Molecular Ecology (2005) **14**, 1445–1456 doi: 10.1111/j.1365-294X.2005.02501.x

Postglacial expansion of the southern red-backed vole (*Clethrionomys gapperi*) in North America

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Abstract

Dynamic climatic oscillations of the Pleistocene dramatically changed the distributions of high latitude species. Molecular investigations of a variety of organisms show that processes of postglacial colonization of boreal regions were more complex than initially thought. Phylogeographical and coalescent analyses were conducted on partial sequences of the cytochrome *b* gene (600 bp) from 64 individuals of *Clethrionomys gapperi* from North Carolina, Pennsylvania, Minnesota, Idaho, Washington, British Columbia, Northwest Territories, and Alaska to test hypotheses relating to Pleistocene refugia and postglacial colonization routes. Three divergent clades (east, west, central) were identified with highest net divergence (d_A = 5.2%) between the eastern and western clades. Populations from the recently deglaciated higher latitudes of Canada and Alaska are closely related to lower latitude populations of the central clade (d_A = 1.2%) suggesting recent expansion from this midwestern region. No representatives from the east or west clade were found at latitudes higher than 50°N, indicating that postglacial colonization occurred through a midcontinental route. The high latitude population from the Northwest Territories exhibited demographic patterns and genetic diversity consistent with a stable noncolonizing population. This population is found near the Mackenzie range, where the two continental ice sheets were believed to have coalesced. Molecular variation observed in this population may be the result of leading edge population diversifying in the continental corridor or may reflect the signal of a high latitude refugial population.

Keywords: *Clethrionomys gapperi*, colonization, continental corridor, cytochrome *b*, glacial refugia

Received 28 September 2004; revision accepted 20 January 2005

Introduction

Climatic oscillations in the last 2 million years (Myr) caused dramatic shifts in the ranges of taxa with spatial effects largely dependent on latitude and topography. A growing body of research is focusing on how climate change has structured the genetic variation of high latitude mammals of North America (Wooding & Ward 1997; Arbogast 1999; Cook *et al*. 2001; Stone *et al*. 2002; Fedorov *et al*. 2003). Pleistocene events are hypothesized to have stimulated intraspecific diversification by separating populations through formation of glacial barriers and by shifting the location of suitable habitat (Hewitt 1996). In contrast, homogenization of northern populations through long-distance postglacial dispersal is also hypothesized (Hewitt 1996). Concordant genetic signals of population expansion into deglaciated areas have been found across taxa with widely different life histories, perhaps reflecting the common role the glacial vicariant events had in shaping community composition at higher latitudes (Lessa *et al*. 2003). To appreciate the significance of contemporary geographical variation at high latitudes, we need to develop a better understanding of the degree to which vicariance in distinctive glacial refugia affected intraspecific diversification and how organisms subsequently recolonized once heavily glaciated regions. These northern ecological communities were assembled following the retreat of glaciers, and the generality of processes of diversification and recolonization is only now being examined.

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Phylogeographical studies of a variety of northwestern North American species are testing hypotheses relating to the timing, routes, and concordance of postglacial colonizers into high latitudes and are revealing differential contributions that refugial areas made in shaping the composition of this boreal region (Conroy & Cook 2000; Stone & Cook 2000). High latitude species are often found to be comprised of several divergent lineages, but the nuances of how individual species responded to climate change differ, thus providing an opportunitily to examine how closely associated species (either evolutionarily or ecologically) respond to environmental change. For many of these species, two distinct colonization routes have been proposed that led from the south into the high latitudes of northwestern North America as the continental ice sheets retreated. A continental corridor route was centred upon the eastern edges of the Rocky Mountains in central western Canada where the Laurentide and Cordilleran ice sheets once coalesced (Pielou 1991), while a coastal route existed on the western side of the coastal mountain range of British Columbia and southeast Alaska.

In addition to identifying multiple routes of colonization, uncovering the mode and tempo of dispersal of postglacial colonizers is essential in determining how ecological conditions, habitat distributions, and physical barriers shaped contemporary biotic communities in the northern hemisphere. Typically, once appropriate habitat becomes available, postglacial colonizers will expand rapidly into a region (Hewitt 1996). Long-distance dispersers at the leading edge are usually responsible for inhabiting a new area. The pioneer model (Nichols & Hewitt 1994) leaves a signature of reduced allele diversity and genetic homogeneity (Ibrahim *et al*. 1996) and has been invoked for a number of northern species (Hewitt 1996; Bernatchez & Wilson 1998). In contrast, slower colonization into a region should allow populations to retain genetic diversity because of shorter dispersal distances and larger effective population sizes (Hewitt 1996). This phalanx model (Ibrahim *et al*. 1996) of dispersal is often indicative of some form of barrier to colonization. Typically, a genetic signature of the pioneer model is expected in postglacial colonizers. However, exceptions to this model are being uncovered in Arctic adapted species such as lemmings (Fedorov *et al*. 1999; Fedorov *et al*. 2003).

The southern red-backed vole (*Clethrionomys gapperi*) occupies later successional coniferous, deciduous, and mixed-wood forests (Merritt 1981) of the Hudsonian and Canadian life zones (Fig. 1). The fossil record for this species dates to the middle Pleistocene in North America (Graham 1976; Gromov & Polyakov 1977). During the late Pleistocene, the Cordilleran and Laurentide ice sheets covered most of its current distribution, and populations were believed to persist in refugia south of the continental ice (Fig. 2; Hibbard *et al*. 1965; Macpherson 1965). A phylogenetic analysis of several species of red-backed voles identified two divergent clades of *C. gapperi* that apparently reflect eastern and western refugial populations (Cook *et al*. 2004). This study expands on those preliminary analyses and further tests hypotheses related to refugial diversification and routes of postglacial colonization. Specifically, we examined sequence variation of the cytochrome *b* gene from eight populations of *C. gapperi* across their distribution

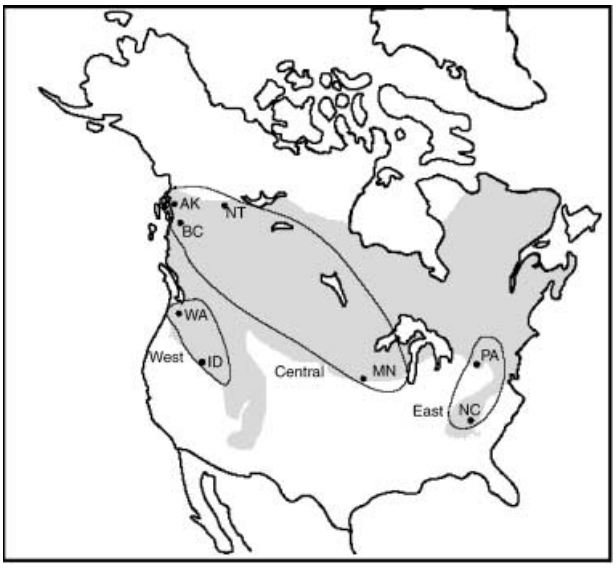

Fig. 1 Distribution map of *C. gapperi* with sampling localities and major clades (west, central, east) shown.

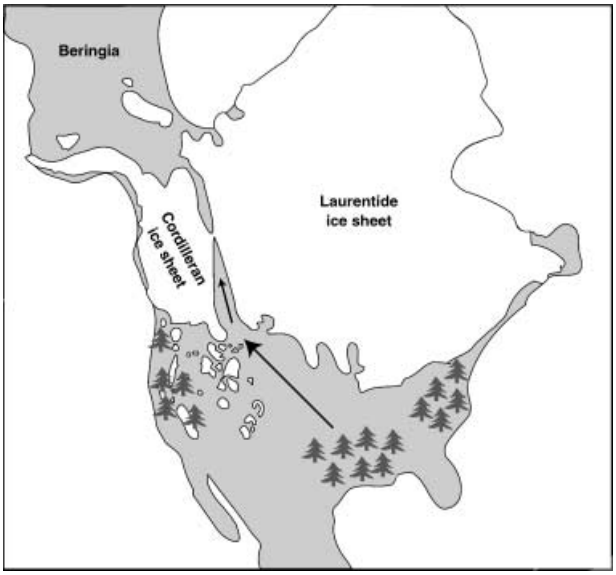

Fig. 2 Map of North American ice sheets *c*. 13 000 YBP modified from Pielou (1991). Potential forest refugia and the hypothesized colonization route of *C. gapperi* into the higher latitudes of North America are identified.

to address (i) the origin of high latitude populations; (ii) whether postglacial expansion northward into the high latitudes of northwestern North America occurred through coastal and continental routes; and (iii) whether spatial genetic patterns are concordant with other North American forest-associated mammalian taxa.

Materials and methods

Sampling

We sequenced 58 *Clethrionomys gapperi* from North Carolina, $n = 5$; Minnesota, $n = 9$; Washington, $n = 11$; British Columbia, $n = 9$; Northwest Territories, $n = 11$; Idaho, $n = 4$; and Alaska, $n = 9$ and added individuals from Pennsylvania, $n = 2$ (GenBank Accession nos AY309433 and AY309434); North Carolina, $n = 2$ (AY309429 and AY309430); and Minnesota, $n = 2$ (AY309431 and AY309432). Individuals from Minnesota, Alaska, British Columbia, and Northwest Territories were sampled from localities that spanned a similar spatial range. We also included two sequences of the other North American species, *C. californicus* (AY309422 and AY309423) and *C. rutilus* (AY309426 and AY309427) in our phylogeographical analyses. *C. californicus* has been considered conspecific with *C. gapperi* (Gromov & Polyakov 1977). One sequence each for *Ondatra zibethicus* (AF119277), *Eothenomys melanogaster* (AB017254), and *C. rufocanus* (AY309418) was included as out-groups (Conroy & Cook 1999; Suzuki *et al*. 1999; Cook *et al*. 2004, respectively). Voucher specimens (Appendix) for all the individuals we sequenced are deposited at the University of Alaska Museum of the North (UAM).

DNA extraction and sequencing

Total genomic DNA was extracted from frozen liver samples using a modified salt extraction method (Miller *et al*. 1988; Fleming & Cook 2002). The first 600 bp of the cytochrome *b* (cyt *b*) gene were amplified by the polymerase chain reaction (PCR) following the laboratory methods of Cook *et al*. (2004), using the primer combination *MVZ 05* (Smith & Patton 1993) and *CLETH 06* (5′-CCTGTTGGG-TTGTTGGATCCTG-3′). All reactions included negative controls. PCR products were purified using polyethylene glycol (PEG) precipitation (Bernstein & Abbot 1987). Purified PCR products were cycle sequenced using the *Taq* DyeDeoxy Terminator Cycle Sequencing Kit and the Big Dye Terminator Cycle Sequencing Ready Reaction Mix 3.0 (Applied Biosystems). Automated sequencing of both heavy and light strands was conducted using the Applied Biosystems 373 and 3100 DNA sequencers. Sequences were aligned and compared manually using SEQUENCE NAVIGATOR version 1.01 (Applied Biosystems). All sequences were deposited in GenBank (Accession nos AY952146–AY952203).

Phylogeographical analyses

MODELTEST version 3.06 (Posada & Crandall 1998) was run on the complete data set to determine the model of DNA substitution through a hierarchical likelihood ratio test. The Hasegawa-Kishino-Yano (HKY) model (Hasegawa *et al*. 1985) plus gamma was chosen as the simplest model that best fits these data. Parameters estimated from this model were ti/tv = 7.4007 and gamma = 0.1545. The computer program PAUP version 4.0b10 (Swofford 2002) was used to reconstruct phylogenetic relationships under a distance framework (neighbour-joining) with bootstrap analysis (1000 replicates) to determine nodal support. A Bayesian reconstruction was conducted using MRBAYES (Huelsenbeck & Ronquist 2001) with four Markov chain Monte Carlo (MCMC) chains running simultaneously for one million generations sampling every 1000 generations. Four runs were conducted with each starting from a random tree. Stationarity of the chains was assessed through the graphical output, and the initial 100 000 generations (and 10 000 trees) from each run were discarded to allow time for the chains to become stationary. A consensus of all the remaining trees from the four runs was computed to assess nodal strength.

To test for constancy in rates of cytochrome *b* evolution among lineages, we reconstructed phylogenetic relationships using maximum likelihood and constrained the phylogeny to a molecular clock in PAUP version 4.0b10 (Swofford 2002). Twice the difference of the resulting log-likelihood scores from the constrained and unconstrained trees were used as the test statistic and compared to a χ^2 distribution.

Molecular diversity and demographic analyses

Measures of molecular diversity and demographic analyses were performed on *C. gapperi* populations except for Idaho and Pennsylvania (because of small sample sizes). Haplotype (h) and mean nucleotide diversities (π) within each population were calculated based on the pairwise differences matrix using ARLEQUIN version 2.000 (Rogers & Harpending 1992; Schneider *et al*. 2000). The distribution of the number of nucleotide differences between pairs of haplotypes (mismatch distribution) was computed for each population to test the null hypothesis of recent population expansion in ARLEQUIN version 2.000 (Schneider *et al*. 2000). Tajima's (1989) test was used to test for deviation from selective neutrality and population equilibrium. Net sequence divergence (d_A; Nei 1987) was computed between the major clades.

Historical population dynamics

The software package FLUCTUATE version 1.3 (Kuhner *et al*. 1998) was used to test for exponential population expansion

or decline. FLUCTUATE implements coalescence theory and maximum likelihood to estimate present-day θ ($\theta = 2\mu N_F$) and exponential growth rate (g; Kuhner *et al*. 1998) where μ is the mutation rate per nucleotide and N_F is the effective population size of females. FLUCTUATE was run separately on each of the six populations with transition and transversion ratio of 7:4 (as determined through MODELTEST). We ran 10 short chains of 2000 steps, each starting with Watterson's estimate of θ (Watterson 1975). Ten long chains of 20 000 steps were run, sampling every 20 chains.

Results

Cytochrome b *sequence data*

Compositional sequence bias was observed common to mammalian cytochrome *b* genes (Irwin *et al*. 1991). Average base frequencies among all *Clethrionomys gapperi* were A (29.5%); C (29.6%); G (13.4%); and T (27.5%). Nucleotide substitutions were observed at 75 sites. The third position substitutions were most abundant (85%) and the second the least abundant (5.3%). The distribution of amino acid variation is consistent with the model of cytochrome *b* (Irwin *et al*. 1991).

Phylogeographical structure

Phylogenetic reconstruction using the Bayesian statistics (not shown) and neighbour-joining distance method produced similar topologies. Three major clades ($d_A = 3.3\%$) of *C. gapperi* were revealed: east (east of the Appalachians), west (west of the Rockies), and central (Fig. 3). Individuals from the higher latitudes of Canada and Alaska formed a subclade within the central clade (Fig. 3) with 1.2% net divergence between these two groups. The east and west clades were the most divergent ($d_A = 5.2\%$). The central clade was most closely related to the west clade ($d_A = 3.3\%$). Haplotypes were shared across populations only among individuals from higher latitudes of Canada and Alaska.

Likelihood scores of trees constructed with and without molecular clock constraints were not significantly different ($\chi^2 = 55.7$, d.f. = 70, $P > 0.05$), indicating that these cytochrome *b* sequences evolved at a relatively constant rate.

Demographic history

Mismatch distribution showed a unimodal frequency distribution of pairwise differences for two of the three high latitude populations (British Columbia and Alaska; Fig. 4), indicating a historical demographic expansion in these deglaciated regions (Rogers & Harpending 1992).

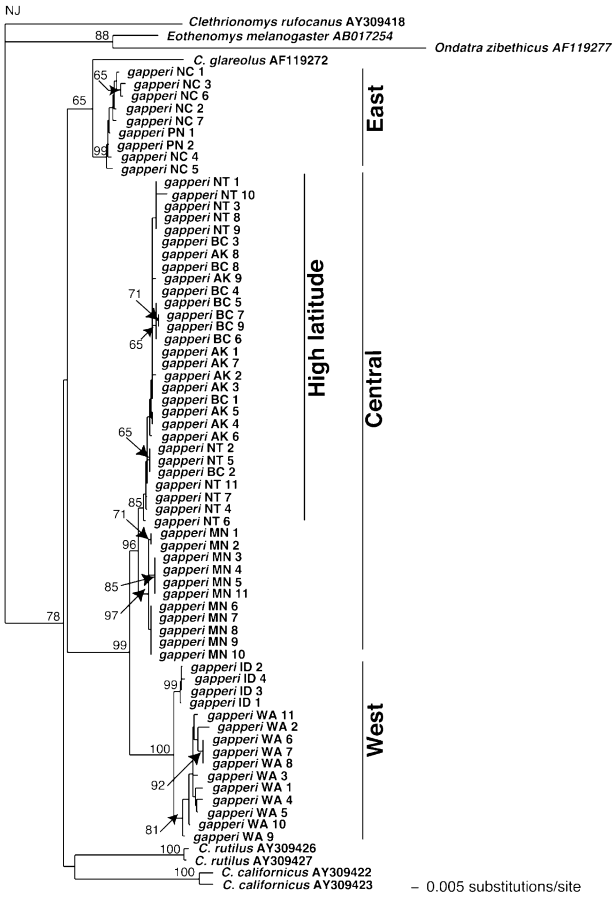

Fig. 3 Neighbour-joining tree of *C. gapperi* and their outgroups. Phylogeny reconstruction was conducted using the HKY + gamma model of sequence evolution. Numbers above the branches are bootstrap support (1000 replicates).

Expected and observed frequencies of pairwise differences were similar in these populations, indicating recent population growth. However, unimodal distribution of pairwise differences can be the result of non-neutral selective effects as well as demographic effects (i.e. population expansion and bottleneck). In contrast, Northwest Territories had a trimodal distribution and the expected and observed frequencies of pairwise distributions were not similar. North Carolina, Washington, and Minnesota also had multimodal distributions, indicating stationary populations (Harpending *et al*. 1998).

Tajima *D* values ranged from largely positive (east coast samples) to slightly negative (Table 1).

Results of the exponential expansion model using FLUCTUATE 1.3 showed a significant increase in population size in Alaska, Washington, and North Carolina populations (Table 1). British Columbia had a slight growth rate, but standard errors around the growth estimates for Minnesota and Northwest Territories encompassed zero.

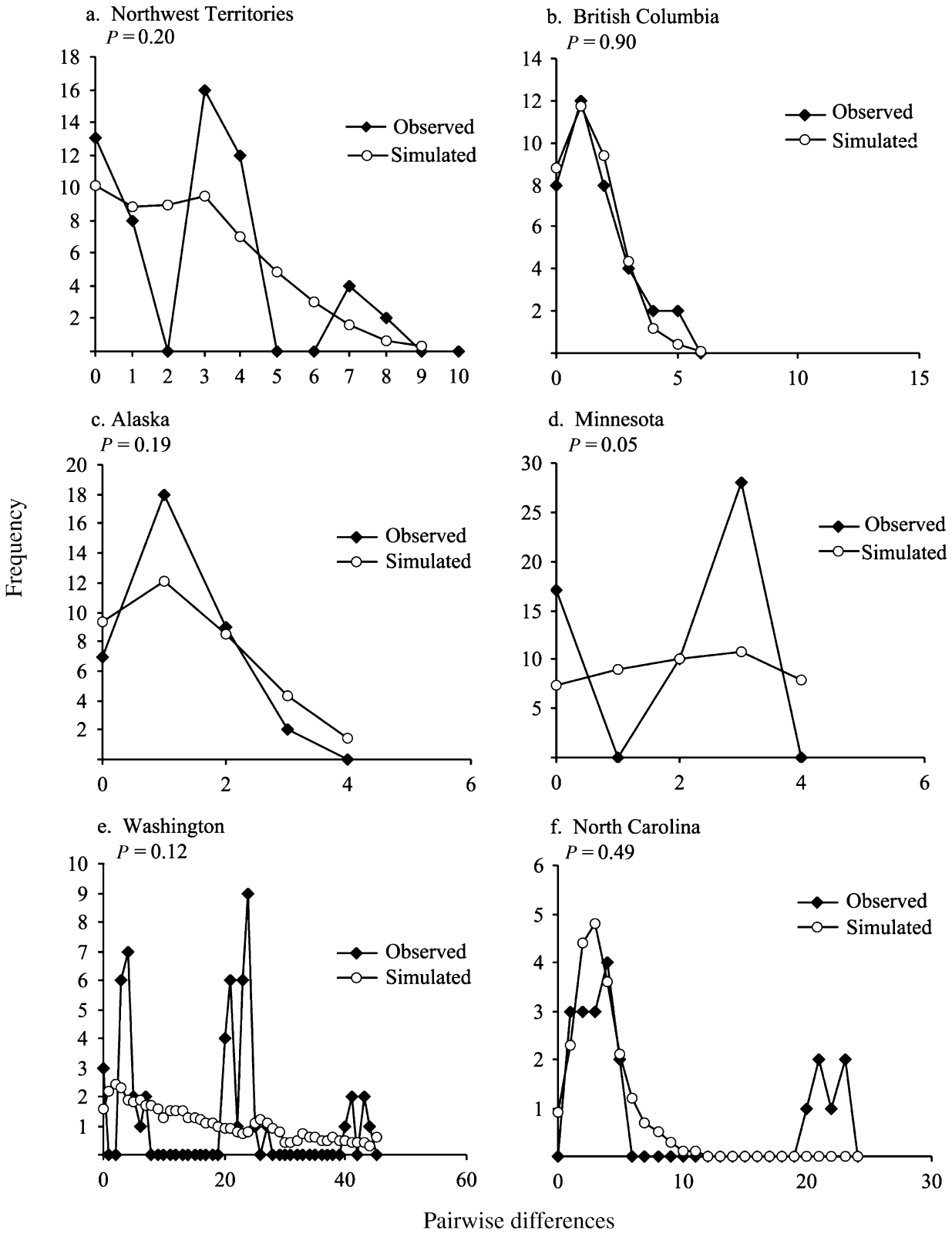

Fig. 4 Mismatch distributions of *C. gapperi* from six sampling localities (Pennsylvania and Idaho were not included because of small sample sizes). Solid diamonds represent the observed distribution of pairwise differences, and open circles represent the expected distribution, assuming stepwise population expansion. *P* values are the probability that the variance of the simulated distribution is greater than the empirical distribution.

Table 1 Descriptive statistics of genetic variation and parameter estimates. Number of individuals (*n*), number of haplotypes (*Nh*), number of segregating sites (*S*), haplotype diversity (*h*) and standard error (SE), nucleotide diversity (π) and standard error (SE), growth (g) and standard deviation (SD). Pennsylvania (PA) and Idaho (ID) samples are not included due to sample size ($n = 2$ and $n = 4$, respectively)

Locality	Clade	*n*	*Nh*	*S*	*h*	SE (*h*)	π	SE (π)	Tajima's *D* test	g	SD (g)
Northwest Territories	High latitude	11	5	8	0.818	0.083	0.0045	0.0029	−0.061	−128.74	289.1
British Columbia	High latitude	9	4	5	0.778	0.110	0.0027	0.0019	−0.526	570.1	404.2
Alaska	High latitude	9	5	4	0.805	0.119	0.0019	0.0015	−0.842	10 000	2850.2
Minnesota	Central	11	3	4	0.691	0.086	0.0031	0.0021	1.408	149.61	378.7
Washington	West	11	9	20	0.946	0.066	0.0079	0.0044	−1.611	2180.2	121.4
North Carolina	East	7	7	9	1.0	0.076	0.0138	0.0083	6.67	2548.7	523.6

Discussion

By comparing phylogeographical patterns across forest associated taxa, we can test specific hypotheses related to the location of Pleistocene refugia and the role refugia had in shaping community structure at high latitudes (Hewitt 1996). Differential success of colonization from these refugia into the high latitudes reveals how organisms responded as environments changed. Through comparative phylogeographical studies, we can begin to detect whether regional diversity reflects common biogeographical histories. If species do share biogeographical histories, comparative phylogeographical studies will provide opportunities to test general principles related to community assembly and dynamics (Hewitt 2000).

Phylogeography of Clethrionomys gapperi

Southern red-backed voles form three distinct and well supported mitochondrial clades: east of the Appalachians (east clade), between the Rocky and Appalachian mountains (central clade), and west of the Rockies (West clade; Fig. 1). Initial work (Cook *et al*. 2004) uncovered two clades, but their sampling did not extend west of Minnesota in the continental United States. The magnitude of genetic differentiation between east and west clades is similar to patterns observed in other forest-associated taxa such as black bears (*Ursus americanus*; Wooding & Ward 1997) and marten (*Martes americana*; Stone *et al*. 2002). Based on Delcourt & Delcourt (1987), Wooding & Ward (1997) proposed that eastern and western forest refugia existed prior to 120 000 YBP resulting in long-term separation of forest-associated species. This disjunction is reflected in high levels of genetic differentiation between the west and east clades ($d_A = 5.2\%$) and suggests that separation has persisted for several glacial cycles, with diversification initiated well before the Last Glacial Maximum (Klicka & Zink 1997; Avise *et al*. 1998). We did not attempt; however, to date the divergence using only one gene.

A third divergent clade (central) is sister to the west clade. Late Wisconsin fossils of *C. gapperi* are documented from Iowa, Illinois, Missouri, Kansas, Arkansas, and Tennessee, south of their current distribution (Graham 1976; Faunmap Working Group 1994), and the central clade may represent descendants of these refugial populations. White spruce (*Picea glauca*) pollen is documented from the south–central area of the continent 18 000 YBP, and its movement north into central Minnesota is documented at 13 000 YBP (Birks 1976; Ritchie & Macdonald 1986), thus potentially providing habitat for this forest-associated species.

Alternatively, the central clade may be of western origin. Because the geological history of the Rocky Mountains is dynamic and distinct biogeoclimatic zones exist, differentiation of the central clade from the west clade may be a result of individuals from multiple western refugia expanding into the midwest. The Bitterroot Mountains in the Rockies have been hypothesized to be an area of primary allopatric diversification for forest-associated taxa (Brunsfeld *et al*. 2001). During full glacial events, forests were further isolated as increased mesic conditions displaced sub-alpine forests (Barnosky *et al*. 1987). Phylogeographical studies focused on organisms in northwest North America are demonstrating cryptic variation across the Rocky Mountains (Li & Adams 1989; Arbogast 1999; Brunsfeld *et al*. 2001; Good & Sullivan 2001; Demboski & Sullivan 2003). Additional sampling from the Rockies and midwest is needed to determine the geographical extent of the central clade, and to further test these hypotheses.

Postglacial colonization and population expansion

The sister relationship of the Minnesota and all high latitude populations surveyed indicates that colonization of Alaska, Northwest Territories, and British Columbia occurred through an interior continental route from ancestral populations residing east of the Rocky Mountains (Fig. 2). Successful colonization by ancestors of the central clade likely relates to the timing and rate of retreat of the continental ice sheets. As the eastern edge of the Cordilleran ice sheet retreated around 14 000 YBP (Anderson & Borns 1994; Mandryk 1996; Dyke *et al*. 2002), a corridor between

the two continental ice sheets opened, creating a potential route of colonization into the high latitudes of North America (Fig. 2). Postglacial pollen records from within this proposed corridor (Mackenzie River basin) show northward spread of spruce (*Picea* sp.) forests replacing the herb and shrub vegetation around 10 000–8500 YBP, linking the high latitude refugium, Beringia, with the plains south of the ice sheets (Macdonald 1987).

Analyses of historical demographic measures suggest recent expansion of *C. gapperi* into the previously glaciated high latitudes of British Columbia and Alaska. Populations that have undergone sudden demographic expansion result in unimodal distributions of pairwise differences (Rogers & Harpending 1992) but homoplasy or selection may also produce unimodal distributions (Bertorelle & Slatkin 1995). Because of the low levels of genetic differentiation within populations, homoplasy is likely not influencing the mismatch analysis. To distinguish whether demographic or selective forces are influencing the mismatch analyses, additional unlinked genes should be analysed. With these issues in mind, we interpret these unimodal distributions and the nearly identical observed and expected mismatch frequencies under a model of expansion, which is consistent with expansion and recent increase in population size. Furthermore, high haplotype and low nucleotide diversity support population expansion. Exponential growth estimates obtained using FLUCTUATE were significantly positive (Table 1) for British Columbia and southeast Alaska.

Northwest Territories, in contrast, did not show a strong signal of recent expansion. Notably, this population had greater nucleotide diversity, and the shape of the mismatch distribution was trimodal. Comparisons of expected and observed mismatch frequencies were not similar, suggesting that this population has not undergone a recent increase in population size. Growth rates estimated using FLUCTUATE were negative and estimates within the 95% confidence interval (CI) encompassed zero (Table 1). Peripheral expanding populations are expected to have reduced diversity when compared to central populations as a result of decreases in population size during founding events (Nei *et al*. 1975). Therefore, reduced genetic variation is expected with recent founding events. However, the mean nucleotide diversity of Northwest Territories was greater than that of Minnesota and demographic estimates indicated that the high latitude Northwest Territories population is stable.

British Columbia and southeast Alaska samples possessed low levels of nucleotide diversity and signals of large population growth, indicative of rapid (pioneer model) colonization (Nichols & Hewitt 1994). In contrast, Northwest Territories samples had higher levels of nucleotide diversity and no signature of expansion suggesting that postglacial colonization into this area may have occurred gradually (phalanx model) or that this is a refugial population. Slower expansion would result in shorter dispersal distances, larger effective population sizes (Hewitt 1996), and retention of higher levels of genetic diversity (phalanx model; Nichols & Hewitt 1994).

Because Northwest Territories samples are from the area where two ice sheets coalesced (Pielou 1991), it is conceivable that gradually receding barriers (either ice sheets or unsuitable habitat) may have slowed the expansion of *C. gapperi* into the region. However, movement of potential habitat (*Picea glauca*) into the corridor happened very suddenly, not in a time-transgressive manner (Ritchie & Macdonald 1986). In addition to vegetational composition, interactions with other species, prior colonizers, and the distribution of necessary habitat affect the success of colonizers. The two different patterns of colonization are not surprising. It would be overly simplistic to think that postglacial colonization across a landscape measuring greater than 2000 km would occur for all taxa under the same model of expansion. These preliminary observations on the southern red-backed vole need to be further tested with additional independent loci and additional populations from within and outside of the continental corridor to understand the complexity of colonization. Additional work could specifically test for concordance of colonization rates of *C. gapperi* and the rapid spread of spruce, and how topographic features affected movement of these colonizers across the landscape.

Comparative patterns of postglacial colonization

When multiple divergent lineages existed south of the ice sheets, postglacial colonization into central Canada and Alaska has been taken place by members from at least two clades representing coastal and continental routes (Conroy & Cook 2000; Stone & Cook 2000; Demboski & Cook 2001), or a coastal, continental, and Beringian route (Fleming & Cook 2002; Small *et al*. 2003). However, postglacial expansion of forest-associated mammals into central Canada and Alaska largely occurs by eastern refugial lineages. Descendents of western refugial lineages generally remain restricted west of the Rocky Mountains (Wooding & Ward 1997; Arbogast 1999; Fleming & Cook 2002; Stone *et al*. 2002) with northward expansion limited to the Pacific coast west of the Coast Ranges. Predominance of eastern lineages in postglacial recolonization of these midcontinental regions has been attributed to the rapid expansion of eastern forests across the continent (Williams *et al*. 1993), which provided suitable habitat for postglacial colonizers.

Clethrionomys gapperi in central Canada and Alaska appears to have descended from only one clade (central) using the continental route. The northern flying squirrel (*Glaucomys sabrinus*; Arbogast 1999) is the only other example of a forest-associated mammal that is represented by only one

lineage (eastern lineage) in the high latitudes of central Canada and Alaska, suggesting that colonization of this species was also restricted to the continental route. The Cordilleran ice sheet persisted longer than the Laurentide ice sheet and is believed to have been a physiographic barrier to some colonizers along the Pacific coast (Westgate *et al*. 1987). Similar ecological requirements of these two species may have precluded their colonization as a result of limited habitat availability along the western coast.

Phylogenetic relationships of C. gapperi *and* C. californicus

Western populations of *C. gapperi* are parapatric with the western red-backed vole, *C. californicus*. Separated from *C. gapperi* by the Columbia River, the western red-backed vole inhabits forests along the western coast from the Columbia River in Oregon to about 100 km north of San Francisco Bay (Hall 1981). No fossils exist for *C. californicus* (Alexander & Verts 1992) and its phylogenetic placement and evolutionary history in relation to other North American red-backed voles is not resolved (Gromov & Polyakov 1977; Cook *et al*. 2004). We included *C. californicus* in this study to determine their relationship in respect to western populations of *C. gapperi*. Although the cytochrome *b* gene of *C. californicus* is highly differentiated from that of *C. gapperi* (uncorrected $P = 7.5$; Fig. 3), how *C. californicus* is related to *C. gapperi* is still unresolved. Very short internodal branch lengths define the different species of *Clethrionomys* (Fig. 3), perhaps reflecting a pulse of speciation, similar to that observed in other arvicoline rodents (Chaline *et al*. 1993; Conroy & Cook 1999).

Conclusions

High levels of genetic variation define three major clades of southern red-backed voles in North America. These results are consistent with the deep fossil record of this species and suggest that allopatric diversification occurred prior to and through the Pleistocene. The presence of the third (central) clade in North America has not been uncovered in previous studies of forest-associated mammals and may reflect the existence of an additional refugial area south of the ice sheets.

Postglacial colonization of central Canada and Alaska by *Clethrionomys gapperi* apparently occurred primarily through a continental route and is consistent with other phylogeographical studies that indicate a continental route of postglacial expansion into the high latitudes of North America. Of particular interest are the high latitude populations of British Columbia and Alaska which showed signals of expansion and population growth — a finding consistent with postglacial colonization. However, the Northwest Territories population exhibited genetic structure and demographic signals of a stable population, reflecting either a northern refugium or the leading edge of postglacial expansion in Canada.

Acknowledgements

This project was funded by the Beringian Coevolution Project (NSF 0196905 and NSF 019605), Arctic Archival Observatory (NSF 9981915), and the BRIN Program of the National Centre for Research Resources (NIH Grant # P20 RR16454). We thank Brian Arbogast, John Bender II, Leigh Dickey, James Harper, Nena MacDonald, Michael MacDonald, Steve MacDonald, Richard Runck, and the government of Northwest Territories (Susan Carrière and Richard Popko) for assistance in the collection of specimens, and Kayce Bell for assistance in the laboratory. We thank Enrique Lessa and Marjorie Matocq for insightful reviews. All specimens used in this research have been deposited at the University of Alaska Museum of the North.

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This study is part of Amy Runck's PhD dissertation which was molecular genetics to examine interspecific interactions of *C. gapperi* and *C. rutilus* in areas of contact across Alaska and Canada. Joseph Cook uses molecular genetic techniques to study ecology, evolution, and conservation of organisms.

Appendix

Localities, sample and voucher identification, and GenBank Accession nos for individuals sequenced

State or province	Sample	Voucher ID	Locality	GenBank Accession no.
North Carolina	NC 1	IF5706	Jackson County	AY952166
	NC 2	IF5707	Swain County	AY952167
	NC 3	IF5709	Swain County	AY952168
	NC 4	IF5710	Haywood County	AY952169
	NC 5	IF5711	Yancey County	AY952170
	NC 6	IF5705	Jackson County	AY309430
	NC 7	IF5708	Swain County	AY309434
Pennsylvania	PA 1	AF26609	no specific locality	AY309433
	PA 2	AF26610	no specific locality	AY309434
Minnesota	MN 1	no voucher	no specific locality	AY309431
	MN 2	UAM77133	no specific locality	AY309432
	MN 3	UAM59438	Brown County	AY952171
	MN 4	UAM59496	Brown County	AY952172
	MN 5	UAM59501	Brown County	AY952173
	MN 6	UAM59507	Brown County	AY952174
	MN 7	UAM59508	Brown County	AY952175
	MN 8	UAM59513	Brown County	AY952176
	MN 9	UAM59518	Brown County	AY952177
	MN 10	UAM59521	Brown County	AY952178
	MN 11	UAM59522	Brown County	AY952179
Idaho	ID 1	UAM53937	Latah County	AY952183
	ID 2	UAM53938	Latah County	AY952180
	ID 3	UAM53939	Latah County	AY952181
	ID 4	UAM53940	Latah County	AY952182
Washington	WA 1	UAM41861	Kittitas County	AY952185
	WA 2	UAM41863	Kittitas County	AY952186
	WA 3	UAM41864	Kittitas County	AY952187
	WA 4	UAM41865	Kittitas County	AY952188
	WA 5	UAM41866	Kittitas County	AY952189
	WA 6	UAM41742	Lewis County	AY952190
	WA 7	UAM41743	Lewis County	AY952191
	WA 8	UAM41744	Lewis County	AY952192
	WA 9	UAM41673	Lewis County	AY952193
	WA 10	UAM41674	Lewis County	AY952194
	WA 11	MSB43646	Clallam County	AY952184
British Columbia	BC 1	UAM59964	Swan Lake	AY952157
	BC 2	UAM59965	Swan Lake	AY952158
	BC 3	UAM59966	Swan Lake	AY952159
	BC 4	UAM59967	Swan Lake	AY952160
	BC 5	UAM59968	Swan Lake	AY952161
	BC 6	UAM59969	Swan Lake	AY952162
	BC 7	UAM59970	Swan Lake	AY952163
	BC 8	UAM59972	Swan Lake	AY952164
	BC 9	UAM59973	Swan Lake	AY952165
Northwest Territories	NT 1	UAM77408	Fort Liard	AY952146
	NT 2	UAM77410	Fort Liard	AY952147
	NT 3	UAM77411	Fort Liard	AY952150
	NT 4	UAM77460	Fort Liard	AY952149
	NT 5	UAM77461	Fort Liard	AY952156
	NT 6	UAM77473	Fort Liard	AY952148
	NT 7	UAM77475	Fort Liard	AY952151
	NT 8	UAM77479	Fort Liard	AY952152
	NT 9	UAM77486	Fort Liard	AY952153
	NT 10	UAM77539	Fort Liard	AY952154
	NT 11	UAM77541	Fort Liard	AY952155

Appendix *Continued*

State or province	Sample	Voucher ID	Locality	GenBank Accession no.
Alaska	AK 1	UAM64039	Duck Point	AY952198
	AK 2	UAM70222	Duck Point	AY952203
	AK 3	UAM70023	Duck Point	AY952197
	AK 4	UAM70025	Duck Point	AY952200
	AK 5	UAM70027	Duck Point	AY952199
	AK 6	UAM70029	Duck Point	AY952202
	AK 7	UAM70051	Duck Point	AY952195
	AK 8	UAM70067	Duck Point	AY952196
	AK 9	UAM70055	Duck Point	AY952201
Outgroup taxa				
Clethrionomys californicus		LHS642	California	AY309422
		LHS541	California	AY309423
Clethrionomys rufocanus		IF5702	Japan	AY309418
Clethrionomys rutilus		UAM20293	Alaska	AY309426
		UAM20357	Alaska	AY309427
Clethrionomys glareolus		UAM30029	Finland	AF119272
Eothenomys melanogaster		HS1503	Taiwan	AB017254
Ondatra zibethicus		UAM34209	Alaska	AF119277