

Phylogeography of a post-glacial colonizer: *Microtus longicaudus* (Rodentia: Muridae)

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Abstract

The molecular phylogeography of *Microtus longicaudus* was investigated with DNA sequences of the mitochondrial cytochrome *b* gene. We used phylogenetic and pairwise distance methods to reconstruct the history of the species with particular emphasis on the Pacific Northwest. Genetic variation across the species was consistent with vicariant events during the Pleistocene and subsequent northern postglacial expansion following the receding Laurentide and Cordilleran ice sheets. The largest break (> 6% uncorrected sequence divergence) was found to exist between populations found southeast of the Colorado River (eastern Arizona, Colorado, Wyoming and New Mexico) and all other western populations. Other well-supported subclades were composed of samples from: (i) the islands and north coast of southeast Alaska; (ii) eastern Alaska, British Columbia, Washington and Oregon; and (iii) northern California, Idaho and Montana. Within subclades, divergence was low. Our results suggest that the close relationships among haplotypes within northern subclades are a result of recent colonization, whereas higher among-subclade divergence is caused by genetic differentiation during prolonged periods of isolation, possibly as a result of mid-Pleistocene climatic events.

Keywords: Alaska, climate, cytochrome *b*, glaciation, long-tailed vole, population

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Introduction

The retreat of the Laurentide and Cordilleran ice sheets in North America at the end of the Pleistocene ($\approx 10\,000$ years ago) provides an opportunity to assess the genetic structure of colonizing populations of organisms. Genetic and phylogeographical structure among post-glacial populations may be impacted by the extent of: (i) population expansion; (ii) vicariant events; (iii) differing dispersal abilities; and (iv) refugial isolation (Marjoram & Donnelly 1994; Slatkin & Hudson 1991; Hewitt 1993, 1996; Ibrahim *et al.* 1996). For example, populations inhabiting recently colonized regions may be predicted to exhibit star-like lineage topology when examined using phylogenetic methods. Furthermore, smooth mismatch distributions in pairwise comparisons of DNA sequences would be expected for recently colonizing populations (e.g. see Slatkin & Hudson 1991; Fedorov *et al.* 1996; Jaarola

& Tegelström 1996; Merilä *et al.* 1997). Genetic divergence (e.g. DNA substitutions) between clades within species is predicted if there has been expansion from separate refugia or source areas. Widely divergent haplotypes that are sympatrically distributed might indicate secondary contact. Populations that have persisted in or near glacial refugia should have deeper among-clade branch lengths than found within expanding populations (Bernatchez & Wilson 1998).

Previously glaciated regions of North America are occupied by organisms that have colonized since the Pleistocene. These populations characteristically have reduced levels of genetic variation when compared with southerly conspecific populations (Sage & Wolff 1986; Hayes & Harrison 1992; Merilä *et al.* 1997; Soltis *et al.* 1997). However, levels of genetic variation in populations in northwestern North America may be confounded by admixture among populations expanding from multiple refugia south of the ice sheets (Soltis *et al.* 1997), or from refugia further north along the Pacific Coast (Heaton *et al.* 1996) or from Beringia (MacDonald & Cook 1996).

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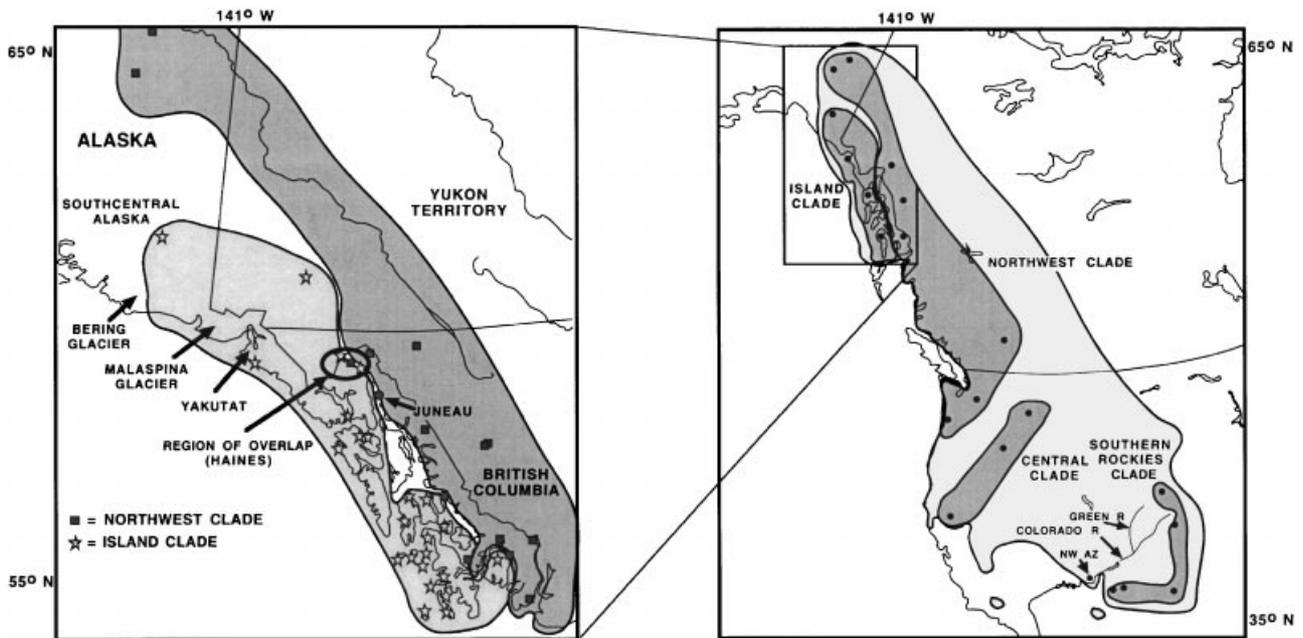


Fig. 1 Map with distribution of sampled localities and general distribution of clades within *Microtus longicaudus*. Lines enclosing clades should not be construed as the actual geographical limit.

Microtus longicaudus

M. longicaudus, the long-tailed vole, is a North American representative of a speciose holarctic genus. This herbivorous rodent occurs in western montane taiga from New Mexico and Arizona, north into British Columbia, Yukon Territory and Alaska (Smolen & Keller 1987). The long-tailed vole occurs in isolated mesic habitats on western and southwestern mountains, but has a more continuous distribution at higher latitudes and along the North Pacific Coast. It shares this distribution with many plants and mammals that apparently expanded northward following glacial retreat. Populations also are isolated in the mountains of the Great Basin (Lomolino *et al.* 1989; Patterson 1995) and the southern Sierras.

The Pleistocene fossil record has been interpreted to indicate that most taxa exhibit independent responses to climatic change (Graham *et al.* 1996). This hypothesis is being tested by a growing body of molecular data at the intraspecific level for a variety of species. In some cases, these studies provide an alternative perspective on the geography of plant and animal movement in the Holocene. For instance, phylogeographical studies (e.g. Bermingham & Martin 1998; Da Silva & Patton 1998; Taberlet *et al.*, 1998) may help to identify or characterize Pleistocene refugia, postglacial invasions and variation within species, previously undetected by morphological studies.

In this study we characterized the genetic variation of postglacial range expansion of long-tailed voles by examining DNA sequence variation across populations.

We focused on populations along the North Pacific Coast within the context of variation across the entire distribution of the species.

Materials and methods

Sampling

Specimens were obtained across the range of *Microtus longicaudus* with a particular emphasis on localities north of 54° North latitude along the Pacific Coast (Fig. 1, Appendix I). This region has a complex biogeographical history (Klein 1965; Scudder & Gessler 1989; MacDonald & Cook 1996; Conroy *et al.* 1999) and has been suggested to support palaeoendemic populations of mammals (Heaton *et al.* 1996). Because many northern populations have descended from periglacial lineages within the last 10 000 years, we investigated genetic variation within and between phylogenetically defined lineages (e.g. Stone & Stoneking 1998) in the northern part of the range. Our goal was to characterize these northern lineages relative to more southern ones, not to assess genetic variation throughout the entire range.

Molecular methods

We examined variation in the mitochondrial cytochrome *b* gene (*cyt-b*) in 111 specimens of *M. longicaudus*. Of those, we sequenced the complete *cyt-b* gene (1143 bases) from 72 specimens and a region of the gene (409 bases) from 39

specimens using methods described in Conroy & Cook (2000). Sequences have been deposited on GenBank with accession nos: AF187160–187230; AF205285–205318. PAUP* (test version 4.0d64; Swofford 1997) was used for genetic and phylogeographical analyses. Variable nucleotides and amino acids, and the synonymous to replacement ratio, were examined both between and within species by using a G-test (McDonald & Kreitman 1991). We rooted phylogenetic trees with *cyt-b* sequences from the closely related species, *M. pennsylvanicus* and *M. montanus* (Conroy & Cook 1999). Neighbour-joining trees (Saitou & Nei 1987) were based on complete *cyt-b* sequences and Kimura 2-parameter distances (Kimura 1980). This distance method corrects for multiple substitutions, but assumes equal base frequencies. However, trees constructed with more complex models (e.g. Tamura & Nei 1993) did not differ in the branching order among major subclades. Only individuals with complete *cyt-b* sequences were used in bootstrap resampling (5000 replicas) to identify well-supported clades. Depth of divergence in *cyt-b* within *M. longicaudus* was compared with 23 other species of *Microtus* (Conroy & Cook 2000).

Clades with at least five individuals and bootstrap support greater than 50% were used for analysis of within-clade diversity. Gene diversity was calculated for all samples and also examined within clades using ARLEQUIN (Excoffier *et al.* 1997). Pairwise mismatches were calculated, plotted and tested against a sudden-expansion model for expanding populations (Watterson 1975; Rogers 1995). We estimated the relative time of divergence of mitochondrial lineages using a maximum-likelihood distance based on interspecific comparisons (Conroy & Cook 2000). We assumed that the deepest phylogenetic splits within the genus *Microtus* should approximate the

oldest branching events in the group. Therefore, we set the genetic distance between *M. gregalis* and *M. oregoni*, the deepest split, to 2.2 million years divergence, based on the fossil record (Repenning *et al.* 1990). χ^2 -tests of rate heterogeneity indicated that species of *Microtus* were evolving in a clock-like manner (Conroy & Cook 2000). We estimated the average pairwise distances within clades under the HKY85 (Hasegawa *et al.* 1985) + Γ model ($\alpha = 0.2159$, transition/transversion (ti/tv) ratio = 2.6) using PAUP*, and then subtracted these distances from between-clade differences for a net divergence time (Edwards 1997; Avise & Walker 1998). We also tested for rate heterogeneity among the complete *cyt-b* sequences within *M. longicaudus* by evaluating maximum-likelihood (ML) trees with and without a molecular clock constraint (Felsenstein 1988).

Results

Nuclear copies of *cyt-b* have been described for a variety of genera of mammals, including *Microtus* (DeWoody *et al.* 1999). Variation across the third (178 variable sites, 78% of all variable sites), first (39 variable sites, 17% of all variable sites) and second (12 variable sites, 5% of all variable sites) positions of codons was distributed as expected for genuine sequences of mammalian *cyt-b* (Table 1). Base composition (A: 31%, C: 27%, G: 13%, T: 29%) was similar to other mammals (Irwin *et al.* 1991; Lessa & Cook 1998) and other species of *Microtus* (Conroy & Cook 1999). The distribution of 45 variable amino acid sites along the gene was consistent with structural and functional models of variation (e.g. Irwin *et al.* 1991). The ratio of replacement to synonymous sites was the same when compared between and within species, suggesting that directional selection on *cyt-b* was minimal (McDonald & Kreitman 1991).

Table 1 Descriptive statistics of genetic variation by major clade

	Southern Rockies	Northwest	Island	Central	Total plus outgroups
Number of individuals	8	25	27	8	71
Polymorphic sites	64	63	104	31	229
Observed transitions	58	55	96	30	211
Observed transversions	7	11	8	1	33
Transition/transversion ratio	8.29	5	12	30	6.39
Substitutions	65	66	104	31	244
C	34.57%	35.94%	36.40%	26.21%	37.66%
T	29.49%	26.79%	26.10%	37.90%	26.30%
A	21.68%	20.13%	22.86%	20.16%	27.20%
G	14.26%	17.14%	14.64%	15.73%	8.83%
Mean pairwise differences	28.12	11.21	12.26	10.07	29.2
(± 1 standard deviation)	± 13.80	± 5.26	± 5.71	± 5.16	± 12.9
Mismatch observed	255.06	22.61	16.43	15.11	264.46
variance					
Nucleotide diversity	0.44 ± 0.25	0.18 ± 0.09	0.12 ± 0.06	0.32 ± 0.19	0.09 ± 0.05

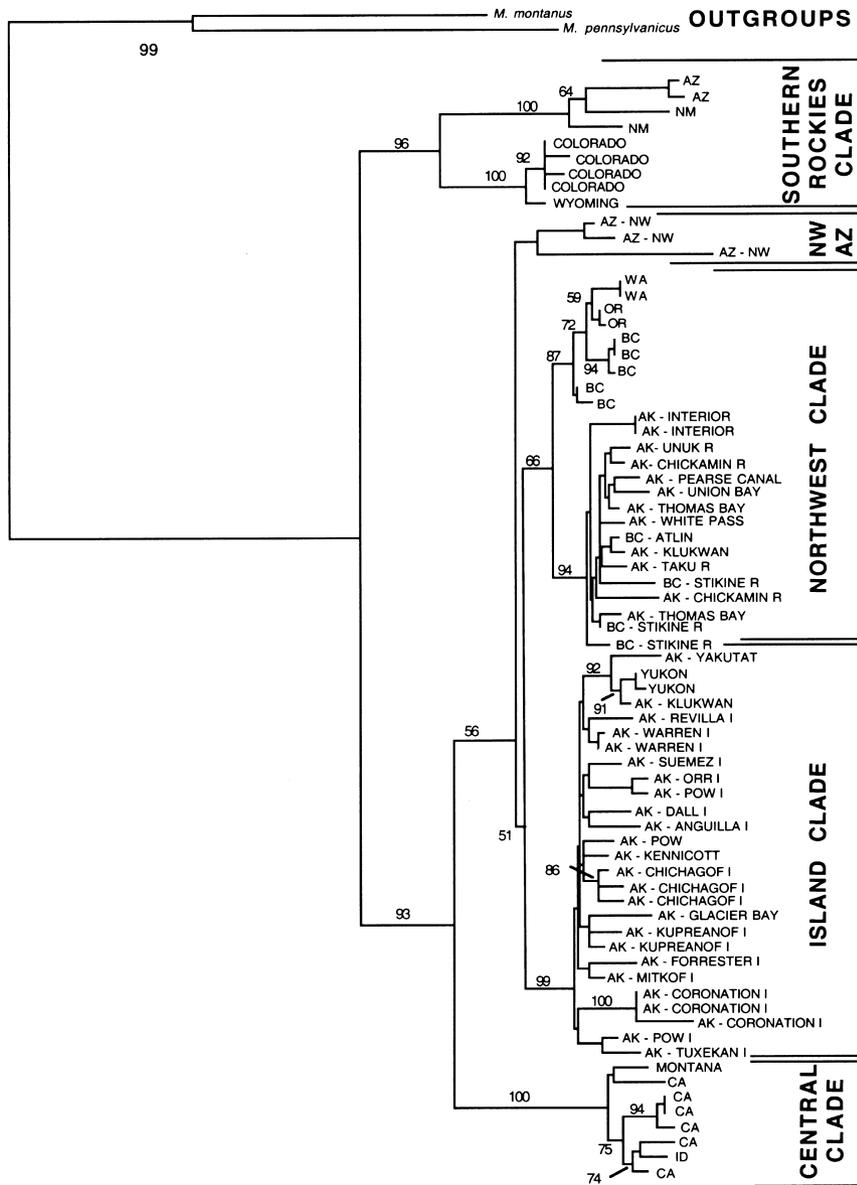


Fig. 2 Neighbour-joining phylogram based on the 2-parameter distance of Kimura (1980) from complete *cyt-b* sequences. Values above or adjacent to branches indicate bootstrap percentages from 5000 iterations.

Parsimony and distance analysis retrieved topologically equivalent trees that differed only at nodes with bootstrap values below 50% (Fig. 2). Five primary clades were identified (Fig. 2). The Southern Rockies clade was highly divergent from all other haplotypes. The Island and Northwest clades apparently contact in the vicinity of Haines, Alaska. Although the similarity of haplotypes from northwest Arizona and other northern haplotypes is remarkable, that area was represented by few specimens ($n = 3$) and was not strongly monophyletic (often intermediate to the Central, Northwest and Island clades). Thus, we focused on the other four clades in our study.

Pairwise distance analysis (Fig. 3) indicated variation both within and among clades (Marjoram & Donnelly 1994). Mean pairwise numbers of differences within

M. longicaudus was 29.2 ± 12.9 ($= 1$ SD). Pairwise analysis suggested that the Central clade and Southern Rockies clade were significantly different from expectations according to the sudden expansion model of Rogers (1995). However, pairwise differences within the Island (12.3 ± 5.7) and Northwest (11.2 ± 5.3) clades were indistinguishable from an expansion model. Mean pairwise difference was lowest in the Central clade (10.1 ± 5.2). The Southern Rockies clade exhibited deeper divergence than all other clades (28.1 ± 13.8). The test of rate heterogeneity indicated that some lineages might be evolving at different rates under the Kimura 2-parameter model. Although this result weakens inferences based on a molecular clock, we explored the relationship between genetic divergence and time since the last common ancestor. Net divergence time for

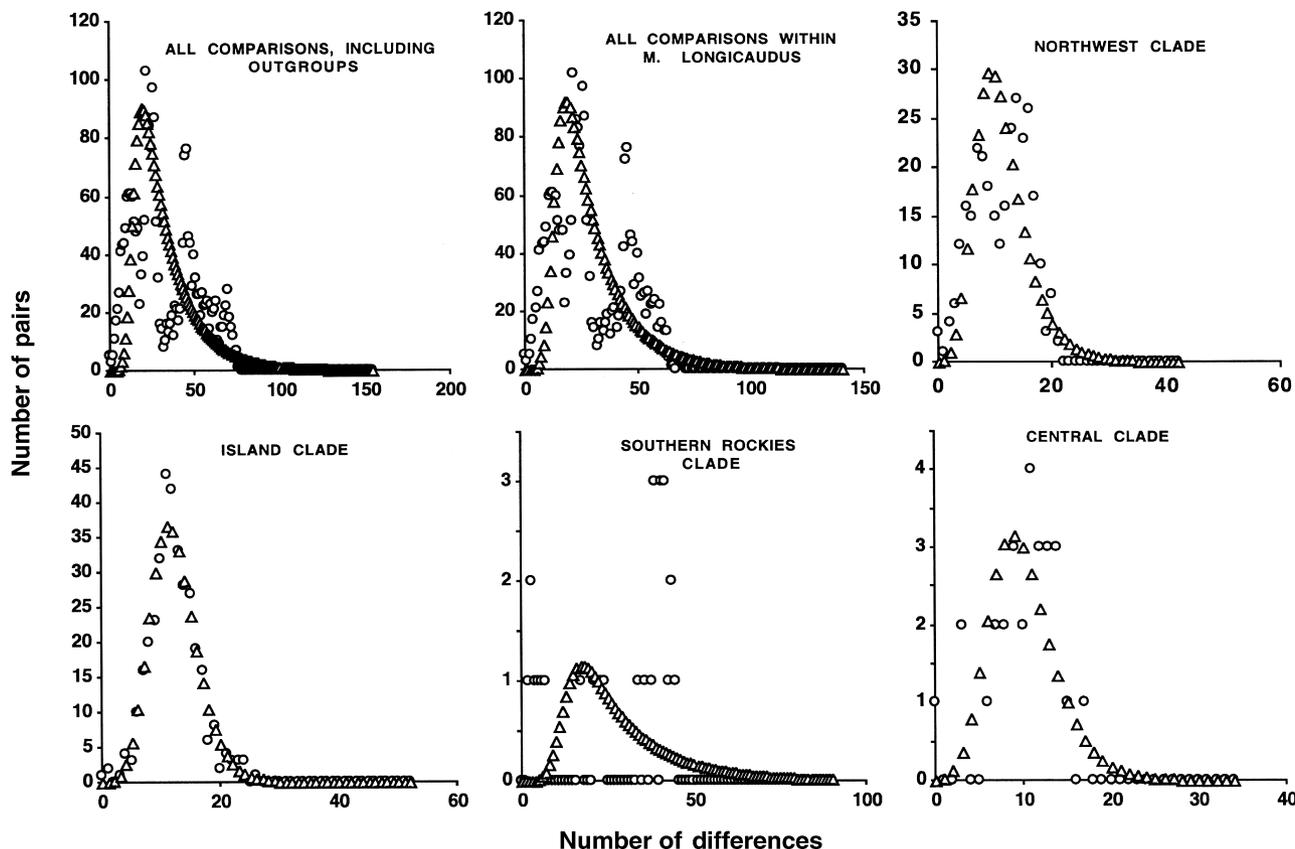


Fig. 3 Charts of pairwise analysis. The x -axis represents the number of nucleotide differences between any pair of sequences and the y -axis represents the number of times that that amount of difference was found in that particular set of sequences. Circles indicate observed differences and triangles indicate predictions under the expansion model of Rogers (1995).

M. longicaudus from other *Microtus* was estimated at 0.92 ± 0.02 (= 1 SD) million years ago (Ma). The Southern Rockies clade diverged from the other clades $\approx 0.34 \pm 0.07$ Ma. The Central clade diverged next at $\approx 0.25 \pm 0.03$ Ma. The Island and Northwest clades were estimated to have diverged most recently, at 0.09 ± 0.02 Ma. By subtracting within-clade variation, these estimates of net divergence reduced gross estimates of divergence among clades by between 20 and 45% (Edwards 1997; Avise & Walker 1998).

Discussion

Climatic change can impact organismal evolution at various temporal and geographical scales. Cycles of fluctuating climate since the Pliocene, for example, may be partially responsible for greater numbers of species of rodents in western North America (Mönkkönen & Viro 1997). Events in the late Pleistocene, however, have primarily influenced variation below the species level (Avise & Walker 1998), and these climatic fluctuations may be responsible for generating distinctive genetic

lineages within *Microtus longicaudus*. Variation in the *cyt-b* gene can be used to start to examine predictions of post-glacial expansion and describe the geographical limits of divergent lineages. Furthermore, these data may provide a framework for further exploration of morphological variation within this widespread species of western North America.

History of colonizations

Pairwise analysis of DNA sequences has been used to recover the history of population movement, particularly in humans (Shields *et al.* 1993; Harpending 1994; Rogers 1995; Rogers & Jorde 1995). Distribution of pairwise mismatches may indicate whether populations have expanded recently into an area or, alternatively, have persisted with a relatively constant size (i.e. not substantially bottlenecked). A bimodal distribution pattern is expected from gene trees with a single major bifurcation (Slatkin & Hudson 1991), such as that seen between the Southern Rockies clade and all others. The pattern of pairwise mismatches within the southern clades departs

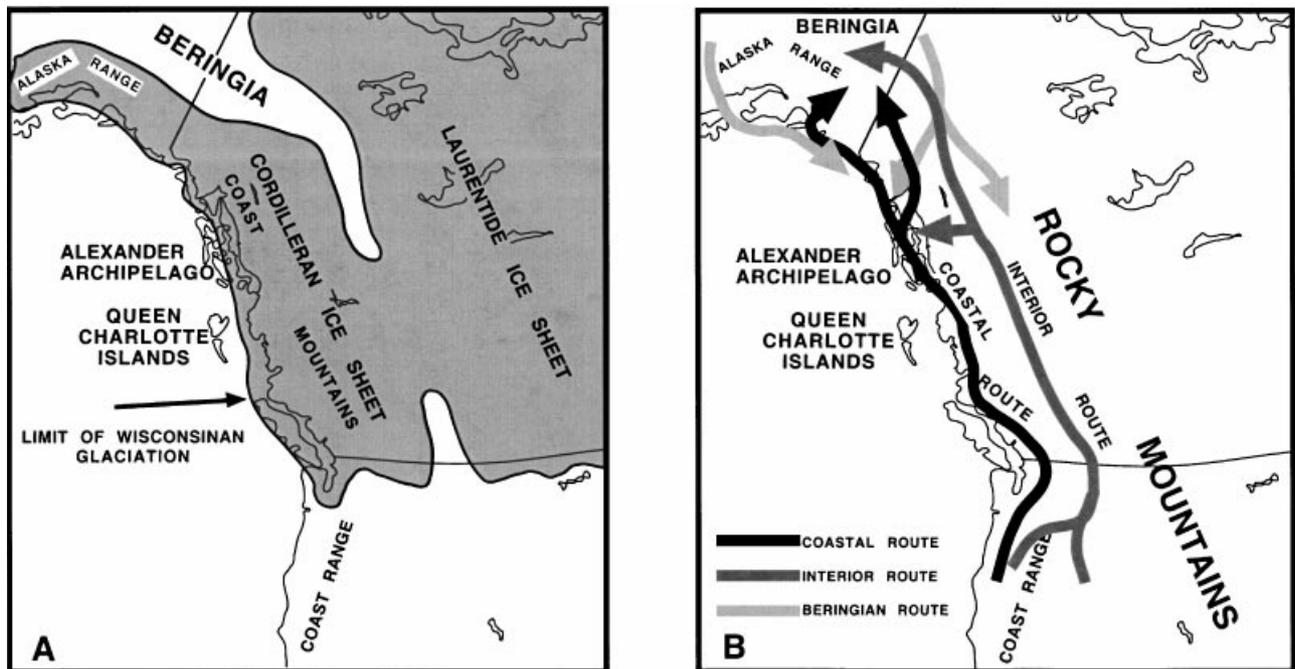


Fig. 4 (A) Limits of peak Wisconsin glacialiation. The limits of the ice sheets have not yet been clearly determined, nor has the timing of deglaciation in many areas (Mann & Hamilton 1995; Mandryk 1996). The ocean depth around southeastern Alaska during peak glaciation and during deglaciation is unknown, but exposed surfaces may have supported refugia or permitted early postglacial invasion. (B) Possible routes of postglacial recolonization from the south along the coast, through the interior, or southward from Beringia.

from expectations of the model of sudden expansion of Rogers (1995), suggesting long-term persistence *in situ*. These clades contrast with two northern clades that have rapidly expanded northward into deglaciated areas and are indistinguishable from a model of expansion. This interpretation agrees with the well-supported branches (Fig. 2) that define the suspected older populations, but star-like topology for the expanding clades (Slatkin & Hudson 1991).

The distribution of pairwise differences in the northern clades (i.e. relatively small differences and a single peak) indicates low variability (Marjoram & Donnelly 1994; Merilä *et al.* 1997). Soltis *et al.* (1997) documented a reduction in lineage diversity among populations of plants in deglaciated areas of the Pacific Northwest. Serial bottlenecking is also thought to be common among animals invading recently deglaciated regions (Sage & Wolff 1986; Merilä *et al.* 1997) and may be the case for *M. longicaudus*. The relatively low DNA divergence within the Island and Northwest clades indicates recent population-level radiation, a pattern consistent with other high-latitude arvicoline rodents (*Myopus schisticolor*, Fedorov *et al.* 1996; *M. agrestis*, Jaarola & Tegelström 1996). This pattern contrasts with southern *M. longicaudus* clades, which exhibit larger genetic differences over equivalent geographical distances; this is consistent with a longer period of isolation and divergence (e.g. on mountain tops).

Our sampling scheme, which emphasized sampling in the northern latitudes, may have contributed to the skewed levels of variation between northern and southern clades (Fig. 3). Although average pairwise differences were less within the two northern clades than within more southern clades, more extensive sampling of populations at lower latitudes is required. Further sampling may also allow tests of spatial distribution of genetic variation expected from different forms of dispersal over extended time periods (e.g. normal vs. leptokurtic, Ibrahim *et al.* 1996).

Postglacial colonization pathways

The continental ice sheets started to deglaciate $\approx 14\,000$ years ago (Mandryk 1996). The long-tailed vole may have colonized these exposed areas through several pathways including: (i) northward coastal expansion (west of the coast mountain ranges) from refugia south of the continental ice sheets; (ii) northward expansion east of the coast mountain range; (iii) expansion from North Pacific coastal refugia; or (iv) expansion southward from the Beringian refugium (Fig. 4B), or any combination of these pathways. The geographically unstructured genetic variation within the northern clades suggests that this expansion was recent.

A northward coastal invasion might have occurred as early as 13 500–10 400 years ago when a rapid retreat of

ice along the coast of British Columbia and southeast Alaska exposed large areas of land (Josenhans *et al.* 1995; Mann & Hamilton 1995). These exposed low-relief areas would have been productive habitats for early-succession generalists (Heusser 1960) such as *M. longicaudus*. In contrast, old-growth associated species, such as the northern flying squirrel, *Glaucomys sabrinus*, probably arrived much later (Demboski *et al.* 1998). The absence of *M. longicaudus* from Queen Charlotte and Vancouver islands, which are isolated by deep-water channels, suggests that these islands were not part of the colonization route used by long-tailed voles or were not accessible to long-tailed voles during the Pleistocene.

An alternative to northward colonization into central southeast Alaska by *M. longicaudus* is the possibility that it persisted in a refugium in this region during the latest glaciation, as has been suggested for *Ursus arctos* (Heaton *et al.* 1996) and some plants (Hansen & Engstrom 1996). Although fossil evidence of long-term occupation of long-tailed voles in southeast Alaska is lacking, this species now occurs on numerous islands of the Alexander Archipelago and in southern interior Alaska. This distribution, combined with the divergence of the Island clade from other Northwest populations, is consistent with isolation in a refugium. Indeed, application of a molecular clock suggested that the Island and Northwest clades diverged $\approx 90\,000$ years ago, long before the last glaciation. *Pinus contorta*, which is often co-distributed with *M. longicaudus*, occurred in southeast Alaska throughout the Holocene and possibly persisted through glacial advances (Hansen & Engstrom 1996), suggesting that other boreal taxa may have been present through these glacial advances in or near southeast Alaska. Although our data are consistent with a refugium in the area, locating this Northwest Coast refugium has been controversial because of a lack of information for midglacial sea levels and paleoenvironments in the region (Demboski *et al.* 1999).

The glacial history of southeastern Alaska has impacted the distribution of genetic lineages. Although the islands are relatively ice-free, numerous glaciers and ice fields still occur south of Juneau in the Coast Mountains of southeastern Alaska and British Columbia (Fig. 1). Absence of the Island clade from this mainland area is consistent with an eastern expansion from the outer edge of the Alexander Archipelago that was halted by mainland glaciers or rising sea level which isolated the islands. The presence of the Island clade in southcentral Alaska could have been facilitated by invasion along exposed beaches and moraines during lowered sea levels (MacDonald & Cook 1996). However, the complexity of neotectonics and rates of deglaciation complicates reconstruction of ocean levels in the Pacific Northwest (Josenhans *et al.* 1995).

All 12 of the Alaskan island populations we examined were placed in a clade separate from all mainland popu-

lations (south of Haines) indicating an early colonization of the archipelago when sea levels were depressed and distances between islands were reduced or nonexistent. Absence of *Microtus* from the nearby Queen Charlotte islands to the south argues against a refugium for long-tailed voles there, as suggested for other taxa (Foster 1965; Scudder & Gessler 1989; Byun *et al.* 1997). Lack of geographical structure among those archipelago lineages, however, is consistent with an initial colonization followed by isolation and subsequent divergence on different islands. The paucity of shared identical haplotypes between localities suggests that modern gene flow is uncommon.

Colonization routes between eastern Beringia (interior Alaska and Yukon Territory) and southern regions in North America were not available until 12 000–15 000 years ago when the Laurentide and Cordilleran ice sheets had melted sufficiently (Mandryk 1996). This corridor has implications for the peopling of North America and expansion of populations from other refugia (Fladmark 1978; Rogers *et al.* 1990; 1991; 1992). The presence of the relatively undifferentiated Northwest clade of long-tailed voles (Oregon to interior Alaska) is consistent with a rapid postglacial colonization along an interior route (Fig. 4), possibly from a single source.

Coastal vs. interior (continental) lineages occur within a number of taxa (mammals, MacDonald & Cook 1996; birds, *Passerella iliaca*, Zink 1994; and trees, *Pinus contorta*, Critchfield 1985). We found that the Northwest and Island clades of *M. longicaudus* overlapped in the vicinity of Haines, Alaska (Fig. 1). This might be a region of overlap for other interior-coast pairs. Coastal and interior forms of *P. contorta* were also found in the region; however, introgression between these forms was not very extensive (Wheeler & Guries 1982).

Southern refugia

Findley & Anderson (1956) suggested that the Wyoming Basin and the valley of the Green River, a tributary of the Colorado River, were a barrier to some mammalian taxa during the late Pleistocene. They suggested that this led to discordant distributions across this barrier for some taxa, and subspecific differentiation or no effect on others, depending on their responses to fluctuations in their habitats. Our data also indicate an east–west phylogeographical break across this region, a deep division that has not been mirrored by taxonomy (Hall 1981) or chromosome differentiation (Judd & Cross 1980). Other taxa that are not taxonomically differentiated across this region should be examined for cryptic genetic differences. Morphological analysis in long-tailed voles (e.g. pattern of occlusal patterns on teeth) might corroborate this break.

The similarity of disjunct boreal mammal communities throughout the southern Rocky Mountains suggests that

these taxa dispersed between areas through the Pleistocene (Davis *et al.* 1988; Luikart & Allendorf 1996). However, this region may have supported distinct Pleistocene refugia for mammals of low vagility, and some small mammals also exhibit large phylogeographical breaks in this region (e.g. *Onychomys leucogaster*, Riddle *et al.* 1993; and *Sciurus aberti*, Lamb *et al.* 1997). Extensive genetic divergence among southern haplotypes in *M. longicaudus* (and other taxa) could have been caused by rare colonization events during cooler periods in the Pleistocene (Lomolino *et al.* 1989; Roy *et al.* 1996), followed by isolation during interglacials (Findley & Anderson 1956). Early phylogeographical divisions in *M. longicaudus* \approx 0.34 Ma correspond to the Kansan glaciation (Winograd *et al.* 1997). Although Pleistocene climatic oscillations may not have precipitated mammalian and avian speciation (Riddle 1995; Klicka & Zink 1997), this period may have spurred many phylogeographical breaks (Avice & Walker 1998). Our divergence estimates, which placed the major phylogeographical break within *M. longicaudus* within the mid-Pleistocene, are consistent with findings for other taxa.

Conclusions

Climatic change during the middle to late Pleistocene had a profound impact on genetic structure within *Microtus longicaudus*. An early separation across the Colorado River led to the accumulation of significant levels of genetic variation that was equivalent to interspecific comparisons in the genus (Conroy & Cook 2000). The retreat of glaciers in the early Holocene led to a rapid, but spatially organized, northward colonization from at least two source areas. Consequently, specimens examined over large geographical areas in the north (e.g. British Columbia to interior Alaska) are very closely related genetically; however, geographical differentiation suggests the possibility of multiple paths of expansion. The timing and geographical origin for the Island clade indicate the possibility of a glacial refugium in or near southeast Alaska.

Similar phylogeographical patterns across species indicate the possibility of common biogeographical processes (e.g. colonization and vicariance) or history. These patterns for *M. longicaudus* should be examined in light of other phylogeographical studies of mammals, plants and birds common to boreal habitats of western North America. Unfortunately, few boreal taxa of North America have been investigated at both northern and southern extremes, by using molecular methods, to examine whether species respond to their environments independently, as some reports have suggested (Zink 1994; Graham *et al.* 1996; Bernatchez & Wilson 1998; Taberlet *et al.* 1998). Investigations of lineage differentiation in other taxa, particularly plants and invertebrates, would provide a robust test of the response of organisms to climatic change.

An appreciation for the genetic response of organisms to widespread northward invasion may also be useful for predicting future responses to global climatic change.

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This research represents part of the doctoral project of C. J. C. Other research by C. J. C. is directed towards study of the evolution of arvicoline rodents and island biogeography. Research in the laboratory of J. A. C. is directed towards questions in historical biogeography, phylogeography and molecular systematics, with an emphasis on mammals. Other investigators in the laboratory of J. A. C. are investigating the phylogeography and systematics of mammals and other vertebrates in western North America.

Appendix I Source/location of specimens

Clethrionomys gapperi: Washington, Clallam County (NK3221)

Microtus montanus: Utah, Salt Lake County (NK55041)

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

M. longicaudus:

Alaska

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

Big Delta Quad, Goodpaster R. (UAM1894)

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

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

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

Juneau Quad, Chichagof I., Game Creek (AF10376); Lynn Canal, Excursion Inlet, W side (AF17242); Lynn Canal, Excursion Inlet, E side (AF17254); Chichagof I., 11 mi SE of Hoonah (AF1809); Glacier Bay, Bartlett Cove, 10 km NW Gustavus Airport (AF3752); Chichagof I., Game Creek (AF6519); Chichagof I., Otter Lake (AF8619, AF8662)

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

McCarthy Quad, near Kennecott (UAM3553)

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

Port Alexander Quad, Kuiu I. (AF3725)

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

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

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

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

Yakutat Quad, Cannon Beach near Yakutat (AF2031)

Arizona

Apache County (NK1924); Coconino County (NK8521, NK8524, NK8525)

California

Siskiyou County (AF22741, AF22849, AF22850, AF22851, AF22852, AF22853)

Colorado

Chaffee County (AF21209, AF21211, AF21212)

Idaho

Latah County (AF22739)

Montana

Carbon County (AF10901)

New Mexico

Sandoval County (NK1719); Cibola County (NK9766)

Oregon

Lincoln County (AF18526), Lane County (AF18528)

Washington

Kittitas County (NK3135)

Wyoming

Carbon County (AF23201)

British Columbia

Salmon R. (AF12713); Stikine R. (AF12847, 12860); near Atlin (AF12909); Sicamous Creek (AF14020, AF18740, AF24886, AF24988, AF24990); Opax Mountain (AF14909)

Yukon Territory

Near Haines Junction (AF10424, AF10426)

Specimens were obtained from the following collections or individuals: Museum of Southwestern Biology, University of New Mexico (NK) and, University of Alaska Museum (UAM or AF). Quad refers to USGS 1 : 250 000 quadrangle.