Journal of Mammalogy, 81(2):344–359, 2000

MOLECULAR SYSTEMATICS OF A HOLARCTIC RODENT (*MICROTUS*: MURIDAE)

CHRIS J. CONROY* AND JOSEPH A. COOK

University of Alaska Museum, 907 Yukon Drive, Fairbanks, AK 99775-6960 and
Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, AK 99775-7000
Present address of CJC: Department of Biological Sciences, Stanford University, Stanford, CA 94305-5020

The Bering Land Bridge was the intermittent connection that allowed exchange of mammals between Asia and North America. Because some mammalian genera are widely distributed on both continents, recovery of phylogenetic histories of species within these genera may help reconstruct the sequence of intercontinental exchanges. We tested phylogenetic and biogeographic hypotheses in the widespread genus *Microtus* through parsimony and likelihood analysis of mtDNA-sequence data. The extant species of *Microtus* in North America are thought to be derived from multiple invasions from Asia or, alternatively, as a single invasion followed by autochthonous speciation. Mitochondrial cytochrome-*b* gene sequences were obtained for 78 individuals representing 24 species of *Microtus*. Data supported 1 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*), plus the Holarctic *M. oeconomus* and several other previously identified clades. *M. gregalis* also was found to be distant from *M. abbreviatus* and *M. miurus,* thus contradicting monophyly of the subgenus *Stenocranius.* Monophyly of North American species was supported, albeit weakly. Basal relationships were not robust, reflecting a single pulse of diversification about 1.3×10^6 years ago. This pulse mirrors the fossil record and may be partially responsible for the unstable taxonomic history.

Key words: Beringia, maximum likelihood, *Microtus,* mitochondrial DNA, parsimony, taiga, vole

---

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 trans-Beringian taxa are poorly known. Molecular phylogenetic studies are beginning to shed light on the history of North American mammals (Fumagalli et al. 1999; Halanych et al. 1999) and plants (Xiang et al. 1998). Because molecular phylogenies provide opportunities to reconstruct the evolutionary history of taxa, they may be used to estimate number and temporal order of invasions (Givnish 1997), thereby providing valuable insights into the biogeographic history of a region.

We focus on the genus *Microtus* (Rodentia: Muridae), a Holarctic group (Fig. 1) that could be important to the interpretation of historical biogeography of the northern continents. Since the late Pliocene, *Microtus* diversified into one of the more speciose mammalian genera (Musser and Carleton 1993) with 65 species recognized in 14 subgenera. This rapid diversification (Reig

* *Correspondent: chris.conroy@stanford.edu*

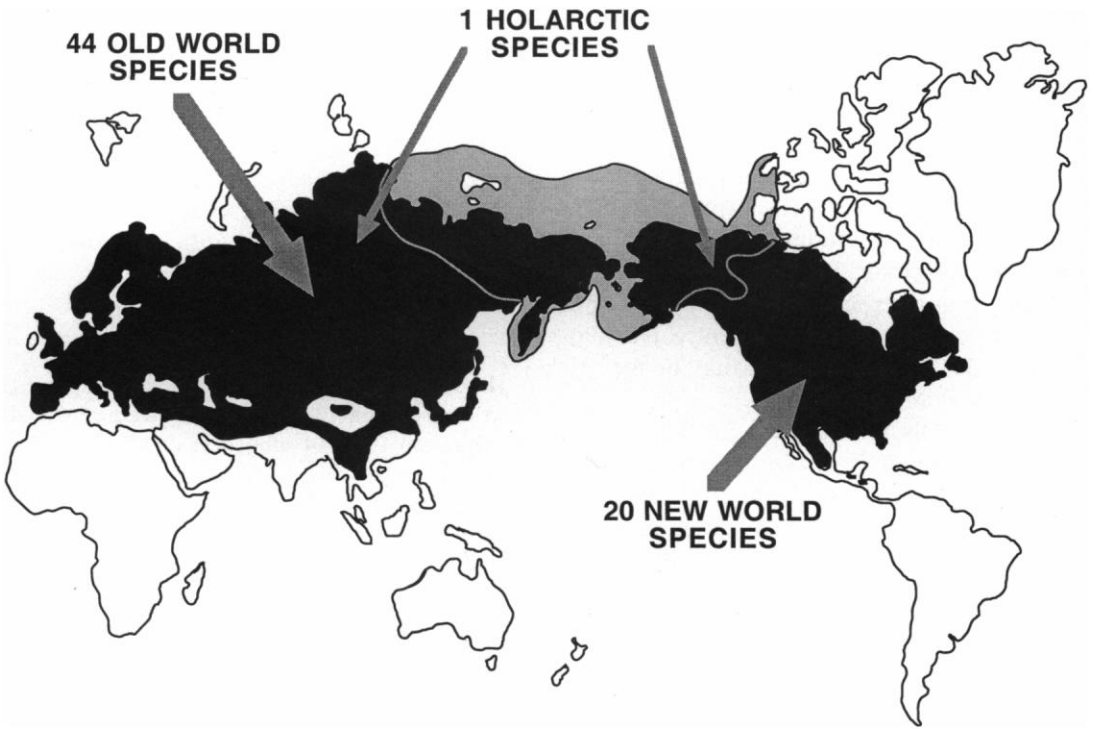

FIG. 1.—Current distribution of *Microtus* (black) and postulated distribution of Beringia (Hopkins et al. 1982) at peak glaciation (gray). Species diversity follows Musser and Carleton (1993), including *Volemys;* distribution of *Microtus* follows Gromov and Polyakov (1977).

1989) may be partially responsible for its chaotic taxonomic history (Anderson 1985; Musser and Carleton 1993) and the enigmatic nature of many relationships within *Microtus.*

Extant species of *Microtus* are distributed throughout grassland, taiga, steppe, and tundra ecosystems of the Northern Hemisphere (Gromov and Polyakov 1992; Hoffmann and Koeppl 1985). The fossil record indicates large fluctuations in distribution associated with climatic change (Graham et al. 1996) with some species invading southerly regions of Eurasia and North America during cold phases (Repenning et al. 1990). During subsequent warm periods, glacial relicts were isolated (e.g., on mountaintops), and those events may be partially responsible for high 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 connected directly 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. These hypotheses have not been thoroughly tested.

Relationships among some species of *Microtus* have been proposed (e.g., as subgenera—Miller 1896), but phylogenies are 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 2 invasions between Old World and New World (endemics + the Holarctic *M. oeconomus*). A phylogeny with multiple sister relationships between particular North American and Asian–European clades would indicate multiple invasions. Further, North American taxa that are related closely to Eurasian sister taxa may reflect recent invasion, whereas deeper relationships may be the result of older invasions.

We used DNA sequences for 24 species to assess monophyly of North American endemics, monophyly of Holarctic subgenus *Stenocranius* (Rausch 1964), and monophyly of each of 2 clades of taiga-dwelling species of *Microtus* in North America (Hoffmann and Koeppl 1985). A discussion of each follows.

First, 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 (Hoffmann and Koeppl 1985; van der Meulen 1978). If all endemic North American species derived from a single invasion, they should be monophyletic. Alternatively, some endemic North America species may have sister species in Eurasia.

Second, phylogenetic relationships among species on separate continents may be obscured by convergent morphological evolution. For example, monophyly of the Holarctic subgenus *Stenocranius* (*M. gregalis, M. miurus,* and *M. abbreviatus*) is based on a shared skull characteristic (narrow cranium). The high degree of morphological convergence in Arvicolinae (Courant et al. 1997), however, cautions that morphology may not always reflect phylogenetic relationships.

Finally, Hoffmann (1981) and Hoffmann and Koeppl (1985) described a model of speciation wherein 2 purported clades of *Microtus* (clade 1: *M. pennsylvanicus, M. montanus,* and *M. townsendii;* clade 2: *M. xanthognathus, M. richardsoni,* and *M. chrotorrhinus*) expanded during interglacials but contracted to 3 separate refugia (western coastal, western montane, and eastern boreal) during glacial periods (Fig. 2). We tested the monophyly of these clades and others. We also conducted statistical tests among competing topologies using likelihood-ratio tests (Huelsenbeck and Rannala 1997).

Materials and Methods

Eight Palearctic species, 15 Nearctic species, and the Holarctic *M. oeconomus* were included to represent 10 of 14 subgenera of *Microtus* (Table 1). Two species of *Clethrionomys* were used as outgroups because Clethrionomyini was found to be sister to *Microtus* in a broader taxonomic survey of Arvicolinae (Conroy and Cook 1999). DNA was extracted via a modified salt method (Medrano et al. 1990) from skin, liver, muscle, or heart tissue that was dried, frozen, or preserved in ethanol. Symmetric PCR (Saiki et al. 1988) was used to amplify the 1,143-bp mitochondrial cytochrome-*b* gene (Conroy and Cook 1999). Sequences were determined on an ABI 373a Stretch DNA sequencer using Prism® dye terminator technology. Sequences for 2 taxa were obtained from Genbank (*Microtus arvalis*—GenBank accession no. U54488; *M. rossiaemeridionalis*—GenBank accession no. U54474). MtDNA was sequenced for 78 individuals, including partial or complete cytochrome-*b* sequences for multiple individuals for 21 of the 24 species. Species represented by multiple samples were all reciprocally monophyletic. Because of computational limitations, phylogenetic analysis included only 1 representative/species (Table 1). All multiple samples from a particular species were closely related. Sequences used in the phylogenetic analysis can be retrieved from GenBank under the accession numbers AF163890–AF163907.

Saturation was examined by plotting maximum-likelihood distance against transitions and transversions across each codon position (Fig. 3). Weighted parsimony searches were rooted with *Clethrionomys glareolus* and *C. gapperi* (Conroy and Cook 1999) and bootstrapped 500 times using a heuristic search, with 500 random additions of taxa for each search, in PAUP*, test version 4.0d59 (D. L. Swofford, pers. comm.).

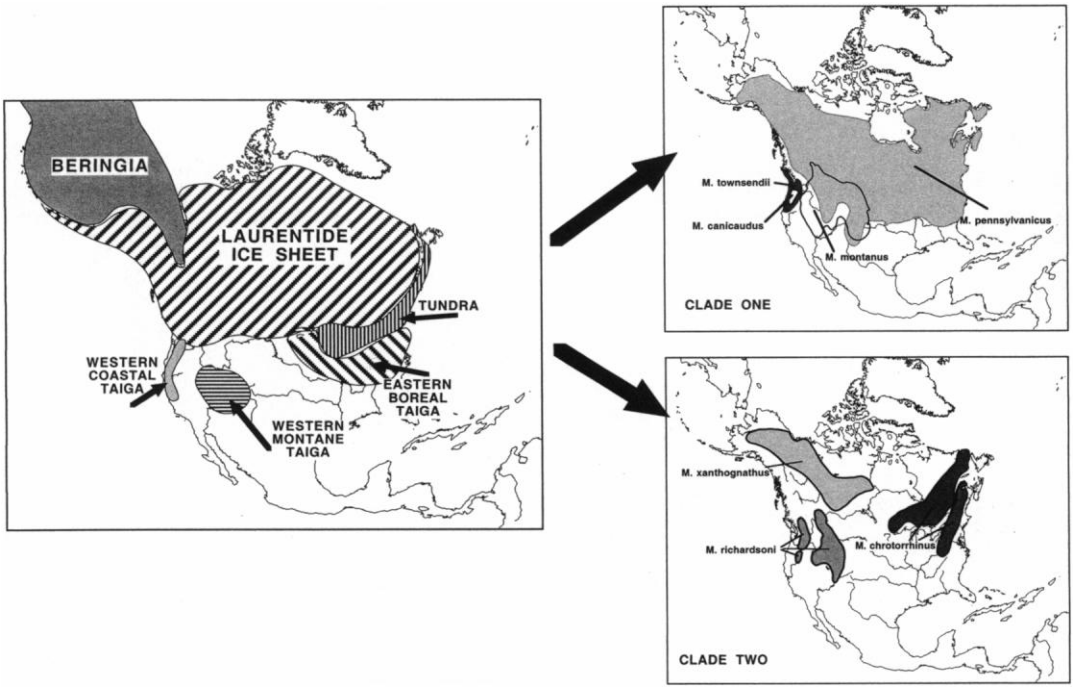

FIG. 2.—Model of taiga biome expansion and contraction through Pleistocene glaciations. Glacial refugia modified from Hoffmann (1981); distribution of species modified from Hoffmann and Koeppl (1985).

A transition to transversion bias of 3.4 (as estimated in the likelihood methods) was used.

Several maximum-likelihood models of DNA evolution were evaluated by estimating likelihood scores and comparing them as distributed under a chi-square distribution with degrees of freedom equal to the number of free parameters between them. Models were JC (Jukes and Cantor 1969), HKY85 (Hasegawa et al. 1985), and GTR (Yang 1994a). The latter 2 models also were run with among-site rate variation based on a gamma distribution (Yang 1994b). Skewness or G_1-statistics were generated from 1,000 random trees (PAUP*), with and without

TABLE 1.—Species of *Microtus* (Musser and Carleton 1993), including *Volemys*, examined in the study; *n* = number of specimens examined; ? 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[a]	*Stenocranius*	*abbreviatus*	4
Microtus	*rossiaemeridionalis*	2[a]	*Stenocranius*	*gregalis*	3
Microtus?	*mexicanus*	2	*Stenocranius*	*muirus*	2
Mynomes	*canicaudus*	4	*Volemys*	*kikuchii*	3
Mynomes	*montanus*	3	*Mynomes*	*townsendii*	2

[a] Obtained from Genbank (*Microtus arvalis*, nos. U54488 and U54489; *M. rossiaemeridionalis*, nos. U54474 and U54477).

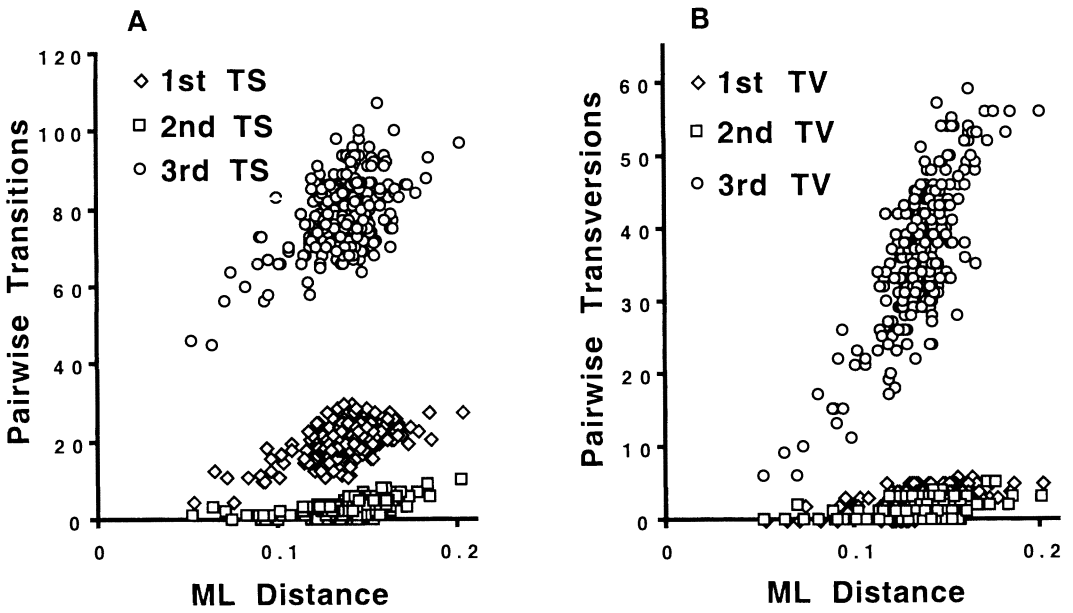

FIG. 3.—Pairwise numbers of A) transitions and B) transversions for 1st, 2nd, and 3rd codon positions plotted against maximum-likelihood (ML) distance (HKY85 + Γ) between species of *Microtus*.

weighting at each position, and compared with values in Hillis and Huelsenbeck (1992) for statistical significance ($\alpha = 0.05$). To evaluate the strength of alternate topologies, a likelihood-ratio test (Kishino and Hasegawa 1989) tested the unconstrained maximum-likelihood tree against maximum-likelihood trees constrained for monophyly of *Stenocranius,* North American monophyly, the 2 clades of taiga voles, and all taiga voles, as well as both maximum parsimony trees. To test the strength of relationships, we also constrained maximum-likelihood trees to exclude 2 well-supported clades and then tested them against the unconstrained maximum-likelihood tree. We also bootstrapped the maximum-likelihood analysis 100 times.

To estimate time of divergence, we used a distance based on the same maximum-likelihood model used for estimating the maximum-likelihood 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 (about 2.1×10^6 years ago—Repenning et al. 1990). Molecular clocks are often subject to error from excessive rate heterogeneity. Therefore, we tested for rate heterogeneity among taxa by evaluating maximum-likelihood trees with and without a molecular clock constraint using a chi-square test (i.e., twice the log-likelihood difference with n = number of taxa minus 2 *d.f.*—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 1,143 base pairs, 459 (40%) were variable, and 361 of those were phylogenetically informative (Table 2). Similar to other studies of mammalian cytochrome-*b* evolution (Irwin et al. 1991; Ma et al. 1993), most polymorphic sites were in 3rd positions (340, 74%), followed by 1st positions (94, 20%) and then 2nd positions (25, 5%). Base pair composition across codon position and between nucleotides (Table 3) was similar to mammals in general (Irwin et al. 1991).

Interspecific distances estimated under the Kimura (1980) 2-parameter model (Ta-

TABLE 2.—Sequence variation and G_1-statistics from 1,000 random trees. G_1-values were significant at $P < 0.01$; significance from table 2 in Hillis and Huelsenbeck (1992). All searches were run 3 times to verify results (data not shown); values from 1st search presented.

Sequence data	1st position	2nd position	3rd position
Numbers of base pairs	381	381	381
Numbers of variable sites	94	25	340
Numbers of parsimony informative variable sites	65	11	285
G_1-statistic with no weights	−0.28	−2.13	−0.39
G_1-statistic with transitions/transversions = 3.4	−0.55	−3.26	−0.98

ble 4) 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). Saturation was not apparent from these plots. G_1-statistics (Table 2) indicated that the data had phylogenetic signal (Hillis and Huelsenbeck 1992).

Phylogenetic results.—Parsimony searches resulted in 2 equally parsimonious trees (Fig. 4A), and the consensus placed *M. gregalis* basal to all others. Several species stemmed from a polytomy above this level. Well-supported sister relationships included *M. abbreviatus* and *M. miurus, M. arvalis* and *M. rossiaemeridionalis, M. californicus* and *M. mexicanus, M. pinetorum* and *M. richardsoni, M. canicaudus* and *M. townsendii, M. montanus* and *M. pennsylvanicus, M. fortis* and *M. middendorffi,* and *M. kikuchii* and *M. oeconomus.* A clade uniting *M. canicaudus, M. townsendii, M. montanus,* and *M. pennsylvanicus* (hereafter the "*pennsylvanicus* clade") was recovered in both maximum-parsimony and maximum-likelihood analyses (Fig. 4B). A clade uniting *M. fortis, M. middendorffi, M. kikuchii, M. oeconomus,* and *M. montebelli* (hereafter the "Asian clade") also was found in maximum-likelihood analyses (Fig. 4B) but was not strongly supported by bootstraps (52% in the maximum-parsimony tree).

Maximum-likelihood searches suggested that the HKY + Γ model was not statistically different from a more complex model (GTR + Γ). Thus, subsequent tests utilized the HKY + Γ model. Maximum-likelihood and 1 of 2 maximum-parsimony trees (not shown) supported monophyly of North American species of *Microtus* (Fig. 4). Most sister relationships identified by maximum parsimony were found with maximum likelihood. No analyses supported monophyly of the subgenus *Stenocranius* or monophyly of the 2nd clade of taiga voles (*M. xanthognathus, M. chrotorrhinus,* and *M. richardsoni*). Only the alternate topology that united all taiga voles was rejected by the likelihood-ratio test (Table 5). All others were insignificantly different from the maximum-likelihood tree.

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 ($\chi^2 = 2.68$) under a molecular clock (Felsenstein 1988). Constraining the oldest interspecific divergence to 2.1×10^6 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

TABLE 3.—Percent nucleotide base composition by codon position and by nucleotide for complete cytochrome-*b* gene sequences averaged across 24 species of *Microtus*.

		Position		
Nucleotide	Overall	1st	2nd	3rd
Guanine	13.0	22.9	12.3	3.7
Adenine	30.7	30.3	20.9	40.9
Thymine	27.2	23.1	41.7	16.8
Cytosine	29.1	23.6	25.0	38.7

TABLE 4.—Kimura pairwise distances (%—Kimura 1980) for 24 species of *Microtus* and 2 outgroup species of *Clethrionomys*.

No.	Species	Species no. 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

of diversification among species about 1.3 $\times$ 10^6 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. Previous studies that used karyotypes and other molecular markers have provided independent assessments of microtine 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 had minimal overlap. Historical implications of relationships supported by our data are summarized in the following.

North American monophyly.—During Pleistocene glacial maxima, ocean levels dropped sufficiently to expose the Bering Land Bridge and unite Beringia (Hopkins et al. 1982). Controversy exists over the nature of Beringia's vegetational composition and climate during and since the Pleistocene (Colinvaux 1996; Elias et al. 1996; Guthrie 1990; Kontrimavichus 1985). Establishing the chronology of invasions between Asia and North America could help to reconstruct environments for the land bridge. Although microtines were a common mammalian component of Beringia (Guthrie 1982), the fossil record has not been investigated well enough to determine their diversity or persistence in this region.

Microtus is thought to have first invaded North America from Asia after the Blancan V glaciation in Laurentia about 2.1 $\times$ 10^6

TABLE 4.—Extended.

Species no.												
13	14	15	16	17	18	19	20	21	22	23	24	25
14.5												
12.8	12.9											
12.3	14.7	14.2										
14.4	10.9	13.8	14.0									
13.9	15.0	14.4	13.6	14.5								
13.5	9.3	13.0	13.9	9.5	14.5							
14.5	16.4	13.8	13.1	14.8	15.2	16.3						
13.7	16.2	14.2	7.6	14.4	14.6	14.8	14.5					
13.0	14.3	12.8	13.6	13.6	13.6	13.9	14.7	14.5				
12.4	13.8	13.9	11.4	14.2	13.6	13.3	14.0	12.9	12.3			
13.9	14.7	14.2	15.0	13.9	14.5	13.4	15.3	14.7	14.9	14.7		
11.6	14.0	12.6	8.3	12.7	13.7	12.8	11.7	9.4	13.8	13.0	14.4	
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

years ago (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 North American taxa (Fig. 4) is consistent with a Eurasian origin for the genus.

From paleontological and zoogeographic data, Hoffmann and Koeppl (1985) suggested that distinct lineages of *Microtus* colonized North America across the Bering Land Bridge in the early, middle, and late Pleistocene (until about 13,000 years ago). Three species hypothesized to be derived from the earliest invasion are *M. californicus* and *M. umbrosus* (Martin 1974) and *M. guatemalensis* (Repenning 1980). Descendants of middle-Pleistocene colonizers 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 colonization of North America than that previously inferred, but weak basal relationships limit our ability to discriminate multiple invasions. Monophyly of the endemic North American species of *Microtus* indicates only 2 invasions (endemics plus *M. oeconomus*) and potentially refutes proposed taxonomic affinities: *M. longicaudus,* a member of Eurasian *Chilotus* (Anderson

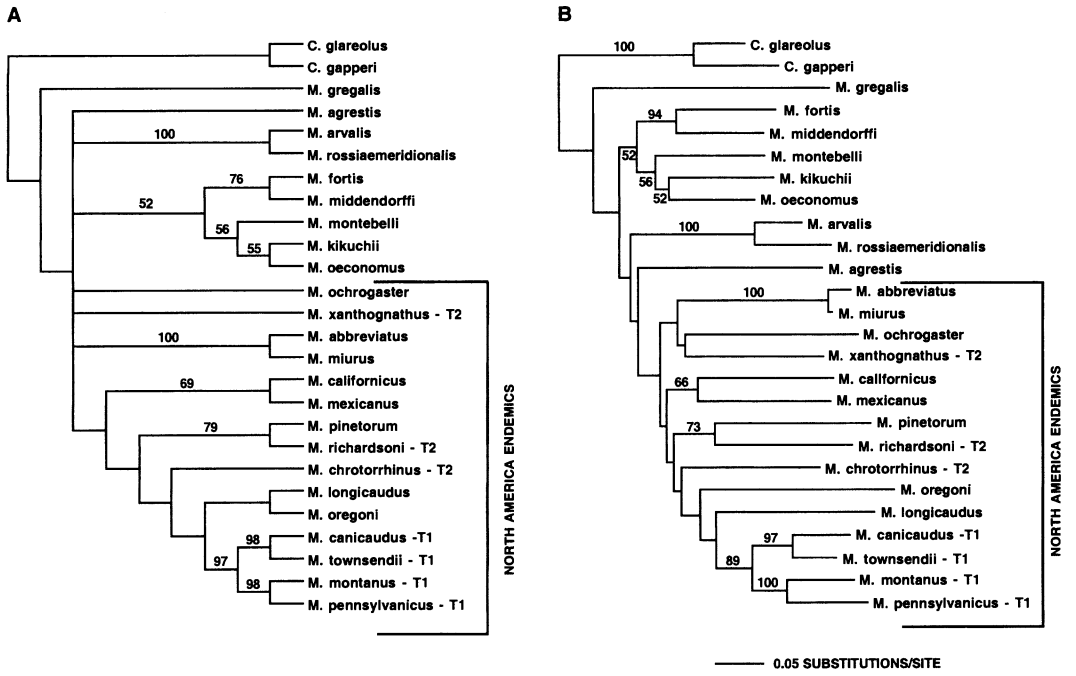

FIG. 4.—A) Consensus tree of 2 equally parsimonious trees for 24 *Microtus* species and 2 *Clethrionomys* species; numbers along branches are bootstrap percentages from 10,000 replicates. B) Maximum-likelihood tree (HKY85 + Γ); T1 and T2 following taxon indicate 1st and 2nd taiga vole clades, respectively; numbers along branches are bootstrap percentages from 100 replicates.

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 allozymic data (Graf 1982), although sampling of taxa was limited.

Because our data address only the history of extant species, there may have been other invasions of North America whose descendants have since gone extinct. For ex-

TABLE 5.—Results of Kishino and Hasegawa (1989) test of tree topolgies (see text for description of tree construction). One topology was significantly different from the maximum likelihood (ML) tree.

Tree	−lnL	Diff−lnL	*SD* (diff.)	T	*P*
ML tree with no constraints (HKY + Γ)	8,919.65				
Asian clade rejected	8,922.19	2.54	14.27	0.18	0.86
Pennsylvanicus clade rejected	8,925.44	5.79	7.98	0.73	0.47
North American monophyly enforced	8,919.81	0.16	6.68	0.02	0.98
Stenocranius monophyly enforced	8,938.32	18.67	14.55	1.28	0.20
Taiga vole clade 1 enforced	8,919.65	0.00	0.00	0.00	1.00
Taiga vole clade 2 enforced	8,932.40	12.75	9.53	1.34	0.18
All taiga voles forced monophyly	8,943.77	24.12	10.26	2.35	0.02[a]
MP tree 1	8,935.72	16.07	11.52	1.39	0.16
MP tree 2	8,921.10	1.45	2.53	0.57	0.57

[a] Significant at $P < 0.05$.

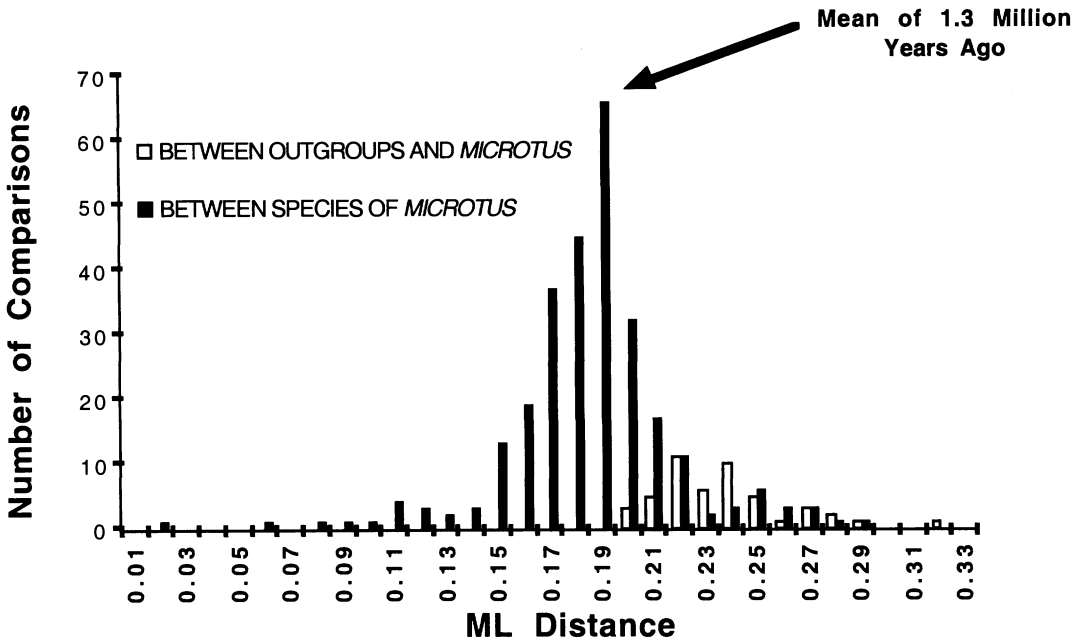

FIG. 5.—Pairwise distances between species of *Microtus* and between all species of *Microtus* and 2 species of *Clethrionomys*. The X-axis is the maximum-likelihood (ML) distance derived from the same model as in the maximum-likelihood tree (see Materials and Methods).

ample, *M. paroperarius* and *M. deceitensis,* now extinct but present in North America in the early Pleistocene (Repenning et al. 1990), share the 4-triangle m1 with *M. oeconomus* (Zakrzewski 1985), a Holarctic species thought to be a late Pleistocene colonizer of North America (Lance and Cook 1998). Because of apparent monophyly of endemic North American species, only 2 invasions may have occurred: the 1st resulting in species restricted to North America and the 2nd in *M. oeconomus* (Lance and Cook 1998). The estimated phylogeny should be tested by including other suspected early colonizers: *M. quasiater, M. oaxacensis, M. umbrosus,* and *M. guatemalensis.*

Albeit weakly supported, the sister relationship between all North American species and the European species *M. agrestis, M. arvalis,* and *M. rossiaemeridionalis* in the maximum-likelihood tree may suggest that these Asian species were isolated in a southern Asian refugium while a northern 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. Although *M. oeconomus* and *M. middendorffi* are distributed widely throughout Asia, *M. kikuchii, M. montebelli* (now both island endemics), and *M. fortis* are distributed south of this corridor and may have been isolated during glacial advances. More thorough sampling of eastern 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. Colonization of North America by a *Stenocranius* ancestor during the Illinoian Age (≈300,000 years ago) was hypothesized to explain their Hol-

arctic distribution (Rausch 1964; Zakrzewski 1985). 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) as reflected by similar morphology and karyotype to *M. miurus* (Rausch and Rausch 1968). 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 (Gromov and Polyakov 1992).

Based on our analyses, it appears that *M. miurus* originated in North America (Fig. 4) and is morphologically convergent with *M. gregalis.* The close sister relationship between *M. abbreviatus* and *M. miurus* based on cytochrome-*b* sequences and chromosomal similarity (Rausch and Rausch 1968) suggests that they may be conspecfic. Morphological and chromosomal similarities between *M. middendorffi* and *M. abbreviatus* (Vorontsov and Lyapunova 1984) appear to be convergent (Figs. 4A–B). Thus, our data support the interpretation of *Stenocranius* as "pseudoamphiberingian" (Vorontsov and Lyapunova 1984). The basal position of *M. gregalis* is consistent with an early Pleistocene origin from the extinct *M. gregaloides* (Chaline 1990; Gromov and Polyakov 1992). *M. miurus* and *M. abbreviatus* were sister to *M. xanthognathus* and included in the North American clade in both maximum-parsimony and maximum-likelihood analyses.

Other relationships within Microtus.—The *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 *pennsylvanicus* clade in the maximum-likelihood tree. *M. canicaudus* was not sister to *M. montanus,* as has been suggested previously (Musser and Carleton 1993), but instead to *M. townsendii.* Modi (1986) also noted significant chromosomal differences between these species. An investigation of the *pennsylvanicus* clade with mtDNA RFLP data (DeBry 1992) suggested that it may not be monophyletic but could not reject the monophyly hypothesis based on a likelihood-ratio test. Our likelihood-ratio test also did not distinguish among these alternatives (Table 5).

The Asian clade has not been recognized previously, although Zagorodnyuk (1990) placed *M. fortis* in the *M. middendorffi* species group of subgenus *Alexandromys.* By retaining *kikuchii* within *Microtus,* we depart from the taxonomy of Musser and Carleton (1993) and Zagorodnyuk (1990), who placed it in a separate genus, *Volemys,* with other species from southeastern Asia (*V. clarkei, V. millicens,* and *V. musseri*). The position of *kikuchii* suggests the need for further sampling of Asian species, including the 3 additional species of *Volemys.* Zagorodnyuk (1990) also suggested that *M. oeconomus* and *M. montebelli* may be sister taxa (Fig. 4) because they share an ancestral form of X–Y chromosome pairing that is not found in other species of *Microtus* (Borodin et al. 1997).

Taiga voles.—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 speciation of 2 taiga-adapted clades of voles in North America to allopatry during Pleistocene glacial phases (Fig. 2). They suggested that ancestors of those clades were widespread during interglacials and then isolated in refugia during glacial advances (Hoffmann 1981; Rand 1948, 1954). For the *pennsylvanicus* clade, refugia were hypothesized for the eastern boreal (*M. pennsylvanicus*),

the western montane (*M. montanus*), and Pacific coastal (*M. canicaudus* and *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 that it is sister to *M. townsendii.*

Other taiga voles were suggested to have arisen in eastern boreal (*M. chrotorrhinus* and *M. xanthognathus*) and western montane (*M. richardsoni*) refugia (Hoffmann and Koeppl 1985). These species were paraphyletic (T2 in Fig. 4), although a phylogeny constrained for their monophyly was not rejected (Table 5). *M. xanthognathus* was sister to the *M. miurus* and *M. abbreviatus* clade, whereas *M. richardsoni* was sister to *M. pinetorum.* The latter relationship was unexpected. *M. pinetorum* is often considered closely related to members of subgenus *Pitymys* of Europe (Gromov and Polyakov 1992) and Mexico (Musser and Carleton 1993). Although *M. richardsoni* has been considered highly divergent, other studies (Conroy and Cook 1999; Jannett 1992, 1997; Matthey 1957; Zakrzewski 1985) support inclusion within *Microtus.*

Use of taiga habitats across these voles may be due to convergence, although a more complete assessment of the genus is needed. A tree constrained for monophyly for the 6 taiga species was rejected (Table 5). Two other species that occur in taiga, *M. longicaudus* and *M. oregoni,* have been considered distinctive because of differences in karyotypes (Modi 1987), allozymes (Moore and Janecek 1990), gonosomal mosaicism in *M. oregoni* (Ohno et al. 1963), and large B chromosome complement in *M. longicaudus* (Judd and Cross 1980).

Basal relationships.—Our data indicate generally weak relationships across basal branches (Fig. 4A). We suggest that rapid diversification early in the evolution of this group resulted in short internodal branches. Because G_1-statistics are significant, saturation is not apparent, and these data have recovered older relationships in arvicolines (Conroy and Cook 1999), we think that the basal relationships most likely reflect rapid diversification.

Pulses of diversification apparently have been repeated throughout the evolution of murid (Conroy and Cook 1999; Smith and Patton 1999) and other rodents (Lessa and Cook 1998). This pulse of speciation in *Microtus* may correspond to an environmental change, such as a period of global warming (Chaline et al. 1993). The paleontological record indicates a rapid appearance of many species of *Microtus* in North America about 500,000 years ago (Hoffmann and Koeppl 1985). Although the timing is not synchronous with our estimate (1.3×10^6 years ago), this discrepancy may suggest that genes and morphology might be recording different aspects of macroevolution in this genus. The relationship between divergence of these markers and divergence of populations is unclear. For example, ancestral polymorphism and fluctuating effective populations, among other factors, may impact estimates of temporal divergence based on molecular markers.

Our estimate of the radiation of *Microtus,* 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). Speciation events that occurred during different glacial cycles should produce a dichotomous phylogeny. However, rapid isolation of multiple refugial populations during a single glacial period should result in polytomous branching. Although a single and severe climatic phenomenon may have been important to speciation in *Microtus,* fine-scale variability on a millennial scale also may 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 1.2×10^6 years ago (Imbrie et al. 1993). However, effects of this shift on mammalian evolution are unstudied. This calibration of the apparent pulse of speciation in *Microtus* should be further tested. For example, 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), and we are currently investigating intraspecific variation of the cytochrome-*b* gene in *Microtus* (Conroy and Cook 2000).

Some of the sister-species relationships that we recovered differ from those proposed based on morphology. Phylogeographic research (Avise et al. 1987) is a logical extension to this work that may help bridge the gap between studies of intra- and interspecific variation, perhaps providing insight into rates of divergence during and since the late Pleistocene (Avise and Walker 1998).

Acknowledgments

Specimens were generously provided by T. Yates and W. Gannon (Museum of Southwestern Biology), J. Patton and A. Yu (Museum of Vertebrate Zoology), J. Kenagy and S. Edwards (Burke Museum), P. Myers (University of Michigan Museum of Zoology), R. Jannett, and K. Martin (Oregon State University and Forest and Rangeland Ecosystem Science Center at Oregon State University). L. Jermiin allowed use of his program K2WuLi, and D. Swofford allowed use of a test version of PAUP*. R. DeBry, J. Demboski, A. DeWoody, M. Fleming, E. Hadley, R. Hoffmann, G. Jarrell, and K. Stone provided comments on earlier drafts. The University of Alaska Fairbanks Graduate School provided a fellowship to C. J. Conroy. Grants from the National Science Foundation, Arctic Institute of North America, the United States Department of Agriculture Forest Service, and United States Fish and Wildlife Service to J. A. Cook and C. J. Conroy supported aspects of the research.

Literature Cited

Anderson, S. 1985. Taxonomy and systematics. Pp. 52–83 in Biology of New World *Microtus* (R. H. Tamarin, ed.). Special Publication, The American Society of Mammalogists 8:1–893.

Avise, J. C., et al. 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Annual Review of Ecology and Systematics 18:489–522.

Avise, J. C., and D. Walker. 1998. Pleistocene phylogeographic effects on avian populations and the speciation process. Proceedings of the Royal Society of London, Series B 265:457–463.

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

Borodin, P. M., M. B. Rogatcheva, K. Koyasu, K. Fukuta, K. Mekada, and S. I. Oda. 1997. Pattern of X-Y chromosome pairing in the Japanese field vole, *Microtus montebelli*. Genome 40:829–833.

Chaline, J. 1990. An approach to studies of fossil arvicolids. Pp. 45–84 in the International symposium on evolution, phylogeny, and biostratigraphy of arvicolids (O. Fejfar and W. D. Heinrich, eds.). Pfeil-Verlag, Prague, Czech Republic.

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

Colinvaux, P. 1996. Low-down on a land bridge. Nature 382:21–23.

Conroy, C. J., and J. A. Cook. 1999. MtDNA evidence for repeated pulses of speciation within arvicoline and murid rodents. Journal of Mammalian Evolution 6:221–245.

Conroy, C. J., and J. A. Cook. 2000. Phylogeography of a post-glacial colonizer: *Microtus longicaudus* (Muridae: Rodentia). Molecular Ecology 9:165–175.

Courant, F., B. David, B. Laurin, and J. Chaline. 1997. Quantification of cranial convergences in arvicolids (Rodentia). Biological Journal of the Linnean Society 62:505–517.

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

Edwards, S. V. 1997. Relevance of microevolutionary processes to higher level molecular systematics. Pp. 251–278 in Avian molecular evolution and systematics (D. P. Mindell, ed.). Academic Press, New York.

Elias, S. A., S. K. Short, C. H. Nelson, and H. H. Birks. 1996. Life and times of the Bering Land Bridge. Nature 382:60–63.

Fedyk, S. 1970. Chromosomes of *Microtus (Stenocranius) gregalis major* (Ognev 1923) and phylogenetic connections between sub-arctic representatives of the genus *Microtus* Schrank, 1798. Acta Theriologica 15:143–152.

Felsenstein, J. 1988. Phylogenies from molecular sequences: inference and reliability. Annual Review of Genetics 22:521–565.

Fumagalli, L., P. Taberlet, D. T. Stewart, L. Gielly, J. Hausser, and P. Vogel. 1999. Molecular phylogeny and evolution of *Sorex* shrews (Soricidae: Insectivora) inferred from mitochondrial DNA sequence data. Molecular Phylogenetics and Evolution 11:222–235.

Givnish, T. J. 1997. Adaptive radiation and molecular systematics: issues and approaches. Pp. 1–54 in Molecular evolution and adaptive radiation (T. J. Givnish and K. J. Sytsma, eds.). Cambridge University Press, Cambridge, United Kingdom.

Graf, J.-D. 1982. Génétique biochimique, zoogéogra-

phie et taxonomie des Arvicolidae (Mammalia, Rodentia). Revue Suisse de Zoologie 89:749–787.

GRAHAM, R. W., ET AL. 1996. Spatial response of mammals to Late Quaternary environmental fluctuations. Science 272:1601–1606.

GROMOV, I. M., AND I. YA. POLYAKOV. 1992. Voles (Microtinae). (D. Siegel-Causey and R. S. Hoffmann, scientific eds.). Onyx Publishers, New Delhi, India 3:1–725.

GUTHRIE, R. D. 1982. Mammals of the mammoth steppe as paleoenvironmental indicators. Pp. 307–326 in Paleoecology of Beringia (D. M. Hopkins, J. V. Matthews, Jr., C. E. Schweger, and S. B. Young, eds.). Academic Press, New York.

GUTHRIE, R. D. 1990. Frozen fauna of the mammoth steppe: the story of Blue Babe. The University of Chicago Press, Chicago, Illinois.

HALANYCH, K. M., J. R. DEMBOSKI, B. J. VAN VUUREN, D. R. KLEIN, AND J. A. COOK. 1999. Cytochrome *b* phylogeny of North American hares and jackrabbits (*Lepus,* Lagomorpha) and the effects of saturation in outgroup taxa. Molecular Phylogenetics and Evolution 11:213–221.

HASEGAWA, M., H. KISHINO, AND T. YANO. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution 22:160–174.

HEWITT, G. M. 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. Biological Journal of the Linnean Society 58:247–276.

HILLIS, D. M., AND J. P. HUELSENBECK. 1992. Signal, noise, and reliability in molecular phylogenetic analyses. Journal of Heredity 83:189–195.

HOFFMANN, R. S. 1981. Different voles for different holes: environmental restrictions on refugial survival of mammals. Pp. 25–45 in Evolution today (G. G. E. Scudder and J. L. Reveal, eds.). Proceedings of the Second International Congress of Systematic and Evolutionary Biology 2:1–486.

HOFFMANN, R. S., AND J. W. KOEPPL. 1985. Zoogeography. Pp. 84–115 in Biology of New World *Microtus* (R. H. Tamarin, ed.). Special Publication, The American Society of Mammalogists 8:1–893.

HOOPER, E. T., AND B. S. HART. 1962. A synopsis of recent North American microtine rodents. Museum of Zoology, University of Michigan, Miscellaneous Publications 120.

HOPKINS, D. M., J. V. MATTHEWS, JR., C. E. SCHWEGER, AND S. B. YOUNG (EDS.). 1982. Paleoecology of Beringia. Academic Press, New York.

HUELSENBECK, J. P., AND B. RANNALA. 1997. Phylogenetic methods come of age: testing hypotheses in an evolutionary context. Science 276:227–232.

IMBRIE, J., ET AL. 1993. On the structure and origin of major glaciation cycles 2. the 100,000-year cycle. Paleoceanography 8:699–735.

IRWIN, D. M., T. D. KOCHER, AND A. C. WILSON. 1991. Evolution of the cytochrome b gene of mammals. Journal of Molecular Evolution 32:128–144.

JANNETT, F. J. 1992. Morphogenic and histologic patterns among the posterolateral glands of microtine rodents. Pp. 125–128 in Chemical signals in vertebrates 6 (R. L. Doty and D. Muller-Schwarze, eds.). Plenum Publishing, New York.

JANNETT, F. J. 1997. The uncut m1 and systematic position of the North American water vole (*Microtus richardsoni*). The American Midland Naturalist 137: 173–177.

JUDD, S. R., AND S. P. CROSS. 1980. Chromosomal variation in *Microtus longicaudus* (Merriam). The Murrelet 61:2–5.

JUKES, T. H., AND C. R. CANTOR. 1969. Evolution of protein molecules. Pp. 21–132 in Mammalian protein metabolism (H. N. Munro, ed.). Academic Press, New York.

KASCHENKO, N. F. 1901. *Stenocranius i Platycranius,* dva novye podroda sibirskikh' polevok'. Ezhegodnik' Zoologicheskogo Muzeia Imperatorskaia Akademii Nauk 6:165–206.

KIMURA, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16:111–120.

KISHINO, H., AND M. HASEGAWA. 1989. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. Journal of Molecular Evolution 29:170–179.

KONTRIMAVICHUS, V. L. (ED.). 1985. Beringia in the Cenozoic era. Amerind Publishing Company Pvt. Ltd., New Delhi, India.

KORTH, W. W. 1994. The Tertiary record of rodents in North America. Plenum Publishing, New York.

KURTÉN, B. 1968. Pleistocene mammals of Europe. Aldine Publishing, Chicago, Illinois.

LANCE, E. W., AND J. A. COOK. 1998. Biogeography of tundra voles (*Microtus oeconomus*) of Beringia and the southern coast of Alaska. Journal of Mammalogy 79:53–65.

LESSA, E. P., AND J. A. COOK. 1998. The molecular phylogenetics of tuco-tucos (genus *Ctenomys,* Rodentia: Octodontidae) suggests an early burst of speciation. Molecular Phylogenetics and Evolution 9: 88–99.

MA, D.-P., A. ZHARKIKH, D. GRAUR, J. L. VANDEBERG, AND W.-H. LI. 1993. Structure and evolution of opossum, guinea pig, and porcupine cytochrome b genes. Journal of Molecular Evolution 36:327–334.

MARTIN, R. A. 1974. Fossil mammals from the Coleman IIA fauna, Sumter County. Pp. 35–99 in Pleistocene mammals of Florida (S. D. Webb, ed.). The University Presses of Florida, Gainesville.

MATTHEY, R. 1957. Cytologie comparée, systématique et phylogénie des Microtinae (Rodentia—Muridae). Revue Suisse de Zoologie 64:39–71.

MEDRANO, J. F., E. AASEN, AND L. SPARROW. 1990. DNA extraction from nucleated red blood cells. Biotechniques 8:43.

MILLER, G. S., JR. 1896. The genera and subgenera of voles and lemmings. North American Fauna 12:1–84.

MILLER, G. S., JR. 1899. A new vole from Hall Island, Bering Sea. Proceedings of the Biological Society of Washington 13:13–14.

MODI, W. S. 1986. Karyotype differentiation among two sibling species pairs of New World microtine rodents. Journal of Mammalogy 67:159–165.

MODI, W. S. 1987. Phylogenetic analyses of the chro-

mosomal banding patterns among the Nearctic Arvicolidae. Systematic Zoology 36:109–136.

MODI, W. S. 1996. Phylogenetic history of LINE-1 among Arvicolid rodents. Molecular Biology and Evolution 13:633–641.

MOORE, D. W., AND L. L. JANECEK. 1990. Genic relationships among North American *Microtus* (Mammalia:Rodentia). Annals of Carnegie Museum 59: 249–259.

MUSE, S. W., AND B. S. WEIR. 1992. Testing for equality of evolutionary rates. Genetics 132:269–276.

MUSSER, G. G., AND M. D. CARLETON. 1993. Family Muridae. Pp. 501–756 in Mammal species of the world: a taxonomic and geographic reference (D. E. Wilson and D. M. Reeder, eds.). Smithsonian Institution Press, Washington, D.C.

NADLER, C. F., N. M. ZHURKEVICH, R. S. HOFFMANN, A. I. KOZLOVSKII, L. DEUTSCH, AND C. F. NADLER, JR. 1978. Biochemical relationships of the Holarctic vole genera *Clethrionomys, Microtus,* and *Arvicola* (Rodentia: Arvicolinae). Canadian Journal of Zoology 56:1564–1575.

OHNO, S., J. JAINCHIL, AND C. STENIUS. 1963. The creeping vole as a gonosomic mosaic, the OY/XY construction in the male. Cytogenetics 2:232–239.

RAND, A. L. 1948. Glaciation, an isolating factor in speciation. Evolution 2:314–321.

RAND, A. L. 1954. The ice age and mammal speciation in North America. Arctic 7:31–35.

RAUSCH, R. L. 1964. The specific status of the narrow-skulled vole (subgenus *Stenocranius* Kashchenko) in North America. Zeitschrift für Säugetierkunde 29: 343–358.

RAUSCH, R. L. 1994. Transberingian dispersal of cestodes in mammals. International Journal for Parasitology 24:1203–1212.

RAUSCH, R. L., AND V. R. RAUSCH. 1968. On the biology and systematic position of *Microtus abbreviatus* Miller, a vole endemic to the St. Matthew Islands, Bering Sea. Zeitschrift für Säugetierkunde 33: 65–99.

REIG, O. 1989. Karyotypic repatterning as one triggering factor in cases of explosive speciation. Pp. 246–290 in Evolutionary biology of transient populations (A. Fontdevila, ed.). Springer-Verlag, Berlin, Germany.

REPENNING, C. A. 1980. Faunal exchanges between Siberia and North America. Canadian Journal of Anthropology 1:37–44.

REPENNING, C. A., O. FEJFAR, AND W. D. HEINRICH. 1990. Arvicolid rodent biochronology of the Northern hemisphere. Pp. 385–418 in International symposium on evolution, phylogeny, and biostratigraphy of arvicolids (Rodentia, Mammalia) (O. Fejfar and W. D. Heinrich, eds.). Pfeil-Verlag, Prague, Czech Republic.

ROY, K., J. W. VALENTINE, D. JABLONSKI, AND S. M. KIDWELL. 1996. Scales of climatic variability and time averaging in Pleistocene biotas: implications for ecology and evolution. Trends in Ecology and Evolution 11:458–463.

SAIKI, R. K., ET AL. 1988. Primer-directed enzymatic amplifications of DNA with a thermostable DNA polymerase. Science 239:487–491.

SMITH, M. F., AND J. L. PATTON. 1999. Phylogenetic relationships and the radiation of sigmodontine rodents in South America: evidence from cytochrome b. Journal of Mammalian Evolution 6:89–128.

VAN DER MEULEN, A. J. 1978. *Microtus* and *Pitymys* (Arvicolidae) from Cumberland Cave, Maryland, with a comparison of some New and Old World species. Annals of the Carnegie Museum 47:101–145.

VORONTSOV, N. N., AND E. A. LYAPUNOVA. 1984. Genetics and problems of trans-Beringian connections of Holarctic mammals. Pp. 441–463 in Beringia in the Cenozoic Era (V. L. Kontrimavichus, ed.). Amerind Publishing Company Pvt. Ltd., New Delhi, India.

WU, C.-I., AND W.-H. LI. 1985. Evidence for higher rates of nucleotide substitutions in rodents than in man. Proceedings of the National Academy of Sciences 82:1741–1745.

XIANG, Q.-Y., D. E. SOLTIS, AND P. S. SOLTIS. 1998. The eastern Asian and eastern and western North American floristic disjunction: congruent phylogenetic patterns in seven diverse genera. Molecular Phylogenetics and Evolution 10:178–190.

YANG, Z. 1994a. Estimating the pattern of nucleotide substitutions. Journal of Molecular Evolution 39: 105–111.

YANG, Z. 1994b. Maximum likelihood phylogenetic estimation from DNA sequences with variable rates over sites: approximate methods. Journal of Molecular Evolution 39:306–314.

ZAGORODNYUK, I. V. 1990. Kariotipicheskaya izmenchivost' i sistematika serykh polevok (Rodentia, Arvicolini). Soobshchenie 1. Vidovoi sostav i khromosomnye chisla. Vestnik Zoologii 2:26–37.

ZAKRZEWSKI, R. J. 1985. The fossil record. Pp. 1–51 in Biology of New World *Microtus* (R. H. Tamarin, ed.). Special Publication, The American Society of Mammalogists 8:1–893.

Submitted 31 August 1998. Accepted 21 September 1999.

Associate Editor was Brett Riddle.

APPENDIX I

Specimens examined.—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 U.S. Geological Survey 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 (AF31001), Tanacross Quad (AF10290).