Historical biogeography at the crossroads of the northern continents: molecular phylogenetics of red-backed voles (Rodentia: Arvicolinae)

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Abstract

Evolutionary relationships of red-backed voles and their relatives were examined and used to test biogeographic hypotheses. Sequences of the mitochondrial cytochrome *b* gene were obtained for 25 individuals representing *Alticola macrotis*, *Clethrionomys californicus*, *C. gapperi*, *C. glareolus*, *C. rutilus*, and *C. rufocanus*. These were combined with 21 partial sequences from GenBank for *C. regulus*, *C. rex*, *C. rufocanus*, *C. rutilus*, *Eothenomys imaizumii*, *E. melanogaster*, *Phaulomys andersoni*, and *P. smithii*. Complete sequences of three species of *Microtus* (*M. montanus*, *M. oeconomus*, and *M. pennsylvanicus*), representative species of other arvicoline genera (*Myopus*, *Synaptomys*, *Arvicola*, *Ellobius*, *Ondatra*, *Lemmus*, *Dicrostonyx*, and *Phenacomys*), and a sigmodontine representative (*Peromyscus*) were included as outgroups. We used maximum parsimony, maximum likelihood, distance, and Bayesian based methods and conducted statistical tests on proposed hypotheses of phylogenetic relationships and biogeographic histories. A close relationship of species representing the genera *Alticola*, *Clethrionomys*, and *Eothenomys* was supported (Clethrionomyini); however, the genus *Clethrionomys* was paraphyletic with respect to both *Alticola* and *Eothenomys*. Three major clades were identified as Asian (*Eothenomys andersoni*, *E. smithii*, *C. rex*, *C. regulus*, and *C. rufocanus*), Trans-beringian (*Alticola macrotis*, *C. californicus*, *C. gapperi*, *C. glarelolus*, and *C. rutilus*), and Taiwanese (*E. melanogaster*). These results are consistent with the fossil record which indicates an initial diversification in Asia followed by colonization of the Nearctic on at least two occasions. The holarctic species, *C. rutilus*, appears to have either reinvaded Asia from North America or colonized North America more recently (late Pleistocene) than the two species of *Clethrionomys* (*C. gapperi* and *C. californicus*) that are endemic to North America (early to mid-Pleistocene). Finally, *C. gapperi*, appears to be comprised of an eastern and a western species, the former with affinities to the Asian *C. glareolus* and the latter more closely related to *C. californicus*.
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1. Introduction

Faunal exchanges across the Bering Land Bridge had a significant impact on the biogeography of the Northern Hemisphere and were instrumental in defining the structure of communities in the Nearctic and Palearctic. Our primary understanding of the historical biogeography of this nexus between the northern continents has been developed through paleontology, palynology, and geomorphology (e.g., Elias et al., 1996; Hopkins et al., 1982; Marincovich and Gladenkov, 1999); however, a growing number of studies now are using molecular phylogenetics to trace the history of faunal exchange in the region (e.g., Fedorov, 1999; Zink et al., 1995). By elucidating evolutionary relationships among trans-Beringian species complexes or phylogeographic variation within taxa distributed across the Holarctic (e.g., Hundertmark et al., 2002), we are refining our views of the sequence and timing of colonization events and the impact of repeated inundation of the Bering Land Bridge (e.g., Conroy and Cook, 1999; Hoberg, 1995). Testable hypotheses have been proposed related to the

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impact of geologic events in this region on diversification of mammals (Hoffmann, 1981; Sher, 1999) and other fauna (e.g., Hoberg et al., in press; Rausch, 1994) and flora (e.g., Abbott and Brochmann, 2003).

In addition to Beringia's impact on the intercontinental movement of organisms, the region may have played a primary role in the diversification of high latitude biota (Elias et al., 1996; Guthrie and Matthews, 1971). Glacial advances created a large refugium, Beringida (Sher, 1999), and a number of boreal taxa have been described as endemic to that region (Murray, 1981). The status of some of these purported endemics is now being re-evaluated using molecular approaches (e.g., Conroy and Cook, 2000; Fedorov et al., 2003).

Red-backed voles have been used to infer the history of northern paleoenvironments because they are intimately tied to boreal forests and tundra. For example, Graham (1976) interpreted Late Wisconsin environmental gradients in eastern North America partially within the context of the fossil distribution of *Clethrionomys gapperi*. Because repeated glacial advances during the Pleistocene likely left a genetic signature in high latitude taxa (Hewitt, 1996), species of *Clethrionomys* provide an opportunity to test the possible influence of hypothesized glacial refugia at high latitudes on biotic diversity. Similarly, warm periods may have isolated boreal taxa in southern alpine refugia further contributing to diversification.

The taxonomy of red-backed voles remains problematic. Within North America, species of *Clethrionomys* are difficult to distinguish morphologically, a problem that also has plagued specialists working with Asian species (e.g., Suzuki et al., 1999). For example, a long-standing debate has centered on the possibility that *C. rutilus* and *C. gapperi* may be conspecific because of apparent convergence of otherwise diagnostic morphological characters in areas where their ranges contact (Bee and Hall, 1956; Youngman, 1975). These species are suspected to be widely parapatric from Hudson Bay, Canada west to the Pacific Coast in southern Alaska (Hall, 1981; Hall and Cockrum, 1953). A similar case is found further south, where the definition of distributional limits of *C. gapperi* and *C. californicus* has been debated (Johnson and Ostenson, 1959).

By examining variation in DNA sequences of the mitochondrial cytochrome *b* gene, we begin to assess the taxonomic limits and phylogenetic relationships of this boreal group in an effort to explore the pattern and timing of faunal exchange across the crossroads of the northern continents and the potential impact of past climatic events on differentiation. In this paper, we examine the evolutionary relationships of 12 species of four genera of red-backed voles (Muridae: Clethrionomyini), a widespread Holarctic group (Fig. 1). We particularly focus on how the phylogeny impacts our perception of the colonization history of the three species of *Clethrionomys* that occur in North America.

2. Materials and methods

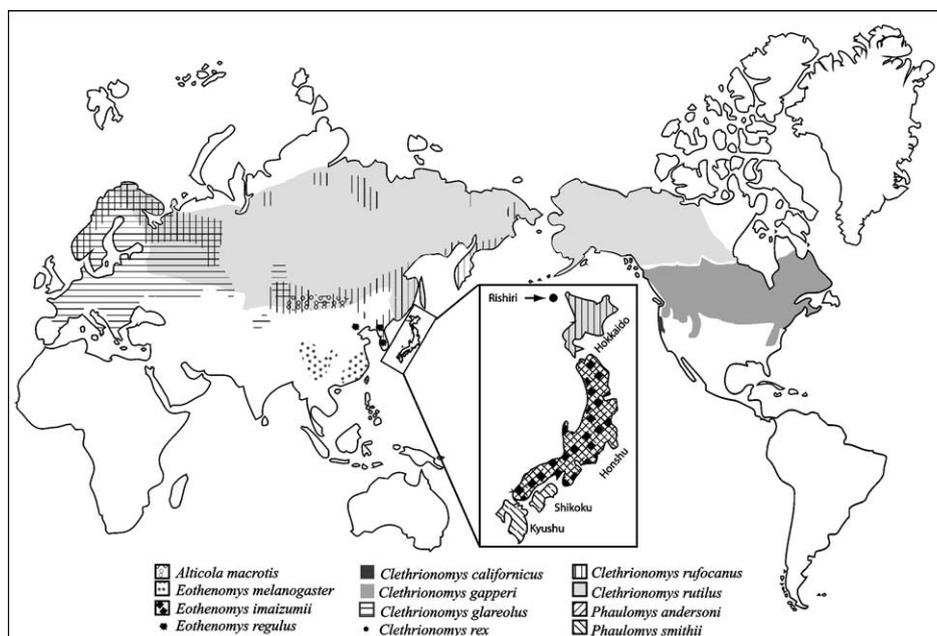We follow the taxonomy of Musser and Carleton (1993), except where noted.

Fig. 1. Map depicting the worldwide distribution of the 12 species of red-backed voles we investigated (adapted from Corbett 1978).

2.1. Specimens examined

Complete sequences of the cytochrome *b* gene (1143 base pairs) were obtained (Table 1) for a total of 25 individuals (including one partial sequence) representing *C. gapperi* ($n = 7$), *C. rutilus* ($n = 5$), *C. rufocanus* ($n = 7$), *C. glareolus* ($n = 3$), *C. californicus* ($n = 2$), and *Alticola macrotis* ($n = 1$). The sequences for these individuals have been submitted to GenBank (Accession Nos. AY309411–AY309435). In addition, complete sequences for one *C. glareolus*, two *C. rutilus*, one *C. rufocanus*, and one *Alticola macrotis* from GenBank were used. To develop a more extensive view of intraspecific variation, 21 partial sequences (402 bp) of cytochrome *b* (Suzuki et al., 1999) were included from eight Asian species of red-backed voles (Table 1) including *C. regulus* ($n = 2$), *C. rufocanus* ($n = 5$), *C. rutilus* ($n = 2$), *Eothenomys imaizumii* ($n = 1$), *E. melanogaster* ($n = 1$), *C. rex* ($n = 2$), and *Phaulomys andersoni* ($n = 3$), and *P. smithii* ($n = 5$). Species of the genus *Microtus* form a clade that is sister to the Clethrionomyini (Conroy and Cook, 1999), so we chose sequences from three widely divergent species (*M. oeconomus*, *M. pennsylvanicus*, and *M. montanus*; Conroy and Cook, 2000) that were representative of sequence variation for the outgroup. For higher level analyses, we included one representative each for eight genera of arvicolines, as well as one sigmodontine (*Peromyscus*).

2.2. DNA extraction, amplification, and sequencing

Subsamples of frozen tissues or dried skins were subjected to proteinase K digestion, NaCl precipitation of proteins, and DNA precipitation with ethanol, following a modification of the Miller et al. (1988) protocol (Fleming and Cook, 2002). Partial segments of the cytochrome *b* (cyt *b*) genes were amplified by the polymerase chain reaction (PCR; usually 30 cycles of alternating denaturation at 93 °C for 1 min, annealing at 45 °C for 1 min, and extension at 72 °C for 1.5 min) using several primer combinations (Conroy and Cook, 1999). All PCR experiments included negative controls.

Aliquots (5 μl) of the PCR products and negative controls were visualized in agarose minigels. Each remaining product was precipitated with polyethylene glycol, recovered by vacuum centrifugation, and resuspended in 1× TE buffer. The clean products were used as template in cycle sequencing utilizing a Perkin–Elmer kit (Fst-RR, 402119). Cycle sequencing products were purified with Sephadex G-50 (Sigma) in reusable columns (Princeton Separations) and run on 5% polyacrylamide gels using an automated sequencer (ABI 373). Both heavy and light strands were sequenced and compared in all cases.

2.3. Sequence and phylogenetic analysis

Partial sequences were examined, assembled based on overlapping regions and aligned manually using the Sequence Navigator program (Applied Biosystems, version 1.01). Nucleotide composition and numbers of variable and phylogenetically informative sites for nucleotides were obtained using PAUP* (version 4.0d65, Swofford, 1998). Phylogenetic analyses used maximum parsimony (MP), distance (NJ), and likelihood (ML) frameworks using PAUP*, and Bayesian analyses were conducted in MrBayes (Huelsenbeck and Ronquist, 2001).

2.4. Higher level analyses

We tested the validity and composition of the tribe Clethrionomyini by incorporating 12 outgroup taxa (Table 1) representing the Arvicolinae (genera *Arvicola*, *Dicrostonyx*, *Ellobius*, *Lemmus*, *Myopus*, *Ondatra*, *Phenacomys*, *Synaptomys*, and three species of *Microtus*) and the Sigmodontinae (*Peromyscus*) for which complete cytochrome *b* sequences had been generated previously (Conroy and Cook, 1999).

Maximum parsimony analyses were performed using a simple addition of sequences with 1000 random additions to avoid suboptimal islands of trees. MP analyses were unweighted or included a weighting bias of 2:5:1 for first, second, and third positions, respectively, and a 10:1 weighting bias towards transversions over transitions (Lessa and Cook, 1998). Major relationships identified by weighted and unweighted methods did not differ so bootstrap analyses (500 iterations of branch and bound search) were conducted using unweighted MP.

A hierarchical-likelihood ratio test was conducted in Modeltest 3.04 (Posada and Crandall, 1998) to determine the simplest model of sequence evolution that best fits the sequence data. The general time reversible (GTR; Rodríguez et al., 1990) plus invariant sites ($I = 0.6349$) and gamma distribution of variable sites (1.6489) was chosen as it was significantly better than models with fewer parameters. The neighbor-joining algorithm using distances from GTR + I + gamma model was used to reconstruct evolutionary relationships. Support for the relationships was assessed in neighbor-joining bootstrap analysis (1000 replicas).

The Bayesian approach to phylogeny reconstruction (Mau et al., 1999; Rannala and Yang, 1996; Yang and Rannala, 1997) was used to reconstruct the evolutionary relationship using the program MrBayes (Huelsenbeck and Ronquist, 2001). The GTR + invariable sites, plus gamma distribution model of DNA substitution was used with the Markov chain started from a random tree with random branch lengths. Four Markov Chain Monte Carlo (MCMC) chains were run simultaneously

Table 1
List of specimens examined

Taxa	Source	GenBank
Ingroup		
Alticola macrotis		
1 Russia, Providenya	AF 7474	AY309411
2 Russia, Stokovo	AF 3791	AF 119273
Clethrionomys californicus		
1 California, Cabin Creek	LHS 642	AY309422
2 California, Cabin Creek	LHS 541	AY309423
Clethrionomys gapperi		
Alaska, Foggy Bay	AF 4270	AY309435
1 Minnesota	AF17697	AY309432
2 Minnesota	no voucher	AY309431
1 North Carolina, Beartrail Ridge	IF 5705	AY309430
2 North Carolina, Silers Bald	IF 5708	AY309429
1 Pennsylvania (partial sequence)	AF 22609	AY309433
2 Pennsylvania	AF 22610	AY309434
Clethrionomys glareolus		
1 Finland, Sotkomo	AF 3133	AF119272
2 Finland, Sotkomo	UAM 30029	AY309419
1 United Kingdom, Wales	UAM 24464	AY309420
2 United Kingdom, Wales	UAM 24466	AY309421
Clethrionomys rex (*C. rufocanus*, *Eothenomys rex*)		
1 Japan, Rishiri	HS229	AB017239
2 Japan, Rishiri	HS494	AB017240
Clethrionomys rufocanus		
1 Finland, Kilpisjarvi	AF 3153	AY309413
2 Finland, Kilpisjarvi	AF 3157	AY309414
1 Japan, Hokkaido, Ishikari-shi	IF 5700	AY309416
2 Japan, Hokkaido, Ishikari-shi	IF 5701	AY309417
3 Japan, Hokkaido, Ishikari-shi	IF 5702	AY309418
4 Japan, Rishiri	HS 227	AB017232
5 Japan, Hokkaido	HS 272	AB017233
6 Japan, Hokkaido, Naganuma	HS 231	AB017234
1 Russia, Kola Peninsula	AF 15465	AY309415
2 Russia, Kola Peninsula	AF 15469	AF272640
3 Russia, Magadan	AF 6590	AY309412
4 Russia, Sakhalin	HS 639	AB017235
5 Russia, Vladivostok	HS 943	AB017236
Clethrionomys rutilus		
1 Alaska, Cordova	AF 1995	AY309426
2 Alaska, Yakutat	AF 2041	AY309427
3 Alaska, Healy	AF 4853	AF119274
1 Finland, Sotkamo	AF 3130	AF272638
2 Finland, Kilpisjarvi	AF 3151	AY309428
Japan, Hokkaido, Bekkai	HS 444	AB017241
1 Russia, Novosibirsk	HS 1165	AB017242
2 Russia, Stokovo	AF 3764	AY309424
3 Russia, Stokovo	AF3765	AY309425
Eothenomys imaizumii (*Phaulomys andersoni*), Japan, Honshu	HS495	AB017246
Eothenomys melanogaster, Taiwan	HS1503	AB017254
Eothenomys regulus (*C. regulus*)		
1 Korea, Solak	HS680	AB017237
2 Korea, Mt. Chiri	HS276	AB017238
Phaulomys andersoni (*E. andersoni*)		
1 Japan, Honshu, Aomori	HS388	AB017243
2 Japan, Honshu, Fukushima	HS335	AB017244
3 Japan, Hokkaido, Nikko	HS332	AB017245

Table 1 (*continued*)

Taxa	Source	GenBank
Phaulomys smithii (*E. smithii*)		
1 Japan, Honshu, Kyoto	HS258	AB017249
2 Japan, Honshu, Mt. Tsurgi	HS262	AB017250
3 Japan, Honshu, Mt. Tsurgi	HS1032	AB017251
4 Japan, Kyushu, Fukuoka	HS1504	AB017252
5 Japan, Kyushu, Miyazaki	HS79	AB017253
Outgroups		
Arvicola terrestris, Finland	AF 22737	AF 119269
Dicrostonyx groenlandicus, Alaska	AF 2246	AF 119268
Ellobius tancrei, Tadjikistan	AF 27782	AF 119270
Lemmus trimucronatus, Alaska	AF 7421	AF 119276
Myopus schisticolor, Finland	UAM 30030	AF 119263
Ondatra zibethicus, Alaska	AF 7445	AF 119277
Phenacomys intermedius, British Columbia	AF 12726	AF 119260
Synaptomys borealis, Alaska	AF 1196	AF 119259
Microtus montanus, Utah	NK55041	AF 119280
Microtus oeconomus, Russia	HEH040	AF 163902
Microtus pennsylvanicus, New Mexico	NK11205	AF 119279
Peromyscus keeni, Alaska	AF 17750	AF 119261

Classification follows Musser and Carleton (1993), except that *Clethrionomys rex* is recognized as a synonym of *Clethrionomys rufocanus* and *Eothenomys imaizumii* is a synonym of *Phaulomys andersoni*.

for one million generations with the resulting trees sampled at every tenth generation (saving 100,000 trees). The first 100,000 generations (10,000 trees) were discarded as burn in (chain had not become stationary) and only the results from the last 900,000 generations (90,000 trees) were used to compute a consensus tree. We ran three additional analyses starting with random trees. Consensus of all of the post-burn in generations (3600,000 generations resulting in 360,000 trees) was computed from all four runs.

2.5. Relationships among species of red-backed voles

The phylogenetic relationships of the 12 species (49 individuals) of red-backed voles of the genera *Alticola*, *Clethrionomys*, *Eothenomys*, and *Phaulomys* (sometimes considered *Eothenomys*) was evaluated within ML. To avoid computational constraints under the ML framework, we reduced the sample size by including only a single sequence to represent all individuals that differed by less than 2.75% ($n = 17$; uncorrected P distance values) and two outgroup taxa. Modeltest was used in conjunction with PAUP* to hierarchically test among nested DNA substitution models with parameters estimated from a MP tree (unweighted heuristic search). This method selected the GTR model of DNA substitution (Rodríguez et al., 1990) with invariant sites and variable sites distributed according to a gamma distribution. Assumed proportion of invariant sites, I, was 0.5696. α, the shape parameter, was estimated to be 0.8405.

These parameters were used for a heuristic search for a ML topology. In additional heuristic searches, we included constraints for (1) monophyly of *Clethrionomys*,

(2) monophyly of North American endemic taxa and *C. rutilus*, and (3) monophyly of *C. gapperi*. We then performed a Shimodaira–Hasegawa test (Shimodaira and Hasegawa, 1999) in PAUP* with full optimization with 1000 bootstrap replicates. Due to multiple comparisons ($n = 3$), a sequential Bonferroni correction was used to adjust α to 0.017. We also conducted a heuristic search constrained to a molecular clock.

3. Results

3.1. Compositional biases, and patterns and levels of variation

All sequences obtained follow the patterns of compositional bias common among mammalian mitochondrial cytochrome *b* (e.g., Irwin et al., 1991). Average base frequencies for all positions were: A (30.1%); C (29.7%); G (13.0%); and T (27.1%) for 29 taxa of Clethrionomyini with complete cyt *b* sequences. There is a deficiency in guanines in the light strand of all individuals (12.5–13.6%). Observed substitutions were most abundant in third positions and least common in second positions. Observed, uncorrected transitions outnumber transversions in pairwise comparisons of haplotypes that exhibit relatively little divergence (e.g., 9:1 in cyt *b* for the comparison of two samples of *C. rutilus*). These substitutions and biases are expected for functional mitochondrial cyt *b* genes. For *C. rutilus* and *C. rufocanus*, sequences generated in our laboratory and those available from GenBank were comparable and clustered together in all analyses.

3.2. Higher level relationships

The four (*Clethrionomys*, *Eothenomys*, and *Phaulomys* and *Alticola*) genera of Clethrionomyini (Musser and Carleton, 1993) consistently formed a clade when examined under MP, distance, and Bayesian frameworks (Fig. 2). *Clethrionomys* were found to be paraphyletic with respect to *Alticola* and *Eothenomys*. Haplotypes of *C. rufocanus* formed a clade with species of *Eothenomys* (except *E. melanogaster*) and *Phaulomys*. All other species of *Clethrionomys* formed a clade that included *Alticola macrotis*. Individuals of *C. gapperi*

from eastern North America were more closely associated with *C. glareolus* than with *C. gapperi* from Minnesota and Alaska. Samples of *E. imaizumii* were closely related to species of *Phaulomys*, a result that may be consistent with Musser and Carleton's (1993) suggestion that this species should be considered *P. andersoni*.

3.3. Relationships among species of red-backed voles

Distance values for pairwise comparisons ranged from 0 for identical sequences of two samples of *C. gapperi* to 0.10849 (uncorrected *p*) between *C. rufocanus*

Fig. 2. Bayesian estimate (using GTR + *I* + gamma) of the phylogenetic relationship of 12 species of Clethrionomyini and 11 other members of the Arvicolinae. A representative of the Sigmodontinae (*Peromyscus keeni*) was included as outgroup. This is a consensus tree of four runs of 1,000,000 generations after discarding the burn in generations (100,000 generations). Numbers above the branches are the posterior probabilities that the clade is correct. MP and NJ trees are not shown, but had topologies consistent with the well-supported clades identified by the Bayesian assessment.

from Russia (IF5702) and *C. californicus*. There was a significant difference between the best topology and one enforcing monophyly of *Clethrionomys* (to the exclusion of *Alticola*, *Eothenomys* and *Phaulomys*) and a significant difference between the best topology and one enforcing monophyly of North American representatives. However, a topology constrained to monophyly of *C. gapperi* was not significantly different from the ML tree (Table 2, Fig. 3).

A clock-constrained tree (ln = −4583.44156) was compared with the best ML tree with a χ^2 test (difference of 15.05; 17 df) and was not significantly different ($\alpha = 0.05$).

Table 2
Comparison of the optimal (unconstrained) maximum likelihood tree score (−4568.37136) with likelihood scores from constrained trees using the Shimodaira–Hasegawa test (Shimodaira and Hasegawa, 1999)

Constraint-monophyly	Best tree score	P value
Clethrionomys	−4609.17446	<0.010*
North American *Clethrionomys*	−4620.50724	<0.001*
Clethrionomys gapperi	−4571.33859	0.538

* $P < 0.017$ (α adjusted for multiple comparisons).
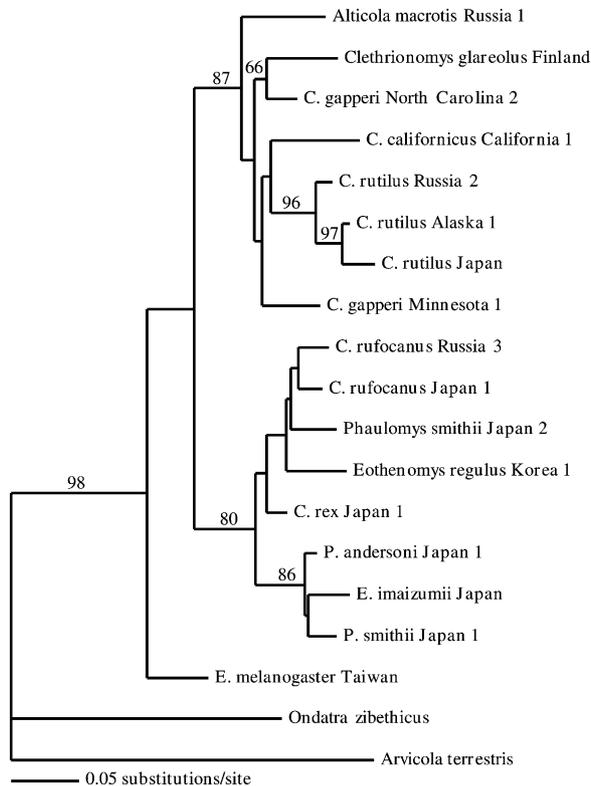
Fig. 3. ML tree constructed from cytochrome *b* sequences for 12 species of the Clethrionomyini and two outgroup taxa. Multiple specimens of *C. gapperi*, *C. rutilus*, *C. rufocanus*, and *P. smithii* were included. Phylogeny was constructed using GTR + *I* + gamma mode of sequence evolution. Bootstrap support > 65 is shown above the branches.

4. Discussion

4.1. Transcontinental exchange

The dynamic climate and landscapes of the Holarctic during the Pleistocene affected the movement and evolution of boreal organisms. As glacial advances lowered sea levels, the exposed Bering Land Bridge and surrounding unglaciated region called Beringia (Hultén, 1937) became the high latitude crossroads between Asia and North America for a number of organisms. Colonization across the region was not symmetric with most organisms suspected to have expanded eastward into North America due to differences in the extent of glaciation along the eastern and western borders of Beringia (Rausch, 1994). While western Beringia had a leaky boundary with the remainder of eastern Asia, movement out of western Beringia into the lower latitudes of North America was blocked by the coalescence of the Laurentide and Cordilleran ice sheets (Pielou, 1991). Exceptions to this generalization have been proposed (Fedorov, 1999; Steppan et al., 1999).

A more detailed view of the timing and extent of glacial advances (Arkhipov et al., 1986a,b; Bespalyy, 1984; Mann and Hamilton, 1995) in Beringia, combined with interpretation of fossil invertebrates (Elias et al., 1996, 2000) and extensive paleobotanical work (Ager, 1982; Bliss and Richards, 1982; Colinvaux and West, 1984; Giterman et al., 1982; Ritchie and Cwynar, 1982) has amplified our understanding of Beringia during the Cenozoic (West, 1996). The extensive Quaternary record of fossil holarctic mammals also has played a prominent role in interpreting intercontinental movement and paleoenvironments in the region (e.g., Guthrie, 1990). Those studies and others (e.g., Hoffmann, 1985) have challenged the early characterization of Beringia as barren tundra; suggesting instead that this region supported a diversity of habitats, including steppe-tundra, taiga, dry upland tundra, and wet lowland tundra (but see Elias et al., 1996; Guthrie, 2001). Diversification of red-backed voles appears to be intimately tied to this dynamic environmental history.

The mitochondrial phylogeny combined with the fossil record for red-backed voles provides a framework for assessing the extent and relative timing of colonization events. We found three primary clades within the Clethrionomyini; the Asian clade (*Eothenomys*); Transberingian clade; and the basal *E. melanogaster* of Taiwan (Fig. 2).

Fossils for *Alticola* date to the early Middle Pleistocene in the Trans-Baikal region (Gromov and Polyakov, 1977). The earliest fossils of *Clethrionomys* are from approximately 2.5 million years ago in France (Chaline and Graf, 1988) or Moldavia (Gromov and Polyakov, 1977). Hence, an Old World origin for the earliest species of the genus has been accepted by most

paleontologists. *C. rufocanus* (and apparently species of the genus *Eothenomys*) was isolated in eastern Asia from other red-backed vole species by the beginning of the middle Pleistocene (~700,000 years ago; Chaline and Graf, 1988).

The earliest colonization of North America was by a progenitor of *C. gapperi*. Based on the fossil record, estimates of the timing of this first colonization vary considerably, ranging from the early Pleistocene (Repenning et al., 1990), to the end of the middle Pleistocene (Hibbard et al., 1965), to about the Late Irvingtonian/Rancholabrean (Kurtén and Anderson, 1980). The close relationship of eastern North American *C. gapperi* and *C. glareolus* is consistent with Hinton's (1926, 267) statement that these species are nearly indistinguishable morphologically. Earliest Old World fossils of *C. glareolus* are from the early middle Pleistocene of central and western Europe (Gromov and Polyakov, 1977). Given the phylogeny for North American species of *Clethrionomys*, we suspect a late Pliocene/early Pleistocene colonization of North America by an ancestral form of *C. gapperi* that was sister to *Alticola*. The paraphyletic relationship of *C. gapperi* with respect to *C. glareolus* is intriguing and suggests the possibility of retention of ancestral lineages or a secondary colonization of Asia and Europe by ancestors of *C. glareolus*.

The colonization pattern for *C. rutilus* is also not resolved. The earliest fossils of *C. rutilus* reported from Siberia and eastern Asia are from the late Pleistocene (Gromov and Polyakov, 1977), but this species is not documented from North America until the Holocene. This preliminary molecular view of the species indicates little genetic differentiation across the Bering Straits reflecting the Late Pleistocene connection (Bering Land Bridge) across the continents. Although the existing fossil record indicates a recent colonization of North America, larger sample sizes, additional markers, and a coalescent analysis of genetic variation might allow a test for a signal of population expansion.

Phylogenetic investigations of several bird taxa (Zink et al., 1995), voles of the genus *Microtus* (Brunhoff et al., 2003; Conroy and Cook, 2000), marmots (Steppan et al., 1999), arctic hares (Halanych et al., 1999), and lemmings (Fedorov, 1999; Fedorov et al., 2003) have begun to elucidate the relationships of trans-Beringian organisms. Examination of details of genetic divergence among these taxa is beginning to provide a view of the relative timing of colonization or vicariant events. In a few cases, species suspected to be close relatives based on morphological characters appear to be only distantly related based on DNA sequences. For example, Conroy and Cook (2000) evaluated the narrow-skull voles of the genus *Microtus* (subgenus *Stenocranius*) and concluded that the Siberian *M. gregalis* is not closely related to the New World *M. miurus* contrary to previous suggestions of a close sister-taxon relationship.

4.2. Evolutionary relationships of the red-backed voles of North America

The phylogenetic relationships and geographic limits of North American species of *Clethrionomys* have long been debated (e.g., Bee and Hall, 1956). These species inhabit boreal biomes and, with the exception of holarctic *C. rutilus*, are endemic to North America (Fig. 1). Several investigators have questioned the validity of particular species pairs based on apparent intergradation of morphologic and biochemical characters in zones of contact. For example, although *C. rutilus* and *C. gapperi* are not sister taxa, they are difficult to distinguish. These have distinctive biogeographic histories and have contacted each other within the Holocene across a broad east/west zone that spans western and central Canada. Variation in pelage and other morphologic characters is minor (Hall, 1981) and seems to converge where these species contact (Bee and Hall, 1956; Youngman, 1975). A protein electrophoresis study (Canham and Cameron, 1972) aimed at distinguishing *C. gapperi* and *C. rutilus* did not equivocally identify either species. Mitochondrial introgression in a zone of overlap between these species has been documented in Southeast Alaska (Runck, 2001). Introgression apparently is not uncommon among species of *Clethrionomys* (Grant, 1974; Suzuki et al., 1999; Tegelström, 1987) and may further complicate the delineation of species in *Clethrionomys*.

C. rutilus apparently is a post-glacial colonizer of North America from Asia based on the fossil record and close genetic relationships with Far Eastern Siberian populations (Iwasa et al., 2002, this study). Although little genetic differentiation exists among populations of *C. rutilus* that have been sampled in North America, substantial phylogeographic structure has been identified in this red-backed vole species in Asia (Iwasa et al., 2002). In contrast, *C. gapperi* has a much longer history in North America where this species shows significant phylogeographic structure.

Based on the mitochondrial DNA sequences, *C. gapperi* may be paraphyletic with respect to *C. glareolus* and *C. californicus*. Members of the western clade of *C. gapperi* are more closely related to *C. californicus*, while the eastern clade forms a sister group relationship with *C. glareolus*, a palearctic species. The position of *C. californicus* is unresolved. The potentially close relationship of *C. glareolus* and *C. gapperi* has been previously suspected. Grant (1974) produced hybrids between *C. gapperi* (representative of eastern clade) and *C. glareolus* and suggested they were semispecies. Clearly, more extensive geographic sampling is needed to understand the significance of the apparent paraphyly of *C. gapperi*. The east/west break in *C. gapperi* appears to mimic geographic discontinuities identified in other forest species such as black bear

(Stone and Cook, 2000; Wooding and Ward, 1997) and marten (Stone et al., 2002) and other species (e.g., Cook et al., 2001). Our limited sampling indicates that members of the eastern clade apparently spread northward following the retreating Laurentide ice-sheet (MacPherson, 1965). In Asia, substantial intraspecific variation also has been uncovered in *C. rufocanus* and *C. rutilus* (Iwasa et al., 2000, 2002) and some of those major phylogeographic discontinuities may parallel breaks found in other taxa (Brunhoff et al., 2003).

4.3. Taxonomy of red-backed voles

This preliminary view of the evolutionary history of red-backed voles has implications at various taxonomic levels. Relationships among genera in the Clethrionomyini are not well resolved, but they apparently form a closely related clade (Gromov and Polyakov, 1977). Of all the species of red-backed voles that we analyzed, *Eothenomys melanogaster* is the most distinctive. The paraphyly of species of *Clethrionomys*, with respect to *Alticola*, *Phaulomys*, and *Eothenomys* (and paraphyly of species of *Eothenomys* with respect to *Clethrionomys*), suggests that either these genera as currently circumscribed are invalid (and all red-backed voles should be called *Clethrionomys*), or that the genus *Clethrionomys* should be realigned into at least two distinct genera. Hooper and Hart (1962) and Corbet (1978, 1984) previously suggested that *Eothenomys* was congeneric with *Clethrionomys*. Gilëva et al. (1989) also demonstrated paraphyly between *Clethrionomys* and *Alticola*, but we included only a single species of *Alticola* in our analyses. With respect to these questions, our results expand the conclusions of Suzuki et al. (1999) who examined evolutionary relationships of three species of *Clethrionomys* and four species of *Eothenomys* from areas surrounding the Sea of Japan using nuclear ribosomal DNA and the partial cyt *b* sequences included in our study.

Collectively, these analyses indicate that *Clethrionomys*, *Eothenomys*, *Phaulomys*, and *Alticola* shared a common ancestor (i.e., the Clethrionomyini may represent a monophyletic clade). Within this primary group, one subclade includes only *Eothenomys melanogaster* of Taiwan, another subclade includes Asian species of *Eothenomys*, *Phaulomys*, and *Clethrionomys*, and a third is comprised of palearctic *Alticola macrotis* and *C. glareolus*, the holarctic *C. rutilus*, and the two species of *Clethrionomys* endemic to North America. *Phaulomys* was proposed to include *E. smithii* and *E. andersoni*, but as noted by Suzuki et al. (1999), *E. smithii* is apparently paraphyletic. We suggest that all of these species of red-backed voles might be placed in the single genus *Clethrionomys* (including species of *Eothenomys* and *Phaulomys*). Further, the close relationship between *E. regulus*, *E. andersoni*, *C. rufocanus*, and *P. smithii* was previously identified by Iwasa and Suzuki (2002a) on the basis sequence variation in the X-linked gene *G6pd*. Detailed analyses of the evolutionary relationships of *E. andersoni* and *P. smithii* (reported as *E. smithii*) were completed by Iwasa and Suzuki (2002b).

This work has also clarified that *C. gapperi* is specifically distinct from *C. rutilus*, a conclusion that some investigators had questioned (Bee and Hall, 1956; Youngman, 1975). Additionally, *C. gapperi*, as currently recognized (Hall, 1981) appears to consist of at least two species.

4.4. Conclusions

These analyses of cyt *b* data support the monophyly of a group that includes the red-backed vole genera *Alticola*, *Clethrionomys*, and *Eothenomys*; however, *Clethrionomys* is found to be paraphyletic with respect to both *Alticola* and *Eothenomys*. Three major clades were identified as Asian (*Eothenomys andersoni*, *E. smithii*, *Clethrionomys regulus*, *C. rex*, and *C. rufocanus*), Holarctic (*Alticola macrotis*, *C. gapperi*, *C. glareolus*, and *C. rutilus*), and Taiwanese (*E. melanogaster*). Additional datasets, especially nuclear DNA, should be developed to test these conclusions before the taxonomy is changed to reflect evolutionary relationships. The distinctive *E. melanogaster* is based on a mitochondrial sequence from a single specimen and more individuals must be sequenced. These results are consistent with the fossil record which indicates an initial diversification of red-backed voles in Asia. That diversification was followed by colonization eastward across the Bering Straits on at least two occasions. The holarctic species, *C. rutilus*, appears to have colonized North America much more recently (late Pleistocene) than the two species of *Clethrionomys* that are endemic to North America. Finally, *C. gapperi*, appears to be comprised of two distinct eastern and western species, the former with affinities to the Asian *C. glareolus* and the latter more closely related to *C. californicus* of western North America. This east/west split is comparable to phylogeographic patterns discovered in other forest associated species in North America.

References

Abbott, R.J., Brochmann, C., 2003. History and evolution of the arctic flora: in the footsteps of Eric Hultén. Mol. Ecol. 12, 299–313.

Ager, T.A., 1982. Vegetational history of western Alaska during the Wisconsin glacial interval and the Holocene. In: Hopkins, D.M. et al. (Eds.), Paleoecology of Beringia. Academic Press, New York, pp. 75–93.

Arkhipov, S.A., Bespaly, V.G., Faustova, M.A., Glushkova, O.Y., Isaeva, L.L., Velichko, A.A., 1986a. Ice-sheet reconstructions. Quaternary Sci. Rev. 5, 475–483.

Arkhipov, S.A., Isayeva, L.L., Bespalyy, V.G., Glushkova, O., 1986b. Glaciation of Siberia and north-east USSR. Quaternary Sci. Rev. 5, 463–474.

Bee, J.W., Hall, E.R., 1956. Mammals of northern Alaska. Misc. Publ. Mus. Nat. Hist. Univ., Kansas 8, 1–309.

Bespalyy, V.G., 1984. Late Pleistocene mountain glaciation in northeastern USSR. In: Velichko, A.A. (Ed.), Late Quaternary Environments of the Soviet Union. University of Minnesota Press, Minneapolis, pp. 31–33.

Bliss, L.C., Richards, J.H., 1982. Present-day Arctic vegetation and ecosystems as a predictive tool for the arctic-steppe mammoth biome. In: Hopkins, D.M. et al. (Eds.), Paleoecology of Beringia. Academic Press, New York, pp. 241–257.

Brunhoff, C., Galbreath, K.E., Fedorov, V., Cook, J.A., Jaarola, M., 2003. Holarctic phylogeography of the root vole (*Microtus oeconomus*): implications for late Quaternary biogeography of high latitudes. Mol. Ecol.

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

Colinvaux, P.A., West, F.H., 1984. The Beringian ecosystem. Q. Rev. Archaeol. 5, 10–16.

Cook, J.A., Bidlack, A.L., Conroy, C.J., Demboski, J.R., Fleming, M.A., Runck, A.M., Stone, K.D., MacDonald, S.O., 2001. A phylogeographic perspective on endemism in the Alexander Archipelago of the North Pacific. Biol. Conserv. 97, 215–227.

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

Conroy, C.J., Cook, J.A., 2000. Molecular systematics of a holarctic rodent (*Microtus*: Muridae). J. Mammal. 81, 344–359.

Corbet, G.B., 1978. The Mammals of the Palearctic Region: A Taxonomic Review, British Museum (London), UK.

Corbet, G.B., 1984. The Mammals of the Palearctic Region: A Taxonomic Review, Supplement, British Museum (London), UK.

Elias, S.A., Berman, D., Alfimov, A., 2000. Late Pleistocene beetle faunas of Beringia: where east met west. J. Biogeol. 27, 1349–1363.

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

Fedorov, F.B., 1999. Contrasting mitochondrial DNA diversity estimates in two sympatric genera of Arctic lemmings (*Dicrostonyx* and *Lemmus*) indicate different responses to Quaternary environmental fluctuations. Proc. R. Soc. London B 266, 621–626.

Fedorov, V.B., Goropashnaya, A.V., Jaarola, M., Cook, J.A., 2003. Phylogeography of lemmings (*Lemmus*): no evidence for postglacial colonization of Arctic from the Beringian refugium. Mol. Ecol. 12, 725–732.

Fleming, M.A., Cook, J.A., 2002. Phylogeography of endemic ermine (*Mustela erminea*) in southeast Alaska. Mol. Ecol. 11, 795–807.

Gilëva, E.A., Rybnikov, D.E., Miroshnichenko, G.P., 1989. DNA-DNA hybridization and phylogenetic relationships in two genera of voles, *Alticola*, and *Clethrionomys* (Microtinae-Rodentia). Doklady Akademii Nauk SSSR 311, 477–480, in Russian.

Giterman, R.E., Sher, A.V., Matthews Jr., J.V., 1982. Comparison of the development of tundra-steppe environments in west and east Beringia: pollen and macrofossil evidence from key sections. In: Hopkins, D.M. et al. (Eds.), Paleoecology of Beringia. Academic Press, New York, pp. 43–73.

Graham, R.W., 1976. Late Wisconsin mammalian faunas and environmental gradients of the eastern United States. Paleobiology 2, 343–350.

Grant, P.R., 1974. Reproductive compatibility of voles from separate continents (Mammalia: *Clethrionomys*). Journal of Zoology, London 174, 245–254.

Gromov, I.M., Polyakov, I.Ya., 1977. Voles (Microtinae), Fauna of the USSR, Mammals, vol. III, No. 8., Smithsonian Institution Libraries and National Science Foundation, Washington, DC, p. 725.

Guthrie, R.D., 1990. Frozen Fauna of the Mammoth Steppe. University of Chicago Press, Chicago.

Guthrie, R.D., 2001. Origin and causes of the mammoth steppe: a story of cloud cover, woolly mammal tooth pits, buckles, and inside-out Beringia. Quaternary Sci. Rev. 20, 549–574.

Guthrie, R.D., Matthews Jr., J.V., 1971. The Cape Deceit Fauna: early Pleistocene mammalian assemblage from the Alaska Arctic. Quaternary Res. 1, 474–510.

Halanych, K.M., Demboski, J.R., van Vuuren, B.J., Klein, D.R., Cook, J.A., 1999. Cytochrome *b* phylogeny of North American hares and jackrabbits (*Lepus*, Lagomorpha) and the effects of mutational saturation in outgroup taxa. Mol. Phylogenet. Evol. 11, 213–221.

Hall, E.R., 1981. The Mammals of North America, second ed. John Wiley and Sons, New York.

Hall, E.R., Cockrum, E.L., 1953. A synopsis of the North American microtine rodents. Univ. Kansas Publ., Mus. Nat. Hist. 5, 373–498.

Hewitt, G.M., 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. Biol. J. Linn. Soc. 58, 247–276.

Hibbard, C.W., Ray, D.E., Savage, D.E., Taylor, D.W., Guilday, J.E., 1965. Quaternary mammals of North America. In: Wright Jr., H.E., Frey, D.G. (Eds.), The Quaternary of the United States, pt. II. Zoogeography and Evolution. Princeton University Press, Princeton, NJ, pp. 509–525.

Hinton, M.A.C., 1926. Monograph of the voles and lemming (Microtinae) living extinct, vol. 1, Trust. Brit. Mus., London.

Hoberg, E.P., 1995. Historical biogeography and modes of speciation across high-latitude seas of the Holarctic: concepts for host–parasite coevolution among the Phocini (Phocidae) and Tetrabothriidae (Eucestoda). Can. J. Zool. 73, 45–57.

Hoberg, E.P., Kutz, S.J., Galbreath, K.E., Cook, J., in press. Arctic biodiversity: from discovery to faunal baselines—revealing the history of a dynamic system. J. Parasitol.

Hoffmann, R.S., 1985. An ecological and zoogeographical analysis of animal migration across the Bering Land Bridge during the Quaternary Period. In: Kontrimavichus, V.L. (Ed.), Beringia in the Cenozoic. Russian Translation Series. A.A. Balkema, Rotterdam, pp. 464–481.

Hoffmann, R.S., 1981. Different voles for different holes: environmental restrictions on refugial survival of mammals. In: Scudder, G.G.E., Reveal, J.L. (Eds.), Evolution Today. Proceedings of the Second International Congress of Systematic and Evolutionary Biology, pp. 25–45.

Hooper, E.T., Hart, B.S., 1962. In: A synopsis of Recent North American microtine rodents, vol. 120. Miscellaneous Publications, Museum of Zoology, University of Michigan, pp. 1–68.

Hopkins, D.M., Matthews Jr., J.V., Schweager, C.E., Young, S.B., 1982. Paleoecology of Beringia. Academic Press, New York.

Huelsenbeck, J.P., Ronquist, F., 2001. MrBayes: Bayesian inference of phylogeny. Bioinformatics 17, 754–755.

Hultén, E., 1937. Outline of the History of Arctic and Boreal Biota during the Quaternary Period. Borförlag Aktiebolaget Thule, Stockholm.

Hundertmark, K.J., Shields, G.F., Udina, I.G., Bowyer, R.T., Danilkin, A.A., Schwartz, C.C., 2002. Mitochrondial phylogeography of moose (*Alces alces*): late Pleistocene divergence and population expansion. Mol. Phylogenet. Evol. 22, 375–387.

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

Iwasa, M.A., Kartavtseva, I.V., Dobrotvorsky, A.K., Panov, V.V., Suzuki, H., 2002. Local differentiation of *Clethrionomys rutilus* in northeastern Asia inferred from mitochondrial gene sequences. Mamm. Biol. (Zeitschrift für Säugetierkunde) 67, 157–166.

Iwasa, M.A., Suzuki, H., 2002a. Evolutionary significance of chromosome changes in northeastern Asiatic red-backed voles inferred with the aid of intron1 sequences of the *G6pd* gene. Chromosome Res. 10, 419–428.

Iwasa, M.A., Suzuki, H., 2002b. Evolutionary networks of maternal and paternal gene lineages in voles (*Eothenomys*) endemic to Japan. J. Mammal. 83, 852–865.

Iwasa, M.A., Utsumi, Y., Nakata, K., Kartavtseva, I.V., Nevedomskaya, I.A., Kondoh, N., Suzuki, H., 2000. Geographic patterns of cytochrome *b* and *SRY* gene lineages in gray-backed voles *Clethrionomys rufocanus* (Mammalia: Rodentia) from Far East Asia including Sakahalin and Hokkaido. Zool. Sci. 17, 477–484.

Johnson, M.L., Ostenson, B.T., 1959. Comments on the nomenclature of some mammals of the Pacific Northwest. J. Mammal. 40, 571–577.

Kurtén, B., Anderson, E., 1980. Pleistocene mammals of North America. Columbia University, New York.

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

MacPherson, A.H., 1965. The origin of diversity in mammals of the Canadian Arctic tundra. Syst. Zool. 14, 153–173.

Mann, D.H., Hamilton, T.D., 1995. Late Pleistocene and Holocene paleoenvironments of the north Pacific coast. Quaternary Sci. Rev. 14, 449–471.

Marincovich Jr., L., Gladenkov, A.Y., 1999. An early opening of the Bering Strait. Nature 397, 149–151.

Mau, B., Newton, M.A., Larget, B., 1999. Bayesian phylogenetic inference via Markov chain Monte Carlo methods. Biometrics. 55, 1–12.

Miller, S.A., Dykes, D.D., Polesky, H.F., 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 16, 1215.

Murray, D.F., 1981. The role of arctic refugia in the evolution of the arctic vascular flora—a Beringian perspective. In: Scudder, G.G.E., Reveal, J.L. (Eds.), Evolution Today. Proceedings of the Second International Congress of Systematic and Evolutionary Biology, pp. 11–20.

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

Pielou, E.C., 1991. After the Ice Age. University of Chicago Press, Chicago.

Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Rannala, B., Yang, Z., 1996. Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. J. Mol. Evol. 43, 304–311.

Rausch, R.L., 1994. Transberingian dispersal of cestodes in mammals. Int. J. Parasitol. 24, 1203–1212.

Repenning, C., Fejfar, O., Heinrich, W.-D., 1990. Arvicolid rodent biochronology of the northern hemisphere. In: Fejfar, O., Heinrich, W. (Eds.), International Symposium on Evolution, Phylogeny, and Biostratigraphy of Arvicolids. Pfeil-Verlag, Prague, pp. 385–418.

Ritchie, J.C., Cwynar, C.C., 1982. The Late Quaternary Vegetation of the North Yukon. In: Hopkins, D.M. et al. (Eds.), Paleoecology of Beringia. Academic Press, New York, pp. 113–126.

Runck, A.M., 2001. Molecular and morphological perspectives in post-glacial colonization of *Clethrionomys rutilus* and *Clethrionomys gapperi* in Southeast Alaska. M.S. thesis, University of Alaska, Fairbanks, 89 pp.

Rodríguez, F., Oliver, J.F., Marín, A., Medina, J.R., 1990. The general stochastic model of nucleotide substitutions. J. Theor. Biol. 142, 485–501.

Sher, A., 1999. Traffic lights at the Beringian crossroads. Nature 397, 103–104.

Shimodaira, H., Hasegawa, M., 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Mol. Biol. Evol. 16, 1114–1116.

Steppan, S.J., Hakhverdyan, M., Lyapunova, E., Vorontsov, N., Fraser, D.G., Hoffmann, R.S., Braun, M.J., 1999. Molecular phylogeny of the marmots (Rodentia: Sciuridae): tests of evolutionary and biogeographic hypotheses. Syst. Biol. 48, 715–734.

Stone, K.D., Cook, J.A., 2000. Phylogeography of black bears (*Ursus americanus*) from the Pacific Northwest. Can. J. Zool. 78, 1–6.

Stone, K., Flynn, R., Cook, J., 2002. Post-glacial colonization of northwestern North America by the forest associated American marten (*Martes americana*). Mol. Ecol. 11, 2049–2064.

Suzuki, H., Iwasa, M., Harada, M., Wakana, S., Sakaizumi, M., Han, S.-H., Kitahara, E., Kimura, Y., Kartavtseva, I., Tsuchiya, K., 1999. Molecular phylogeny of red-backed voles in Far East Asia based on variation in ribosomal and mitochondrial DNA. J. Mammal. 80, 512–521.

Swofford, D.L., 1998. "PAUP*. Phylogenetic Analysis Using Parsimony (* and other methods). Version 4.0b6." Sinauer Associates, Sunderland, MA.

Tegelström, H., 1987. Transfer of mitochondrial DNA from the northern red-backed vole (*Clethrionomys rutilus*) to the bank vole (*C. glareolus*). J. Mol. Evol. 24, 218–227.

West, F.H., 1996. American Beginnings: The Prehistory and Paleoecology of Beringia. Univ. Chicago Press, Chicago.

Wooding, S., Ward, R., 1997. Phylogeography and Pleistocene evolution in the North American black bear. Mol. Biol. Evol. 14, 1096–1105.

Yang, Z., Rannala, B., 1997. Bayesian Phylogenetic Inference Using DNA Sequences: A Markov Chain Monte Carlo Method. Mol. Biol. Evol. 14, 717–724.

Youngman, P.M., 1975. Mammals of the Yukon Territory. National Museum of Canada, Publication in Zoology 10, 1–192.

Zink, R.M., Rohwer, S., Andreev, A.V., Dittmann, D.L., 1995. Trans-Beringian comparisons of mitochondrial DNA differentiation in birds. Condor 97, 639–649.