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Genetic consequences of Pleistocene glaciations for the tundra vole (*Microtus oeconomus*) in Beringia

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Abstract

Repeated glacial events during the Pleistocene fragmented and displaced populations throughout the northern continents. Different models of the effects of these climate-driven events predict distinct phylogeographic and population genetic outcomes for high-latitude faunas. The role of glaciations in (i) promoting intraspecific genetic differentiation and (ii) influencing genetic diversity was tested within a phylogeographic framework using the rodent *Microtus oeconomus*. The spatial focus for the study was Beringia, which spans eastern Siberia and northwestern North America, and was a continental crossroads and potential high arctic refugium during glaciations. Variation in mitochondrial DNA (cytochrome *b* and control region; 214 individuals) and nuclear DNA (ALDH1 intron; 63 individuals) was investigated across the Beringian region. Close genetic relationships among populations on either side of the Bering Strait are consistent with a history of periodic land connections between North America and Asia. A genetic discontinuity observed in western Beringia between members of a Central Asian clade and a Beringian clade is geographically congruent with glacial advances and with phylogeographic discontinuities identified in other organisms. Divergent island populations in southern Alaska were probably initially isolated by glacial vicariance, but subsequent differentiation has resulted from insularity. Tests of the genetic effects of postglacial colonization were largely consistent with expansion accompanied by founder effect bottlenecking, which yields reduced diversity in populations from recently deglaciated areas. Evidence that populations in the Beringian clade share a history of expansion from a low-diversity ancestral population suggests that Beringia was colonized by a small founder population from central Asia, which subsequently expanded in isolation.

Keywords: Beringia, *Microtus oeconomus*, mitochondrial, nuclear DNA, phylogeography, postglacial colonization

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Introduction

During the Pleistocene, glacial advances were interspersed with warm interglacials in cycles that influenced the spatial distribution and demography of high-latitude species (Webb & Bartlein 1992). The genetic consequences of these events, both for broad-scale phylogeographic structure and local population genetic patterns, are not fully understood. With respect to intraspecific differentiation, glacial advances may have either promoted allopatric differentiation by isolating populations in various glacial refugia (Mengel 1964; MacPherson 1965; Hewitt 1999) or hindered differentiation by inducing repeated range shifts that caused population admixture (Coope 1979). At the local population level, postglacial colonization may have caused a loss of genetic diversity through successive founder events (Hewitt 1996), but this pattern might not hold for northern species that inhabit periglacial habitats (Fedorov *et al*. 1999b). Historical scenarios described by these models

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predict distinctive genetic signatures in extant populations, allowing us to arbitrate among models by testing predictions against observed patterns.

Beringia, which spans northeast Siberia, Alaska and northwest Canada, provides an excellent natural laboratory for examining the effects of Pleistocene glaciations and their genetic consequences for northern organisms. As both a glacial refugium and a route of colonization the region played dual roles in influencing the biogeography and genetic diversity of circum-Arctic species (Guthrie & Matthews 1971; Sher 1986). During the ice ages, Beringia was bounded by complex glacial systems that fragmented and condensed populations, isolating most terrestrial organisms from conspecifics outside the refugium. Moreover, lowered sea levels during glacial periods exposed the continental shelf between North America and Asia, permitting an exchange of species between continents.

In this study, the influence of Pleistocene glaciations on the genetic structure of a Holarctic rodent, *Microtus oeconomus*, is examined. If glaciers promoted allopatric differentiation by fragmenting populations into isolated refugia, phylogeographic breaks that are spatially congruent with past glaciations are expected. Matching patterns would also be predicted in independent molecular markers and in co-distributed organisms (Riddle 1996). Conversely, if glacial movements actually inhibited genetic differentiation by promoting the mixing of populations, then there should be no association between phylogeographic breaks and historically glaciated areas. Other barriers to gene flow, such as the Bering Strait, would still be expected to play a significant role in driving differentiation. Independent molecular markers are examined from the mitochondrial and nuclear genomes to assess spatial congruence between phylogeographic patterns of differentiation and historically glaciated regions of eastern Asia and northwestern North America.

The genetic consequences of population expansion following glacial recession are also considered by comparing populations from regions with different glacial histories. If postglacial colonization took place as a series of successive founder events (Hewitt 1996), populations in regions that were recently glaciated are expected to (i) have lower genetic diversity than populations from nonglaciated areas and (ii) exhibit the genetic signature of population expansion from low-diversity founder populations. Populations from nonglaciated areas are not expected to show significant population expansion.

Materials and methods

Study organism

Microtus oeconomus (tundra vole) is a Holarctic rodent that uses a range of habitats, preferring mesic tundra or meadow environments which are currently common throughout the north (Quay 1951; Tast 1966; Getz 1985). Tundra voles are monophyletic with respect to their closest relatives (Brunhoff *et al*. 2003), and are distributed from Europe east to Siberia and into the Nearctic, where their distribution is roughly coincident with the eastern boundary of Beringia. This distribution has been interpreted as evidence that *M. oeconomus* is a relatively recent trans-Beringian immigrant into North America (Rausch 1963; MacPherson 1965), possibly colonizing Beringia and the Nearctic during the penultimate [Illinoian, ≈ 300–130 thousand years ago (Ka); Bowen *et al*. 1986] or latest (Wisconsin, ≈ 80–10 Ka; Bowen *et al*. 1986) glacial period. Recent invasion of North America is consistent with karyotypic (Nadler *et al*. 1976), allozymic (Nadler *et al*. 1978; Lance & Cook 1998), and morphological (Paradiso & Manville 1961) similarities across the Bering Strait. Furthermore, though Palearctic *M. oeconomus* fossils have been found in deposits of Cromerian age (> 350 Ka; Stuart 1982), the oldest reported fossils in North America date to late Illinoian time (≈ 200–130 Ka; Jopling *et al*. 1981; Zakrzewski 1985). Tundra voles currently inhabiting previously glaciated regions in North America originated from populations that persisted in the Beringian refugium during the last glaciation. Neither its current distribution (Fig. 1) nor the fossil record (Zakrzewski 1985) indicate that *M. oeconomus* survived in refugia south of the Laurentide ice sheet in the contiguous United States.

Sampling

The sampling scheme was designed to assess broad phylogeographic patterns while emphasizing areas with diverse glacial histories. A total of 214 specimens were sampled from 30 localities (Appendix 1). In Siberia, specimens were examined from the Chukotka and Kamchatka Peninsulas (three localities), and the upper Kolyma River basin and the Omolon River (10 localities, Fig. 1). The latter localities fall across the traditional western boundary of the Beringian refugium (e.g. Yurtsev 1974), which has a complex glacial history. Specimens were also collected from nearly the entire North American distribution of the species (16 localities, Fig. 1). Particular attention was given to sites in southern Alaska, which were largely buried beneath the Cordilleran Ice Sheet during past glacial advances. To reduce the possibility of sampling closely related individuals from each specific locality, specimens were selected that had been collected at different trapping sites and on different collecting trips.

Three individuals from Finland were sampled to provide sequences for rooting phylogenetic analyses. A more geographically extensive treatment of the Palearctic phylogeography of tundra voles indicates that the Finnish lineage is basal to Beringian lineages (Brunhoff *et al*. 2003).

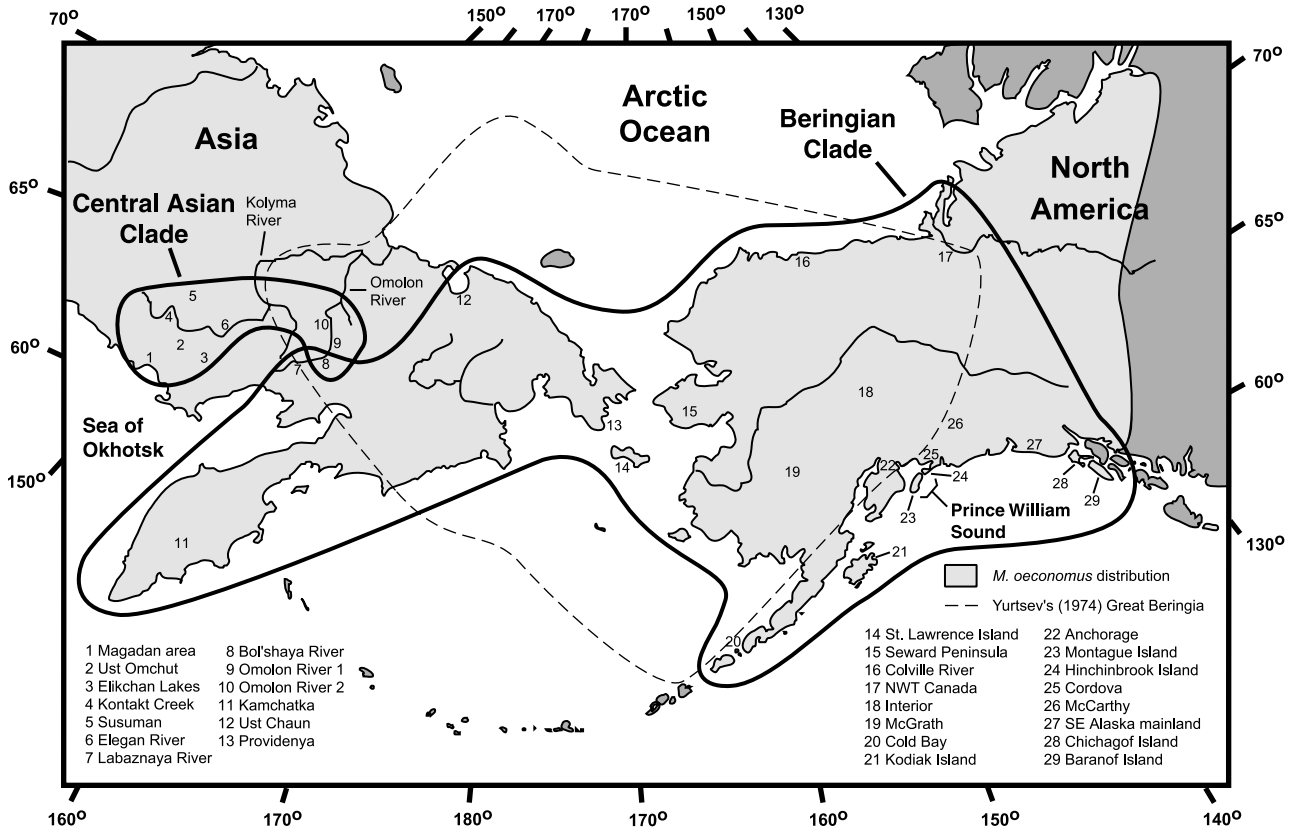

Fig. 1 Distribution of *Microtus oeconomus*, the Central Asian and Beringian clades, and sampling localities in the Beringian region. Numbers designate sampling localities.

Molecular methods

Frozen or alcohol-preserved tissue samples (heart, kidney, skeletal muscle, or liver) were obtained from the University of Alaska Museum Frozen Tissue Collection. Genomic DNA was extracted using a modified sodium chloride extraction protocol (Fleming & Cook 2002), and a region of the mitochondrial DNA (mtDNA) genome was amplified in three overlapping fragments via double-stranded polymerase chain reaction (PCR). This section included the complete cytochrome *b* gene (*cyt-b*), two transfer RNA coding regions, and a portion of the 5′ end of the control region [total fragment length: Beringian clade 1638 base pairs (bp), Central Asian clade 1637–1638 bp; two indels]. Primer sets for *cyt-b* were MVZ05 (Smith & Patton 1993)/Micro06 (5′ GGATTATTTGATCCTGTTTCGT), and Arvic07 (5′ AAAGCCACCCTCACACGATT)/Vole14 (Conroy & Cook 1999). For the control region, primers Micro3 (5′ CTATCATYGTAAATCTCATACCAATCG) and TDKD (Kocher *et al*. 1993) were used. Amplification was performed in 50-μL reaction volumes with the following reagents and concentrations: PCR buffer II (1×; Applied Biosystems Inc.®), primers (1 μM each), dNTP (0.125 mM), $MgCl_2$ (0.16 mM), and *Taq* polymerase (0.005 U/μL). PCR conditions included an initial denaturation (94 °C, 1 min), 35 cycles of denaturation (94 °C, 10 s), annealing (45 °C, 15 s), and extension (72 °C, 45 s), and a final extension (72 °C, 3 min). In addition, 270 bp of a nuclear DNA intron was amplified from 63 voles representing 23 localities (1, 4, 6, 7, 8, 9, 10, 12, 14, 15, 16, 18, 19, 20, 21, 22, 23, 24, 25, 26, 28, 29 and Finland) using primers ALDH1F and ALDH1R (Lyons *et al*. 1997) under the conditions described above, except the $MgCl_2$ concentration for the reaction was 0.08 mM and the annealing temperature was 59 °C. PCR products were sequenced in both directions using a Prism® dye terminator sequencing kit on an ABI 373 automated sequencer (Applied Biosystems Inc.). Sequences were aligned by eye using the program SEQUENCE NAVIGATOR™.

Analyses

To examine phylogeographic structure across Beringia, PAUP* 4.0β10 (Swofford 2000) was used to generate a neighbour-joining tree for the complete set of mtDNA haplotypes from distances calculated using the TrN (Tamura & Nei 1993) + I + Γ nucleotide substitution model. The proportion of invariable sites (I) was 0.7806 and the gamma shape parameter (α) was 1.0108. The model and parameters were

chosen using MODELTEST 3.06 (Posada & Crandall 1998), and they were used throughout the study unless otherwise indicated. Support for the tree topology was evaluated by bootstrapping (5000 replicates).

A molecular clock was applied to examine the relative timing of differentiation events. Conroy & Cook (2000b) used the deepest lineage split within the genus *Microtus* to calculate a *cyt-b* divergence rate of approximately 13% per million years (Myr), which is roughly coincident with upper limits of rate estimates for rodents (e.g. 3.8–11.3%, Martin & Palumbi 1993; 7.5–12%, Arbogast *et al*. 2001). A molecular clock estimate for *cyt-b* in another rodent genus (*Lemmus*), which is largely sympatric with *M. oeconomus* and has similar life-history characteristics, produced a rate of 5%/Myr (Fedorov & Stenseth 2001). Improved fossil dating methods have adjusted this to 7.5%/Myr (V. B. Fedorov, personal communication). The rates for *Lemmus* and *Microtus* represent the best estimates currently available for arvicolid rodents, so both were applied here.

To test for rate heterogeneity among lineages a χ^2 log-likelihood test (Felsenstein 1988) was used to compare maximum-likelihood trees with and without molecular clock constraints for a subset ($N = 26$; Appendix 1) of mtDNA haplotypes. The subset allowed manageable computation times, and it was obtained by computing pairwise uncorrected *p* distances among all haplotypes in the full data set and removing one haplotype from each pair that differed by ≤ 0.005 substitutions per site. MODELTEST selected the TrN + I + Γ model of evolution (I = 0.8235, $\alpha = 2.2286$) for the data subset. Net genetic distance between clades (Edwards 1997) was used to calculate divergence time.

The genetic consequences of postglacial expansion were assessed by comparing populations from the recently glaciated southern part of Alaska (localities 20, 22, 25, 26, 27) to populations from areas that were relatively ice-free during the last glacial maximum (localities 1, 4, 6, 9, 10, 12, 15, 16, 18). Haplotype (Nei 1987) and nucleotide (Nei & Li 1979) diversities were calculated for each population. Diversity estimates for the two sets of populations were compared using a Wilcoxon two-sample test (Sokal & Rohlf 1995). Island populations and those represented by fewer than five individuals were excluded from this analysis.

To test for evidence of recent population growth from low-diversity founder populations in historically glaciated and nonglaciated regions, two methods from the program ARLEQUIN 2.001 (Schneider *et al*. 2000) were used. First, Fu's F_s test (Fu 1997) was performed to test for an excess of rare alleles, which is indicative of recent expansion. Second, pairwise mismatch distributions among individuals were plotted and tested for goodness of fit to a model of sudden expansion using parametric bootstrapping (500 replicates; Schneider & Excoffier 1999). Both methods assume panmixia. Analysis of molecular variance (AMOVA; Excoffier *et al*. 1992) as implemented in ARLEQUIN was used to elucidate the extent of population subdivision within major clades.

In a population that has rapidly expanded from a small ancestral population with low genetic diversity, extant lineages are assumed to coalesce just prior to the initiation of expansion (Rogers & Jorde 1995). The timing of coalescence can be inferred from either the mode of the mismatch distribution (τ) or the mean number of pairwise nucleotide differences (*m*; Rogers & Jorde 1995; Rogers 1995). Both methods were used in this study and an estimate of 2.5 generations/year was applied to calculate expansion times.

Tests of expansion were performed on three sets of populations: (i) Southern Alaska localities 20–22 and 25–29 (23 and 24 were excluded because of evidence that those populations are refugial, with deep colonization histories that are distinct from other southern Alaskan populations; Lance & Cook 1998 and this study), this region was heavily glaciated during the most recent glaciation; (ii) Upper Kolyma River and Magadan (localities 1–6), which was relatively untouched during the last glacial advance but widely impacted by glaciers during the preceding glaciation; and (iii) Omolon River (localities 8–10), which remained ice-free during both of the most recent glacial maxima. Representatives of two clades occur at locality 8 on the Omolon River so only individuals from the Central Asian clade were included in that analysis. To examine the possibility that population growth was not limited to these three sets of populations, the demographic analyses were also applied to all populations in each major clade.

Results

Mitochondrial sequence data

A total of 102 distinct mtDNA haplotypes were identified from 214 specimens (GenBank accession numbers AY305050–AY305263). Base composition of *cyt-b* (C 31%, T 25%, A 31%, G 13%) was consistent with other mammalian *cyt-b* sequences (Irwin *et al*. 1991; Lessa & Cook 1998; Conroy & Cook 2000a,b). The distribution of variation across codon positions (first 21% of all variable sites, second 5%, third 74%) was as expected for genuine, functional *cyt-b* sequences (Lessa & Cook 1998; Conroy & Cook 2000a,b). Likewise, the distribution of 28 variable amino acid sites fitted structural models of variable and conserved regions in *cyt-b* (Irwin *et al*. 1991), and the pattern of variation across the tRNA sequences (conserved) and control region (variable) matched predictions for mammalian mtDNA (Cann *et al*. 1984). Percentages of variable sites for *cyt-b* and control region were roughly equivalent (*cyt-b* 12.5%, control region 11.4%, total fragment 11.6%).

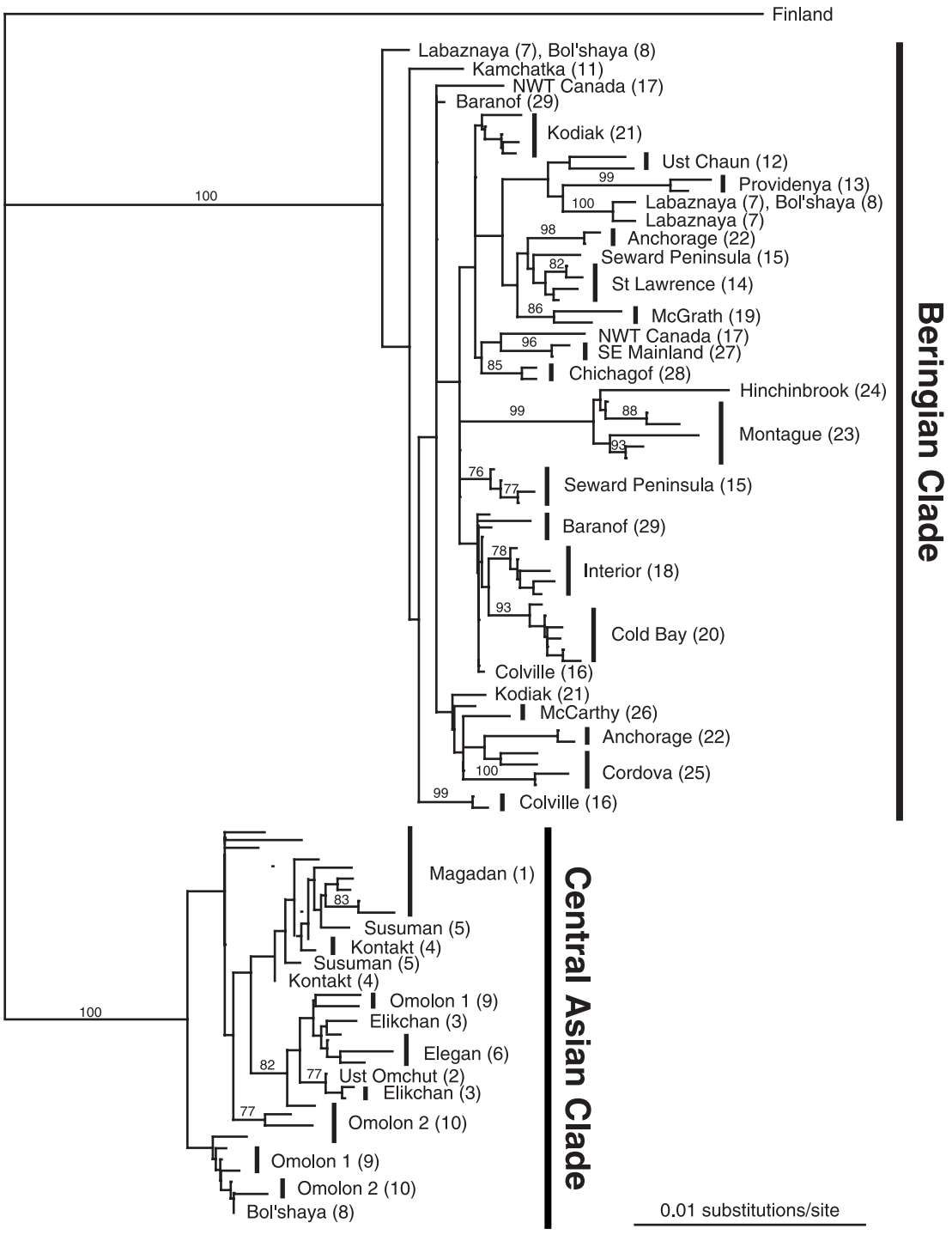

Fig. 2 Neighbour-joining phylogeny of all 102 observed haplotypes using the TrN + I + Γ model of evolution. Numbers above branches are bootstrap values (> 75%) based on 5000 replicates. Numbers in parentheses correspond to locality numbers in Fig. 1.

Phylogeographic structure

The neighbour-joining tree revealed three well-defined clades within *Microtus oeconomus* (Fig. 2). These corresponded to three of four tundra vole clades identified by Brunhoff *et al.* (2003): (i) North European clade (Finland), (ii) Central Asian clade (localities 1–6, 8–10), and (iii) Beringian clade (localities 7, 8, 11–29). We found no evidence of significant differentiation between eastern Siberia and North America across the Bering Strait. Though bootstrap values supported

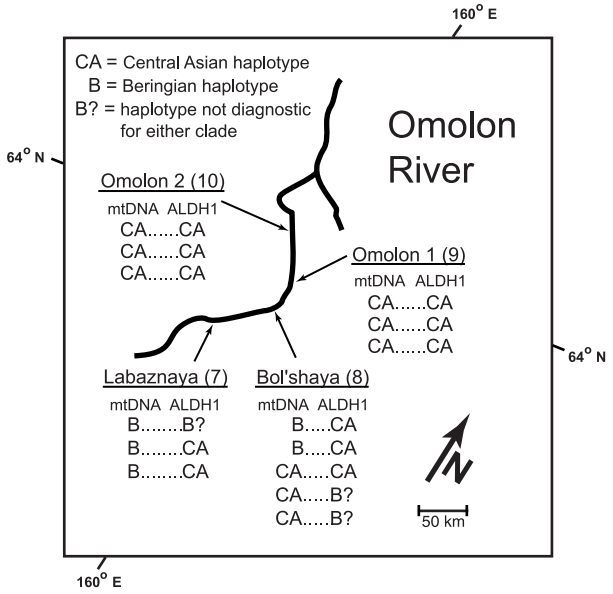

Fig. 3 Distribution of Beringian and Central Asian clade mtDNA and nuclear (ALDH1) haplotypes along the contact zone on the Omolon River. Only the 14 individuals for which both the nuclear and mtDNA markers were sequenced are shown; each one is listed separately. Numbers in parentheses indicate locality numbers. Question marks (B?) indicate nuclear haplotypes that could not be unequivocally assigned to either clade because they matched the haplotype that was shared by members of both clades.

Table 1 Genetic diversity indices for populations from regions that were or were not glaciated during the last glacial maximum. Number of individuals (*n*), number of haplotypes (*Nh*), haplotype (*h*) and nucleotide (π) diversities, and their respective standard errors (SE) are given. Numbers in parentheses correspond to locality numbers given in Fig. 1

Geographical locality	*n*	*Nh*	*h*	SE (*h*)	π (%)	SE (π)
Glaciated						
Cold Bay (20)	10	6	0.778	0.137	0.084	0.064
Anchorage (22)	8	4	0.821	0.101	0.479	0.420
Cordova (25)	10	4	0.711	0.118	0.377	0.315
McCarthy (26)	9	2	0.556	0.090	0.139	0.126
SE Alaska (27)	10	2	0.467	0.132	0.029	0.031
Not recently glaciated						
Magadan (1)	10	9	0.978	0.054	0.360	0.129
Kontakt Creek (4)	11	3	0.473	0.162	0.043	0.053
Elegan River (6)	5	4	0.900	0.161	0.235	0.129
Omolon 1 (9)	11	5	0.709	0.137	0.628	0.389
Omolon 2 (10)	15	6	0.648	0.134	0.331	0.287
Ust Chaun (12)	10	2	0.467	0.132	0.243	0.262
Seward Pen. (15)	10	5	0.822	0.097	0.163	0.198
Colville River (16)	8	3	0.607	0.164	0.329	0.304
Interior (18)	9	4	0.861	0.087	0.132	0.076

some genetic substructure within the Beringian and Central Asian clades, resolution of basal relationships within the clades was poor, and most tip branch lengths were relatively short (< 0.003 substitutions/site). Montague and Hinchinbrook Island populations were a notable exception within the Beringian clade, forming a well-supported monophyletic group (Fig. 2). Voles of the Beringian and Central Asian mtDNA clades were in contact at locality 8 on the Omolon River (Figs 1 and 3). Down-river (northwest) of that location, voles exhibited Central Asian clade haplotypes and up-river (southwest) they represented the Beringian clade.

Two alleles were identified at the nuclear marker ALDH1, which segregated across western Beringia and produced distinct clades in western and eastern Siberia. Fifteen of 19 voles with Central Asian clade mtDNA had a 17-bp nuclear deletion, which was absent in all 39 individuals examined from North America or Siberia east of the Omolon River (i.e. Beringian clade voles). Central Asian clade voles that lacked the deletion were from localities 4, 7 and 8. The high frequency of the nuclear deletion on the Omolon River and its complete absence east of that point suggests that the disjunction between populations representing the two nuclear clades occurs in western Beringia and is geographically congruent with the mitochondrial data. Notably, four of five Omolon River voles with Beringian clade mtDNA had the nuclear deletion, raising the possibility of historical introgression between members of the two mtDNA clades (Fig. 3). Specimens from Finland lacked the deletion.

The maximum-likelihood tree built under a molecular clock constraint did not differ significantly from the unconstrained tree ($P = 0.20$), suggesting that tundra vole lineages are evolving in a clock-like manner. Percentage of variable sites differed little between *cyt-b* and control region, implying roughly equivalent mutation rates for both regions of mtDNA. Therefore, we used all 1638 bp for all analyses, including analyses that incorporated a molecular clock. Net distance between the Beringian and Central Asian clades (0.0296 substitutions per site) yielded divergence times of approximately 227 Ka and 394 Ka using the different divergence rates.

Genetic diversity and expanding populations

Comparison of genetic diversity values (Table 1) between glaciated and nonglaciated regions showed that neither haplotype ($U = 27.5$; one-tailed $P > 0.10$) nor nucleotide ($U = 26$; one-tailed $P > 0.10$) diversity was significantly greater in nonglaciated regions.

The tests of population expansion indicated contrasting demographic histories for the regions with different glacial histories (Fig. 4). Populations from the Omolon River did not possess the genetic signature of recent expansion

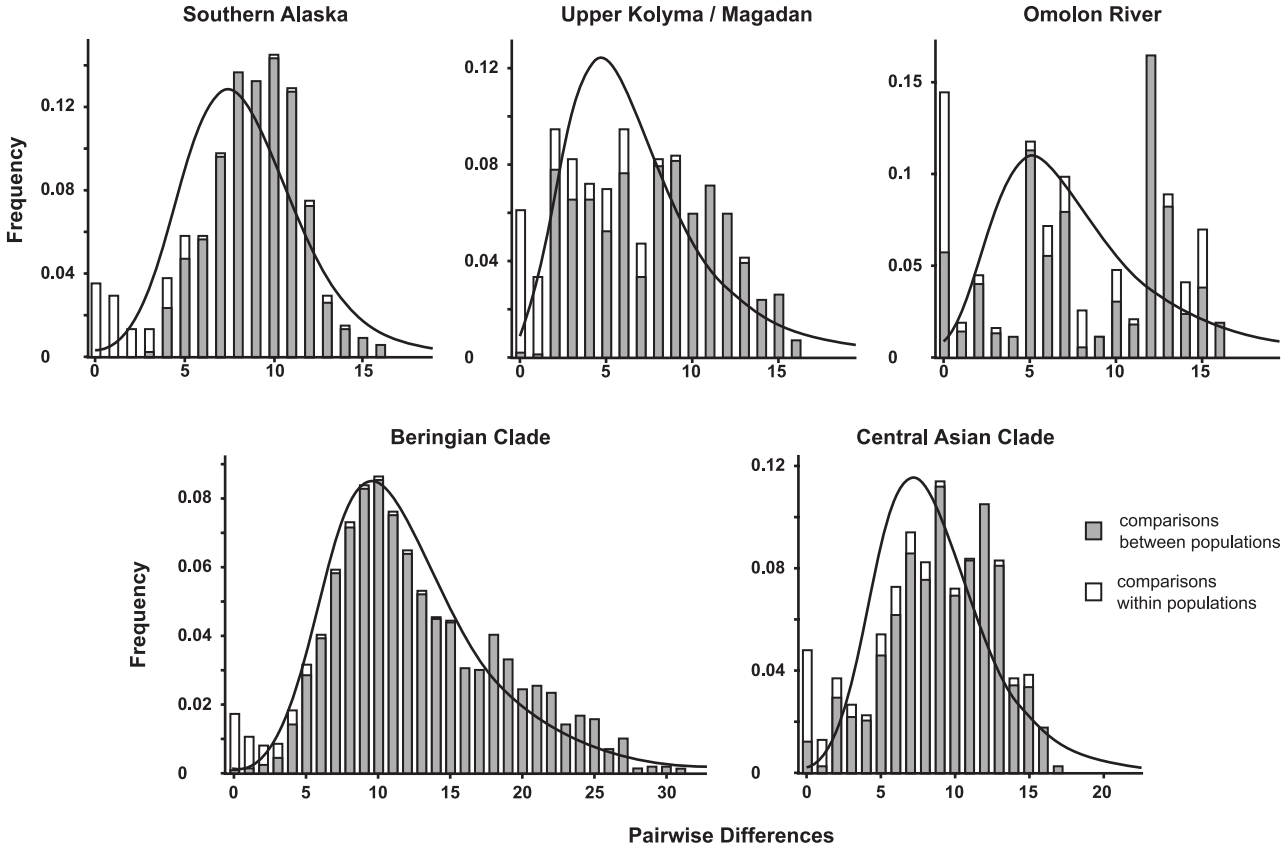

Fig. 4 Pairwise mismatch distributions for five sets of populations with different glacial histories. Vertical bars indicate the frequency of pairwise sequence comparisons that differed by a given number of nucleotides. Within each bar the grey portion indicates comparisons between individuals from different localities, and the white portion indicates comparisons between individuals within localities. The solid line represents the expected distribution under the model of expansion.

($F_s = 1.392$, $P = 0.726$; mismatch distribution test of goodness-of-fit, $P = 0.042$), but populations from the upper Kolyma River and Magadan area did ($F_s = -7.97$, $P = 0.009$; mismatch distribution, $P = 0.876$). A nonsignificant result for the mismatch distribution analysis and a significant result for Fu's F_s test suggest recent population expansion. For the F_s test, $P = 0.02$ is considered to be significant at the $\alpha = 0.05$ level (Fu 1997). Results for populations from recently glaciated areas in southern Alaska were equivocal, with the two tests giving weakly conflicting results at $\alpha = 0.05$ ($F_s = -2.28$, $P = 0.060$; mismatch distribution, $P = 0.096$). Application of the tests to the entire Beringian clade ($F_s = -7.972$, $P = 0.009$; mismatch distribution, $P = 0.672$) and Central Asian clade ($F_s = -10.345$, $P = 0.007$; mismatch distribution, $P = 0.394$) indicated that both possess the genetic signature of expanding populations when considered in their entirety. For populations that did not differ from the expansion model, expansion time estimates were broadly consistent across localities and methods (Table 2).

The AMOVA showed that a significant proportion of genetic variation is partitioned among populations in both the Beringian (74.24%, $P < 0.001$) and Central Asian (49.67%, $P < 0.001$) clades. Population subdivision can confound mismatch distribution analyses by causing incorrect rejection of the population expansion model (Marjoram & Donnelly 1994). This suggests that failure to reject the expansion model, despite population subdivision, is a conservative result. The equivocal result for southern Alaskan populations may have been influenced by subdivision. Clustering of within-population comparisons in the mismatch distribution (Fig. 4) implies restricted gene flow among populations and indicates that those comparisons reflect local population dynamics rather than regional demographic history. The low peak produced by within-population comparisons interrupted the otherwise clear signature of an expanding population, i.e. a smooth, unimodal distribution (Fig. 4). Whether or not subdivision affected the Omolon River population set is less clear, but the stark contrast between its mismatch distribution and those of the other populations, as well as the strong result from the F_s test, are consistent with rejection of the expansion model.

Table 2 Parameter values and expansion time estimates for expanding populations determined by mismatch distribution analyses*

	Beringia clade	95% CI	Southern Alaska	95% CI	Central Asian clade	95% CI	Upper Kolyma and Magadan	95% CI
τ	7.58	4.39–18.5	9.34	6.50–11.2	9.61	5.75–14.4	8.02	3.67–17.5
m	12.4	8.51–19.9	8.29	7.23–10.7	8.77	6.10–11.9	6.80	4.03–11.4
θ_0	5.94	0–20.0	0	0–2.37	0.871	0–3.44	2.01	0–5.23
θ_1	147	40.2–6450	107	58.1–6580	34.80	19.8–2680	13.5	7.57–126
Expansion time estimates (Ka)								
7.5%/Myr divergence rate								
Using τ	61.7	35.7–150	76.0	52.9–91.2	78.3	46.8–117	65.3	29.9–142
Using *m*	101	69.3–162	67.5	58.8–87.1	71.4	49.7–96.9	55.4	32.8–92.8
13%/Myr divergence rate								
Using τ	35.6	20.6–86.9	43.9	30.5–52.6	45.1	27.0–67.7	37.7	17.2–82.2
Using *m*	58.2	40.0–93.4	38.9	34.0–50.2	41.2	28.7–55.9	31.9	18.9–53.6

*Parameters θ_0 and θ_1 are estimates of initial and current effective population size, respectively, scaled by mutation rate (Rogers 1995).

Discussion

Pleistocene glaciations and genetic differentiation

If glacial advances isolated populations, spatial congruence between phylogeographic structure and historical glaciations would be expected. Conversely, if glaciations had an inhibitory effect on differentiation by promoting population admixture, as proposed for high-latitude insects (Coope 1979), populations would be expected to be more genetically homogeneous across regions of historical glacial activity.

In Siberia the two most recent glacial advances, the Zyryanka and Sartan glaciations, generally correspond to the 1st and 2nd Wisconsin glacial periods in North America (≈ 80–55 Ka and ≈ 25–10 Ka, respectively; Arkhipov *et al*. 1986b; Bowen *et al*. 1986). Glacial ice was not a permanent feature of the Beringian landscape during the Pleistocene, and glacial advances differed in magnitude, particularly in Siberia (Fig. 5). During the Zyryanka, glaciers covered approximately 40% of northeast Siberia (Bespalyy 1984; Arkhipov *et al*. 1986a), forming an almost unbroken barrier across the Kolyma uplands from the Sea of Okhotsk in the south to the present day Siberian coast of the Arctic Ocean (Bespalyy 1984; Arkhipov *et al*. 1986b). Beyond this to the north, the continental shelf was exposed because of lowered sea levels and remained ice-free. In contrast, low precipitation during the most recent glaciation (Sartan) prevented glaciers from expanding and coalescing into major ice sheets (Bespalyy 1984; Arkhipov *et al*. 1986b). Large ice-free corridors remained open throughout the glacial maximum, leading some authors to refer to Beringia's western border as 'porous' (e.g. Hoffmann 1981) because of the presumed opportunity for organisms to move between central Eurasia and Beringia. Glaciers retreated with climate warming, sometimes disappearing entirely, and opportunities for postglacial expansion and gene flow among glacially isolated populations probably increased.

Microtus oeconomus does not exhibit well-defined phylogeographic structure within Beringia. The tundra voles on the Prince William Sound islands of Montague (23) and Hinchinbrook (24) are an exception, forming a strongly supported monophyletic group (Fig. 2). Their apparently deeply shared history contrasts with other populations in the Beringian clade, which generally are poorly differentiated. This divergent clade implies that glaciers played a role in driving genetic differentiation. Tundra voles may have been isolated south of the Cordilleran ice sheet (Fig. 5), and then partitioned onto the two Prince William Sound islands after the glaciers retreated and sea levels rose. Isolation and differentiation were probably initiated through glacial vicariance, and subsequently maintained by water barriers.

The close genetic relationship between Siberia and Alaska (Fig. 2) corroborates karyotypic (Nadler *et al*. 1976), allozymic (Nadler *et al*. 1978; Lance & Cook 1998), and morphological (Paradiso & Manville 1961) data. Of the three other rodent species with amphiberingian distributions (*Spermophilus parryii*, *Clethrionomys rutilus*, *Lemmus trimucronatus*), only the phylogeography of *L. trimucronatus* has been examined in detail, and that lemming species also was genetically undifferentiated across the Bering Strait (Fedorov *et al*. 1999b). Cook *et al*. (2003) also report close genetic ties between Asian and North American red-backed voles (*C. rutilus*) and a number of other taxa such as arctic hares (Waltari *et al*. submitted for publication) and moose (Hundertmark *et al*. 2002) do not reflect strong genetic differentiation across this barrier. Populations on either side of the Bering Strait have been separated for at least 10 000 years (Elias *et al*. 1996), providing a qualitative indicator of relative timing of divergence events. Strong

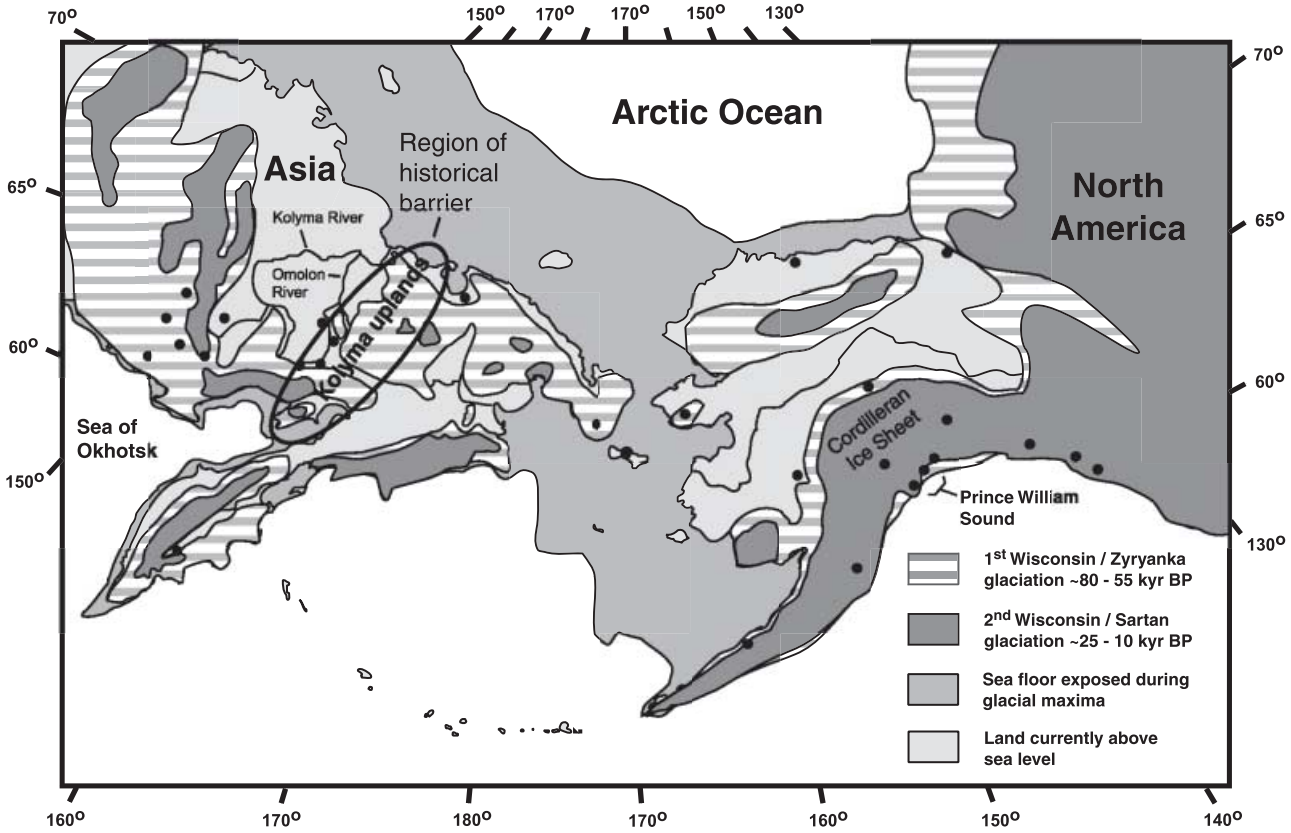

Fig. 5 Maximal extent of the Late Pleistocene glaciations in Beringia and the Bering Land Bridge (modified from Arkhipov *et al*. 1986b; Hamilton *et al*. 1986). The general location of the putative historical barrier in western Beringia is indicated. Black dots denote sampling localities (identified in Fig. 1).

genetic discontinuities found elsewhere probably reflect older isolation events.

The largest genetic break for Beringian tundra voles is located along the Omolon River in the Kolyma uplands (Fig. 1). Strong differentiation between members of the Beringian and Central Asian clades suggests that repeated advances and withdrawals of glaciers along this western border of Beringia did not promote population admixture (Coope 1979). Representatives from the two mitochondrial clades occurred together at only one locality (8), though the nuclear ALDH1 intron hinted at a wider zone of overlap between the western and eastern groups (i.e. voles with Beringian clade mtDNA from two localities possessed the Central Asian nuclear deletion). The presence of a nondeletion haplotype in Central Asian voles could have resulted from introgression between ancestral populations during past glacial periods. Alternatively, the mixture of haplotypes in the Central Asian clade may be the result of incomplete lineage sorting. Further sampling of populations near this zone and surveys of unlinked loci should clarify the extent of introgression and overlap between the clades.

The geographical association between the genetic break in western Beringia and past glaciations seems indicative of an historical glacial barrier that divided the ancestors of the Beringian and Central Asian clades and initiated their divergence. Congruence between the distribution of the Beringian clade and the traditional boundaries of Beringia (Fig. 1) further reinforces this conclusion by implying that the clade originated in the Beringian refugium. This fits the established model of the refugium as a centre of evolution in which populations became isolated and diverged onto unique evolutionary trajectories (Guthrie & Matthews 1971; Sher 1986).

Riddle (1996) describes three corollaries to the hypothesis that relatively deep phylogenetic breaks are associated with biogeographic barriers to gene flow. The first, biotic/abiotic concordance, is met by the spatial congruence between past glaciations and tundra vole phylogeographic structure. This genetic break also roughly coincides with the subdivision between two vegetatively distinct subarctic climatic zones (Lozhkin & Anderson 1995), which may have reinforced the separation of glacially isolated populations during interglacial periods. The second corollary, taxonomic concordance, is also met. Geographically similar genetic discontinuities in other rodent taxa (*Lemmus* and *Dicrostonyx*; Fedorov *et al*. 1999a,b) suggest a shared history

of isolation across a barrier. The possibility that the phylogenetic split in *Lemmus* may have been initiated elsewhere (Fedorov *et al*. 1999b) indicates that the barrier may have been important in maintaining historical isolation as well as driving more recent differentiation. The last corollary is gene-tree concordance, which is demonstrated by the similar spatial distributions of the mtDNA and nuclear ALDH1 clades. Morphometric differences that may have a genetic basis also support the emerging pattern. Eastern Siberian tundra voles are morphologically distinct at the subspecies level from those of the upper Kolyma River region (Chernyavski 1984; Kostenko 2000). The distributions of the eastern and western subspecies are congruent with those of the Beringian and Central Asian molecular clades, respectively. Evidence for an historical barrier in the form of repeated glacial advances in this region strongly implicates glacial isolation as a driving factor behind genetic differentiation.

If a barrier to gene flow was present in western Beringia, the notion that the western boundary of the refugium was 'porous' (Hoffmann 1981) and permitted dispersal is contradicted. Although glaciers did not cover large portions of Siberia during the Sartan glaciation, the Kolyma uplands were probably part of a vast subarctic desert that graded into arctic desert on the exposed continental shelf to the north (Grichuk 1984). Such xeric ecosystems may have been as inhospitable to mesophilous tundra voles as glacial ice, and as effective at preventing gene flow. Furthermore, recent studies of Beringian palaeoenvironments suggest that the centre of Beringia (i.e. the region straddling the Bering Strait) was particularly suited to mesic-adapted species (Elias *et al*. 2000; Guthrie 2001), such as *M. oeconomus*. Differentiation may therefore have been caused by a combination of factors, possibly including an ecologically induced range shift toward central Beringia, coupled with a glacial/ecological barrier in the Kolyma uplands. In addition, lower fitness of hybrids between the members of the Beringian and Central Asian clades may limit population admixture and help maintain isolation at times when the clades come into contact (e.g. Hewitt 1999).

Both timing estimates for the genetic break suggest that tundra voles entered western Beringia no later than the Illinoian period, which placed them in a position to colonize the Nearctic before the Wisconsin glaciations. Once they entered Beringia, expansion across the Bering Land Bridge could have been rapid, particularly if central Beringia was well suited to mesic-adapted species (Elias *et al*. 2000; Guthrie 2001). An Illinoian colonization is consistent with the North American fossil record (Jopling *et al*. 1981; Zakrzewski 1985).

Postglacial colonization and population expansion

Did populations of *M. oeconomus* lose genetic diversity through founder effect bottlenecking during postglacial colonization, or was gene flow between source and founder populations sufficient to maintain high levels of diversity?

Superficially, results from the tests of diversity and demographic analyses are contradictory. Comparisons of haplotype and nucleotide diversity show that populations that recently colonized deglaciated areas (i.e. expanding populations) do not have significantly less variation than populations from areas that were ice-free during the last glaciation (i.e. refugial). This result is consistent with the hypothesis that postglacial expansion by northern species is not associated with a reduction of genetic diversity (Fedorov *et al*. 1999b). However, in the tests for recent expansion, the two sets of populations from areas that were glaciated during the last (southern Alaska) and next to last (upper Kolyma/Magadan area) major glacial advances exhibit the genetic signature of expansion from low-diversity ancestral populations. Those from the Omolon River, a region that has remained free of glaciers for > 130 Ka, do not show an expansion signal, implying instead a long-term, demographically stable population. These demographic results are consistent with a postglacial colonization hypothesis that genetic diversity is lost in expanding populations because of serial bottlenecking (Hewitt 1996).

If expansion accompanied glacial events, relative estimates of expansion times should be consistent with the timing of the most recent major glacial advances. In the upper Kolyma region the last major glaciation was the Zyryanka, which preceded the most recent glaciation in southern Alaska (2nd Wisconsin) by 30 Kyr (Fig. 5). Expansion time estimates for upper Kolyma and southern Alaska populations, however, were roughly equivalent (Table 2). Because of uncertainty in the mutation rate and other unknown variables, timing estimates should be interpreted conservatively, but all estimates for the upper Kolyma and Magadan fall closer to the Zyryanka glaciation than the Sartan and might match expectations derived from the postglacial founder event hypothesis. Expansion times for Alaskan populations do not coincide with glacial history. During North America's last glacial maximum (2nd Wisconsin), southern Alaska was largely buried under the Cordilleran ice sheet (Hamilton *et al*. 1986; Mann & Hamilton 1995; Fig. 5). With the exception of the putative refugial population in Prince William Sound, the ice sheet presumably eradicated all local populations of tundra voles, along with the genetic signature of prior colonization of the region following the 1st Wisconsin glaciation. All expansion time estimates for southern Alaskan populations, however, solidly predate the last advance (Table 2). Furthermore, when the tests of demographic history were applied to the entire Beringian clade they revealed a similar signature of population growth. Cumulatively, these results indicate that Beringian clade populations expanded from a low-diversity ancestral population that predated

the last glacial advance, and the genetic signature of that history was strong enough to be detected when a fraction of the descendent populations was sampled.

Our findings are largely consistent with Hewitt's (1996) founder event model of postglacial colonization. Results of the demographic analyses matched predictions based on that model for two out of three sets of populations, and evidence that Beringian populations share a history of expansion may reconcile the inconsistent expansion time estimate from southern Alaska. Low diversity in source populations could have masked postglacial founder effect in southern Alaska (i.e. source populations had little diversity for colonizing populations to lose). Similar reasoning could reconcile the contradictory result from the tests for differences between diversity estimates of glaciated and nonglaciated regions. Also, the strong signature of expansion from a low-diversity ancestral gene pool could have overwhelmed weaker signatures recorded during postglacial colonization. Finally, high variance associated with coalescence times may cause inconsistencies between expected and calculated expansion times, particularly in a single locus analysis.

Conclusions

Pleistocene glaciations probably promoted intraspecific differentiation in *M. oeconomus*. Phylogeographic structure in Beringian tundra voles is consistent with a history of glacial vicariance that drove genetic differentiation in eastern Siberia and southern Alaska, though other factors may have helped to maintain separation between glacially sundered populations during interglacial periods (e.g. ecological barriers in eastern Siberia or water barriers in southern Alaska). These findings are consistent with suggestions that Pleistocene glacial events promoted intraspecific differentiation (Avise & Walker 1998), and they highlight the importance of Beringia as not only the crossroads of the northern continents, but also as a high-latitude centre of diversification.

The genetic consequences of postglacial colonization by tundra voles remain to be fully clarified, though we find insufficient evidence to reject the well-established postglacial founder event model of colonization (Hewitt 1996) for the alternative model proposed by Fedorov *et al*. (1999b). Tundra voles are adapted to high-latitude environments and probably rapidly colonized periglacial habitats after glacial recession, but the expanding edge of populations might have been subject to founder effect if gene flow from source populations decreased quickly after colonizers became established. There were no haplotypes shared between populations in the Beringian clade, implying low levels of gene flow since populations were established.

An unexpected result of the demographic analyses was evidence that Beringian clade populations share a history of expansion from an ancestral population of low diversity. Beringian populations of another Holarctic taxon, *Alces alces* (moose), show similar evidence for recent (< 30 Ka) demographic expansion from a small group of founders (Hundertmark *et al*. 2002). Given the short history of *M. oeconomus* in Beringia, the expansion signature may reflect the original arrival of a small founder population in the refugium. Evidence for low ancestral diversity indicates that gene flow from central Asia was short-lived, which is consistent with the phylogeographic results and implies that opportunities for movement across western Beringia were rare and ephemeral, despite the region's patchy glacial history. Future work examining other recent immigrants to North America (e.g. the vole *C. rutilus*) for concordant patterns might help to resolve the glacial and ecological context of barrier openings, and clarify the nature of western Beringia's filtering effect on transberingian colonizers.

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This work forms a portion of Kurt Galbreath's master's thesis at the University of Alaska, Fairbanks, conducted under the direction of Joseph Cook. Galbreath is now a doctoral student at Cornell University. Cook, now a Professor at Idaho State University, is moving again to become Curator of Mammals at the Museum of Southwestern Biology. Cook's work focuses on how evolutionary histories of high-latitude organisms have shaped patterns of genetic variation in extant populations.

Appendix I

Specimens listed by locality and University of Alaska Museum AF number. Locality numbers correspond to those used in Fig. 1. Asterisks (*) denote sequences selected for the data subset used in the rate heterogeneity test.

Russia

1. Magadan area: AF6640, AF6691*, AF6693, AF6694, AF6700, AF6713, AF6714*, AF6715, AF6716, AF6728
2. Ust Omchut: AF41301, AF41302*, AF41303
3. Elikchan Lakes: AF41325, AF41330, AF41347
4. Kontakt Creek: AF41103, AF41258, AF41261, AF41262, AF41263, AF41276, AF41278, AF41280, AF41283, AF41285, AF41290
5. Susuman: AF38901, AF38902
6. Elegan River: AF38836, AF38842*, AF38843, AF38854, AF38876
7. Labaznaya River: AF38014, AF38027, AF38032*
8. Bol'shaya River: AF38095, AF38132, AF38137, AF38138, AF38139
9. Omolon River 1: AF38141, AF38148, AF38156, AF38161, AF38163, AF38165*, AF38169, AF38170, AF38171, AF38234, AF38235,
0. Omolon River 2: AF38290, AF38291, AF38349, AF38350, AF38351, AF38356, AF38366, AF38371, AF38376, AF38391, AF38396, AF38397, AF38402, AF38403, AF38405
11. Kamchatka: AF32747*
12. Ust Chaun: AF3758, AF3759, AF3760, AF3761, AF3762*, AF3763, AF3771, AF3772, AF3773, AF3774
13. Providenya: AF7468*, AF7470, AF7472

North America

14. St. Lawrence Island: AF20801, AF20802, AF20805*, AF20807, AF20808, AF20812, AF20817, AF20818, AF20819
15. Seward Peninsula: AF7370, AF7462, AF7463, AF7464, AF36721, AF36722, AF36752, AF36753, AF39706, AF39707
16. Colville River: AF22101, AF22103, AF22104, AF22114, AF22115, AF22117, AF22119, AF22135*
17. Northwest Territories, Canada: AF43634*, AF43794*
18. Interior Alaska: AF347, AF996, AF1092, AF1110*, AF2253, AF18690, AF18705, AF24826, AF28221
19. McGrath: AF31560*, AF31591
20. Cold Bay: AF14978, AF14985, AF14989, AF14991, AF14994, AF14999, AF15678, AF15680*, AF15747, AF15748
21. Kodiak Island: AF794, AF795, AF796, AF797, AF798, AF801, AF835, AF838, AF839, AF840
22. Anchorage: AF8819*, AF8828, AF8831, AF8843*, AF11320, AF11373, AF11380, AF11600
23. Montague Island: AF510, AF513, AF514, AF515*, AF516*, AF517, AF535, AF1951, AF1952, AF1953
24. Hinchinbrook Island: AF458, AF460, AF461*, AF462, AF470, AF476, AF494, AF495, AF496, AF498
25. Cordova: AF452, AF453, AF454, AF455*, AF456, AF505, AF506, AF507* AF1978, AF1979
26. McCarthy: AF3277, AF3278, AF3279, AF3280, AF3284, AF3287, AF3288, AF3289, AF3294
27. Southeast Alaska mainland: AF2032*, AF2033, AF2034, AF2054, AF2055, AF7820, AF7821, AF7822, AF7836, AF7837
28. Chichagof Island: AF16082, AF16083*
29. Baranof Island: AF7601, AF7610, AF7613, AF7657, AF7658, AF7721, AF17071, AF17082, AF17085, AF17133

Finland

Kilpisjärvi: AF1944*, AF1948, AF1949