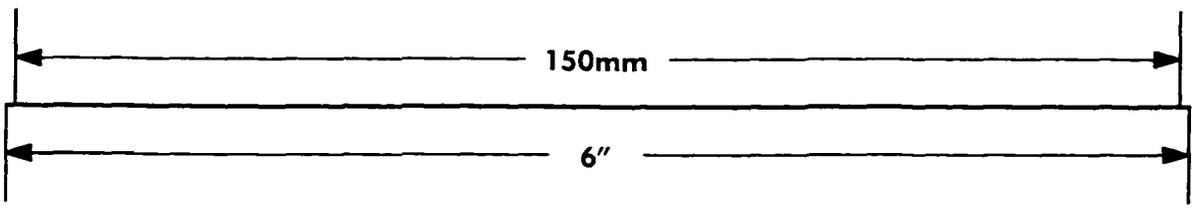


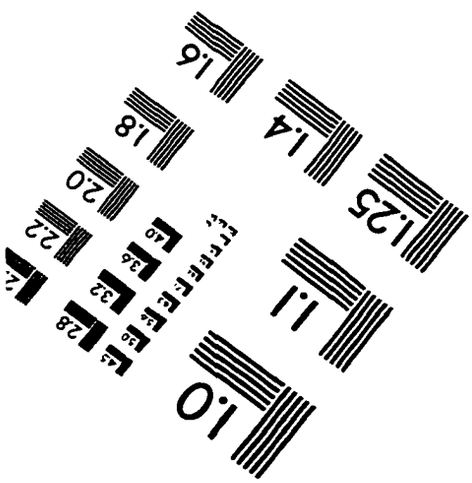


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