
By

Kurt Egan Galbreath

RECOMMENDED:

[Signatures]

Advisory Committee Chair

Edward C
Chair, Department of Biology and Wildlife

APPROVED:

[Signature]
Dean, College of Science, Engineering, and Mathematics

[Signature]
Dean of the Graduate School

Date
GENETIC CONSEQUENCES OF ICE AGES FOR A HOLARCTIC RODENT: PHYLOGEOGRAPHY AND POST-GLACIAL COLONIZATION OF THE TUNDRA VOLE, *MICROTUS OECONOMUS*, IN BERINGIA

A
THESIS

Presented to the Faculty of the University of Alaska Fairbanks in Partial Fulfillment of the Requirements for the Degree of

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By

Kurt Egan Galbreath, B.S.
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I. ABSTRACT

Periodic glacial advances during the Pleistocene fragmented and displaced populations, while lowered sea levels permitted a biotic interchange between Asia and North America via the Bering Land Bridge. The tundra vole (*Microtus oeconomus*), a recent colonizer of North America, is a good model for studying the genetic consequences of these events. Variation in mitochondrial (cytochrome *b* and control region) and nuclear (intron ALDH1) markers was examined within the context of Beringia’s paleoclimatic history to examine the role of glaciations in driving genetic differentiation and structuring patterns of genetic diversity. Genealogical relationships among genetic lineages were also assessed to elucidate probable paths of transberingian gene flow and post-glacial colonization. A deep phylogeographic break in western Beringia separates Beringian and Central Asian clades and may have been initiated by glacial vicariance. Population genetic structure within the Beringian clade has largely been determined by an historical reduction in genetic diversity and subsequent local differentiation. Serial bottlenecking during post-glacial colonization had a minor effect, if any. Female-mediated gene flow among populations has been minimal since the last glacial maximum, but affinities among populations in Siberia and Alaska suggest two latitudinally partitioned routes of gene flow across the Bering Land Bridge. Also, post-glacial colonization of heavily glaciated southcoastal Alaska probably proceeded along coastal routes from the west after glacial recession.
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VII. INTRODUCTION

Repeated cycles of glacial and interglacial periods during the Pleistocene influenced plant and animal distributions, population demographics, and ecological community structure on a global scale (Webb and Bartlein, 1992). Paleoclimatic changes had particularly significant biogeographic repercussions in the Northern Hemisphere. Populations were fragmented and displaced by the movements of massive ice sheets, and fluctuating sea levels alternately exposed and inundated vast expanses of continental shelf. Species responses to these events varied and often reflected the specific ecological requirements of the organism in question. During ice ages, many temperate species were forced southward by advancing glaciers and decreased temperatures at northern latitudes (e.g. Mengel, 1964; Hoffmann, 1981; Webb and Bartlein, 1992; Wooding and Ward, 1997; Taberlet et al., 1998; Hewitt, 1999), while high latitude species generally persisted in one or more isolated glacial refugia (e.g. MacPherson, 1965; Heaton et al., 1996; Fedorov et al., 1999a,b; Conroy and Cook, 2000a).

Significant biogeographic consequences of Pleistocene glaciations imply equally significant genetic consequences. For example, isolation in distinct glacial refugia is regularly invoked to explain patterns of genetic differentiation (e.g. Mengel, 1964; MacPherson, 1965; Hoffmann, 1981; Klicka and Zink, 1997; Avise and Walker, 1998; Taberlet et al., 1998; Hewitt, 1999), and reduced genetic diversity in northern populations may have resulted from serial bottlenecks during post-glacial colonization.
Hypotheses such as these must be tested explicitly in order to understand the full extent of genetic effects of Pleistocene glaciations (Knowles, 2001), which range from population level genetic structuring to deep phylogeographic differentiation that may represent incipient speciation.

Beringia, the region that is centered on the Bering Strait and spans eastern Siberia, Alaska, and northwestern Canada, provides an ideal testing ground for examining the genetic consequences of ice age events. Glaciations (Arkhipov et al., 1986a,b; Hamilton et al., 1986; Mann and Hamilton, 1995), climate-driven ecological shifts (Heusser, 1960; Grichuk, 1984; Ager and Brubaker, 1985; Mann and Hamilton, 1995), and the opening and closing of the Bering Land Bridge (Hopkins, 1959; Elias et al., 1996) all had repeated impacts on the biogeography of Beringia during the Pleistocene. These paleoenvironmental influences potentially produced multiple genetic signals in amphiberingian fauna. Recurring ice ages played counterpoint to ancient isolating events (e.g. initial flooding of the Bering Land Bridge), creating shallow and deep genetic signatures that can be traced by examining intraspecific DNA sequence data in a phylogeographic context (Riddle, 1996).

The tundra vole, *Microtus oeconomus*, is an excellent model organism for examining genetic effects of Pleistocene glaciations in Beringia. Tundra voles are Holarctic in distribution, probably colonizing North America via the Bering Land Bridge during the last (Wisconsin, ca. 80 – 10 thousand years ago; Bowen et al., 1986) or penultimate (Illinoian, ca. 300 – 130 thousand years ago; Bowen et al., 1986) glacial period (Rausch, 1963; MacPherson, 1965; Lance and Cook, 1998). Their short history
in the Nearctic indicates that phylogeographic and population genetic structure should reflect recent colonization and the genetic signature of recent glacial cycles, without being obscured by much older events. Furthermore, previous studies based on karyotypic (Nadler et al., 1976), allozymic (Nadler et al., 1978; Lance and Cook, 1998), and morphological (Paradiso and Manville, 1961; Chernyavski, 1984; Kostenko, 2000) data provide an established phylogeographic framework for developing testable hypotheses.

The primary goals of this thesis are to examine the ways in which Pleistocene ice ages contributed to shaping extant patterns of genetic differentiation and diversity in *M. oeconomus*, and to elucidate the responses of this high latitude species to the Beringian landscape during and after the last glacial maximum. A hierarchical approach is used, which elucidates broad-scale phylogeographic patterns as well as fine-scale population genetic structure. Two major issues are addressed in the first chapter: 1) the role of Pleistocene glaciations in promoting or inhibiting genetic differentiation and 2) the effect of post-glacial colonization on genetic diversity. Chapter 2 largely focuses on hypotheses developed from Lance and Cook’s (1998) study of allozymic variation in Beringian tundra voles. The rapidly evolving mitochondrial DNA sequences used in the present study provide an alternative perspective on 1) post-glacial routes of colonization in southcoastal Alaska, 2) transberingian connections among populations, 3) isolation and differentiation of post-glacially established populations (so-called neoendemics), and 4) congruence of taxonomic designations with phylogenetic structure.
Genetic Consequences of Pleistocene Glaciations for the Tundra Vole

(Microtus oeconomus) in Beringia

ABSTRACT

During the Pleistocene, glaciations in the Beringian region fragmented and displaced populations of the tundra vole (Microtus oeconomus). Different models of the genetic consequences of these climate-driven events predict different phylogeographic and population genetic outcomes. Variation in mitochondrial (cytochrome b and control region) and nuclear (ALDH1 intron) DNA was assessed for 214 individuals to test if glaciations 1) promoted differentiation and 2) led to a reduction in genetic diversity due to post-glacial colonization. Differentiation in western Beringia between a Central Asian clade and Beringian clade is geographically congruent with past glaciations, but maintenance of differentiation during interglacials implies an additional historical barrier to gene flow. Similarly, possible refugial populations in southcoastal Alaska were probably initially isolated by glacial vicariance, but subsequent differentiation has resulted from confinement of the populations to islands. Post-glacial colonization has apparently not been critical to structuring genetic diversity in Beringian populations. Prior to the last glacial maximum, genetic diversity had already been greatly reduced by an unknown event that was not associated with any particular glacial period.

INTRODUCTION

During the Pleistocene, glacial advances were interspersed with warm interglacials in cycles that influenced the spatial distribution and temporal demographic fluctuations of high latitude species (Webb & Bartlein 1992). The genetic consequences of these events, both for broad scale phylogeographic pattern and local population genetic structure, are not well understood. Competing models suggest different outcomes. At the deep phylogeographic level, glacial advances may have either promoted allopatric differentiation by isolating populations in various glacial refugia (Mengel 1964; Hewitt 1999) or hindered differentiation by inducing range shifts that caused population admixture (Coope 1979). At the local population genetic level, post-glacial colonization may have caused populations to undergo 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 predict distinctive genetic signatures in extant populations; therefore, it is possible to arbitrate among the models by testing predictions against observed patterns (Knowles 2001).

Beringia, which spans northeast Siberia, Alaska, and northwest Canada (Fig. 1.1), provides an excellent natural laboratory for examining the impact 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 structuring the biogeography and genetic diversity of circumarctic species (Guthrie & Matthews 1971; Sher 1986). During the ice ages, Beringia was bounded by complex glacial systems that
fragmented and condensed populations, isolating some organisms from conspecifics outside of 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. More species moved from Asia into North America than vice-versa (Rausch 1994), but not all northern species took advantage of the land bridge. Some apparently were prevented from crossing by ecological limitations or competitive exclusion (Hoffmann 1984; Guthrie 2001). During interglacials, the bridge flooded and temporarily halted the flow of migrants between the continents.

The tundra vole, *Microtus oeconomus*, is a Holarctic rodent that utilizes a range of habitats, preferring mesic tundra or meadow environments that are currently common throughout the north (Quay 1951; Tast 1966; Getz 1985). Because of the short generation time of tundra voles (~2–3 per year; H. Henttonen, pers. comm.), the genetic signature of historical events should become fixed in populations rapidly, providing an unambiguous picture of recent influences on population genetic structure. The distribution of tundra voles extends 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, ca. 300–130 thousand years ago, Kya; Bowen *et al.* 1986) or latest (Wisconsin, ca. 80–10 Kya; 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 Kya; Stuart 1982), the oldest reported fossils in North America date to late Illinoian time (ca. 200 – 130 Kya; Jopling et al. 1981; Zakrzewski 1985). The fossil record for tundra voles in North America is quite sparse, however, and should not be viewed as an absolute authority on the timing of colonization. Still, the preponderance of evidence suggests that tundra voles entered North America during one of the most recent glacial periods (i.e. Illinoian or Wisconsin). If so, observed North American phylogeographic structure should only reflect recent paleoclimatic and geologic events. The relatively shallow genetic signature produced by these events will not be confounded by deeper patterns of differentiation underlying them. This "clean phylogeographic slate" should simplify reconstruction of the historical events that determined the genetic structure of the voles.

This study examines the influence of Pleistocene glaciations on the differentiation of tundra vole populations. If glaciers promoted allopatric differentiation by fragmenting a single population into isolated refugia, phylogeographic breaks that are both spatially and temporally congruent with past glaciations would be expected. Matching patterns would also be predicted in independent molecular markers and in other codistributed organisms (Riddle 1996). Conversely, if glacial movements actually inhibited genetic differentiation by promoting mixing of populations, then there should be no association between phylogeographic breaks and historically glaciated areas. Instead, non-glacial barriers to gene flow (e.g. Bering Strait) would be expected to play
a more significant role in driving differentiation. Independent molecular markers from
the mitochondrial and nuclear genomes are examined and a molecular clock is applied in
order to assess spatial and temporal congruence between phylogeographic patterns of
differentiation and historically glaciated regions of western Beringia and southcoastal
Alaska.

The genetic consequences of population expansion following glacial recession
are also examined by comparing populations from regions with different glacial
histories. If post-glacial colonization took place as a series of successive founder events
(Hewitt 1996), populations in regions that were recently glaciated are expected to a)
have lower genetic diversity than populations from non-glaciated areas and b) possess
the genetic signature of a bottleneck that is temporally congruent with the end of the last
 glaciation. However, if populations of *M. oeconomus* display neither evidence of a
bottleneck nor significantly different levels of genetic diversity between recently
 glaciated and non-glaciated regions, the hypothesis that northern faunas did not undergo
post-glacial bottlenecks may be supported (Fedorov *et al.* 1999b). Direct comparisons
of genetic diversity and tests for evidence of recent bottlenecks are applied to sets of
populations from regions with differing glacial histories.
MATERIALS AND METHODS

Sampling

The sampling scheme for the study was designed to assess broad phylogeographic patterns while emphasizing areas with diverse glacial histories. A total of 214 specimens were collected from 30 localities (Appendix I).

In Siberia specimens were examined from the Chukotka and Kamchatka Peninsulas (3 localities), but the primary focus of sampling was the upper Kolyma River basin and along the Omolon River (10 localities, Fig. 1.2), which fall across the traditional western boundary of the Beringian refugium (Yurtsev 1974). Though it is difficult to assign specific borders to a region that was not delineated by obvious geographical boundaries, the Kolyma and Mackenzie Rivers are often cited as Beringia’s western and easternmost limits, respectively. During the ice ages, western Beringia was delineated by glaciers that, though probably formidable barriers to dispersal, were less extensive than those along its eastern border (Arkhipov et al. 1986b). Open corridors between Beringia and Asia’s ice-free central region probably existed even at the height of some glaciations, and during the most recent ice age, the Kolyma/Omolon River drainage was largely untouched by glaciers (Arkhipov et al. 1986a,b). This complex glacial history makes the region ideal for examining how Pleistocene glaciations influenced the phylogeographic and population genetic structure of northern faunas.

Specimens were collected from nearly the entire North American distribution of the species (16 localities, Fig. 1.2), though particular attention was given to southcoastal
sites along the Gulf of Alaska. This region, which was largely buried beneath the Cordilleran Ice Sheet during the glacial advances of the Quaternary, has a complicated geological (Hamilton & Thorson 1983; Molnia 1986; Mann & Hamilton 1995) and biogeographic (Heusser 1960; Klein 1965; Mann & Hamilton 1995; Cook et al. 2001) history. There is strong evidence that ice-free refugia maintained paleoendemic faunas through the last glacial maximum (Heusser 1960; Lindroth 1969, 1971; Heaton et al. 1996), possibly including a population of tundra voles that now inhabits Hinchinbrook Island in Prince William Sound (Lance & Cook 1998).

Three individuals from Finland were included to permit Beringian phylogeographic structure to be understood within the context of the species' broader Holarctic genetic structure (Brunhoff et al. in prep).

**Molecular methods**

Frozen or alcohol preserved tissue samples (heart, kidney, muscle, or liver) were obtained from the University of Alaska Museum Frozen Tissue Collection. Genomic DNA was extracted using a sodium chloride extraction protocol modified from Miller et al. (1988), and a region of the mitochondrial DNA 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 tRNA coding regions, and a portion of the 5’ end of the control region (total fragment length: Beringian clade 1638 bp, Central Asian clade 1637-8 bp; 2 indels). Primer sets for cyt-b were MVZ05 (Smith & Patton 1993) / Micro06 (5'GGATTATTTGATCCTGTTCGT), and Arvic07 (Cook et al. in prep) / Vole14 (Conroy & Cook 1999). For the control region, primers Micro3
(5'CTATCATYGTAAATCCTCATACCAATCG) 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 (1X; Applied Biosystems, Inc.), primers (1 μM each), dNTP (0.125 mM), MgCl₂ (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, a 270 bp nuclear DNA intron was amplified from 63 voles representing 22 localities using primers ALDH1F and ALDH1R (Lyons et al. 1997) under the conditions described above, except the MgCl₂ 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. Sequences were aligned by eye using the program Sequence Navigator™ (Applied Biosystems, Inc.).

Analyses

Extensive directional selection on cyt-b or control region could complicate interpretation of genetic patterns. To test for the possible influence of selection, a G-test was used to compare synonymous and nonsynonymous substitutions that are fixed between species to those that are polymorphic within species (McDonald & Kreitman 1991). The comparison was made using nine M. oeconomus sequences (AF numbers 461, 515, 6640, 7463, 8828, 38014, 38163, 38843, 43794) and nine M. longicaudus sequences obtained from Genbank (Conroy & Cook 2000a; Genbank accession numbers...
AF187171, AF187173, AF187179, AF187182, AF187204, AF187216, AF187227, AF187229, AF187230).

To ensure that *M. oeconomus* is monophyletic, a maximum-likelihood (ML) phylogenetic analysis was performed for a reduced number of individuals of *M. oeconomus* and its four closest relatives: *M. kikuchii, M. montebelli, M. fortis*, and *M. middendorffi* (Conroy & Cook 2000b). Cytochrome *b* sequences for these four taxa (Conroy & Cook 2000b; Genbank accession numbers AF163894, AF163896, AF163898, AF163900) were included with a sample of sequences from the present study (*N* = 26) and analyzed using the GTR (Lanave *et al.* 1984 and others) + I + Γ substitution model. The data subset was obtained by computing pairwise uncorrected *p* distances among all haplotypes from the full data set and removing one haplotype from each pair that differed by 0.005 or fewer substitutions per site. The GTR + I + Γ model of evolution was selected and parameters estimated using the program Modeltest (Posada & Crandall 1998) to arbitrate among 56 different models of evolution with log-likelihood ratio tests. A ML tree was generated using PAUP* version 4.08β (Swofford 2000) and strength of relationships among branches in the tree was assessed by bootstrap resampling (100 replicates).

To examine phylogeographic structure across Beringia, PAUP* (Swofford 2000) was used to generate an unrooted neighbor-joining (NJ) tree for the complete mtDNA data set from distances calculated using the Jukes-Cantor (JC) nucleotide substitution model (Jukes & Cantor 1969). Support for the tree topology was evaluated by bootstrapping (5000 replicates). The JC model is recommended for NJ trees when
pairwise genetic distances among taxa are small (<0.05), regardless of the transition to transversion ratio (Nei & Kumar 2000). When taxa are not strongly differentiated, substitution models with few parameters like the JC model are generally preferable to more complex models because distances estimated by the latter have larger variances. To assess robustness of the topology and branch lengths to the model of evolution, a series of NJ trees was generated using uncorrected p, JC, K2P (Kimura 1980), and HKY85 (Hasegawa et al. 1985) models. All combinations of different gamma shape parameter values (0.5, 1, 2, 10) and proportion of invariable sites values (0, 0.5, 0.75) were used in assessing each model (except for uncorrected p, which assumes equal substitution rates and therefore does not use the gamma shape parameter). The topologies of the trees that were produced by these models were mostly congruent, differing only in tip branching relationships that were poorly supported by bootstrap analysis. Branch lengths differed minimally across methods, indicating that the primary conclusions of this study would not have been altered if different substitution models had been used.

A molecular clock was used to examine temporal congruence between the largest phylogeographic breaks and historic glaciations. Molecular clock calibrations are controversial at best (Hillis et al. 1996; Strauss 1999), and should therefore be cautiously applied to data. However, in lieu of a well-developed fossil record, they may provide the only means of examining 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% My⁻¹. This is much faster than
conventional divergence rate estimates for mammals (e.g. Smith & Patton 1993), and uncertainties in the fossil record suggest that this estimate may be high (C. J. Conroy pers. comm.). It is, however, consistent with evidence that rodents evolve faster than other mammals, and it is only slightly faster than upper limits of other rate estimates for rodents (e.g. 3.8 – 11.3%, Martin & Palumbi 1993; 7.5 – 12%, Arbogast et al. 2001). In contrast, a molecular clock estimate for cyt-b in another rodent genus (*Lemmus*), which is largely sympatric with *M. oeconomus* and has very similar life history characteristics, produced a rate of 5% My\(^{-1}\) (Fedorov & Stenseth 2001). Improved fossil dating methods have since adjusted this to 7.5% My\(^{-1}\) (V. B. Fedorov pers. comm.). Clearly the rates for *Lemmus* and *Microtus* differ, but they are the best estimates currently available for arvicolid rodents. The estimate for *Lemmus* is arguably better supported by fossil evidence and more consistent with other published rates, which suggests that it is more reliable. However, without solid fossil or biogeographical data with which to derive an independent divergence rate estimate for *M. oeconomus*, there is no way to objectively arbitrate between them. Therefore, both rates are applied, with the caveat that more precise rate estimates in the future may refine divergence time estimates and improve resolution of historical events.

To apply a molecular clock, it was first necessary to test for rate heterogeneity among lineages. Maximum-likelihood trees were generated for the mtDNA data subset used above (without the four outgroups) with and without a molecular clock constraint and evaluated using a chi-square log-likelihood test (Felsenstein 1988). The ML model of evolution for this test, TrN (Tamura & Nei 1993) + I + \(\Gamma\), was chosen using Modeltest
(Posada & Crandall 1998). In addition, Takezaki et al.'s (1995) two-cluster and branch length tests were applied to a JC neighbor-joining tree based on all the mtDNA haplotypes. These tests examine rate heterogeneity across all interior nodes of the tree and along individual branches, respectively. The LINTREE software package (Takezaki et al. 1995) was used to implement the tests. For estimating divergence times, net distances between clades were used rather than absolute distances to correct for variation within clades (Edwards 1997). Net distances and their standard errors were calculated with the program MEGA2 (Kumar et al. 2001).

Genetic consequences of post-glacial expansion were assessed by comparing populations from the recently deglaciated southcoastal part of Alaska to Siberian populations from areas that were relatively ice-free during the last glacial maximum. Two measures of genetic diversity (haplotype and nucleotide diversity) were calculated for five Alaskan (Cold Bay, Anchorage, Cordova, McCarthy, SE Alaska mainland) and six Siberian (Magadan, Elegan River, Kontakt Creek, Omolon 1, Omolon 2) populations using the program Arlequin 2.001 (Schneider et al. 2000). A data matrix of pairwise nucleotide differences was used. The diversity estimates for the two sets of populations were compared with a Wilcoxon two-sample test (Sokal & Rohlf 1995). Populations were excluded from this analysis if they were either represented by fewer than five individuals or located on islands. Colonization of islands by a small founding population may be accompanied by a decrease in genetic diversity independent of glacial history, which could bias the comparison.
To test for evidence of recent bottlenecks in populations from historically glaciated and non-glaciated regions, two methods implemented in Arlequin were used. First, Fu's (1997) $F_s$ test was performed to test for an excess of rare alleles, which is indicative of recent expansion from a bottleneck. Second, pairwise mismatch distributions were plotted for populations and tested for goodness of fit against a model of sudden expansion using parametric bootstrapping (500 replicates; Rogers 1995).

Analysis of molecular variance has revealed significant structure among the tundra vole populations examined in this study (Galbreath & Cook in prep.), implying little gene flow among them. Population subdivision that has arisen since expansion can be problematic for reconstructing ancestral population size changes from mismatch distributions. However, in the special case of nearly complete isolation, a mismatch distribution based on individual samples drawn from local populations will match that of a panmictic population descended from the ancestral population of interest (Marjoram & Donnelly 1994). Therefore, to examine population demographic history on a regional scale (e.g. across all southcoastal Alaskan populations), mismatch distributions were generated based on one individual from each local population within the region of interest. For the same analysis on a local scale (e.g. populations along the Omolon River), all individuals were included.

In a population that has rapidly expanded from a bottleneck, extant lineages are assumed to coalesce just prior to the initiation of the expansion (Rogers & Jorde 1995). This time can be inferred via two methods using parameters that are calculated from the mismatch distribution. First, the number of generations ($t$) since the expansion began
can be estimated with the equation \( t = \tau / 2u \), where \( \tau \) is the mode of the mismatch distribution and \( u \) is the mutation rate per generation for the entire mtDNA sequence (Rogers 1995; Rogers & Jorde 1995). Another measure of post-bottleneck expansion time is based on the mean number of pairwise nucleotide differences \( (m; \text{Rogers} \text{ } \& \text{Jorde } 1995) \). The relationship between \( m \) and \( t \) is the same as between \( \tau \) and \( t (t = m / 2u) \).

Calculating post-bottleneck expansion times using \( m \) must be used with caution. It is only effective if the initial bottleneck was prolonged and severe enough to greatly diminish \( m \), and if insufficient time has passed for \( m \) to reach its post-bottleneck equilibrium value (Rogers & Jorde 1995). The results of the analyses of the populations in this study suggest that these potentially confounding issues did not influence the outcome. The latter concern is unlikely to pose a problem given that the calculated expansion time estimates are small relative to the long period of time necessary for \( m \) to reach equilibrium (see Rogers & Jorde 1995). Furthermore, the magnitude of the bottleneck can be addressed by examining the parameters \( \theta_0 \) and \( \theta_1 \), which are, respectively, measures of the pre- and post-expansion female effective population sizes in units of mutational time (general equation: \( \theta = 2Nu; N = \text{female effective population size} \)). In most cases, extremely small \( \theta_0 \) estimates relative to \( \theta_1 \) indicate expansion from substantially reduced population sizes, implying significant bottlenecks (see Results, Table 1.1).

The tests of recent bottlenecks were performed on three sets of populations with different glacial histories: 1) Southcoastal Alaska including Cold Bay, Kodiak, Anchorage, Cordova, McCarthy, southeast mainland, Chichagof, and Baranof
(Montague and Hinchinbrook were excluded because of evidence that the populations there are refugial, Lance & Cook 1998). This region was heavily glaciated during the most recent glaciation (ca. 25 – 10 Kya; Bowen et al. 1986); 2) Upper Kolyma River and Magadan (Magadan area, Ust Omchut, Elikchan Lakes, Kontakt Creek, Susuman), which was relatively untouched during the last glacial advance, but widely impacted by glaciers during the preceding glaciation (ca. 80 – 55 Kya; Arkhipov et al. 1986b); and 3) Omolon River (Bol’shaya River, Omolon River 1, Omolon River 2), which remained ice-free during both of the most recent glacial maxima. If a reduction in genetic diversity is associated with post-glacial colonization, the genetic signature of a bottleneck is expected to be apparent in the first two groups. Furthermore, temporal estimates of these bottlenecks should coincide with the end of the most recent glaciation associated with each group.

Observation of an historical bottleneck in populations from a specific region does not necessarily indicate that the observed bottleneck was limited to that region. It may have affected the ancestors of a wider range of extant populations, but was not revealed because of the narrow focus of sampling on a specific area. To examine the possibility that observed bottlenecks were not limited only to areas with specific glacial histories, the tests of recent bottlenecks were also applied to populations from the entire geographic distributions of the major clades in which bottlenecks were observed. Goodness-of-fit to the models of sudden expansion should be best for the set of populations descended from the original bottleneck event.
RESULTS

_Mitochondrial sequence data_

Nuclear copies of mtDNA sequences (NUMTs) have been identified in species of the genus *Microtus* (DeWoody et al. 1999), and would confound inferences drawn from sequence data. In this study, 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 2000b). 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 2000b). Likewise, the distribution of 28 variable amino acid sites fits structural models of variable and conserved regions in *cyt-b* (Irwin et al. 1991). Furthermore, the pattern of variation across the tRNA sequences (conserved) and control region (variable) matched predictions for mammalian mtDNA (Cann et al. 1984). An unexpected observation was that the percentage of variable sites in *cyt-b* (12.5%) was slightly greater than that of control region (11.5%; total sequence fragment including tRNAs, 11.6%). This differed from expectations that the control region is evolving faster than coding regions of mtDNA (Aquadro & Greenberg 1983; Cann et al. 1984), though it may be due to the magnified effect of stochasticity in the relatively low levels of divergence observed among individuals. When variable sites were calculated separately for two major clades that were identified (see below), differences between *cyt-b* and control region remained slight, but the trend was more consistent with expectations (Beringian clade: *cyt-b* 8.2%, control region 8.4%, total fragment 7.8%; Central Asian clade *cyt-b* 3.4%, control region
5.0%, total fragment 3.5%). There was no significant difference between the ratio of nonsynonymous to synonymous substitutions fixed between species and the ratio for polymorphic substitutions within species (G = 0.575; p = 0.448), indicating that cyt-b is not under strong directional selection (McDonald & Kreitman 1991).

**Phylogeographic structure**

Tundra voles are monophyletic with respect to their closest relatives (Fig. 1.3). Both ML and NJ trees revealed three well-defined clades within *M. oeconomus* (Figs. 1.3 and 1.4), which correspond to three of four tundra vole clades identified by Brunhoff *et al.* (in prep). Relatively deep genetic breaks were apparent among populations from Finland, western Siberia (upper Kolyma River basin, Magadan, and Omolon River: Central Asian clade), and Beringia (eastern Siberia and North America: Beringian clade). There was no evidence of significant differentiation between eastern Siberia and North America across the Bering Strait. Despite the tremendous geographic distance between Finland and other populations, relationships among these three groups were not resolved by bootstrapping. Hence, the genetic break observed in Siberia reflects significant phylogeographic structure across the Holarctic distribution of *M. oeconomus*. Though bootstrap values supported some genetic structuring within the Beringian and Central Asian clades, a basal polytomy was the dominant feature of both, and nearly all branch lengths within the two clades 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 (Figs. 1.3 and 1.4). The Beringian and Central Asian mtDNA clades were in contact at the Bol’shaya location on
the Omolon River (Figs. 1.2 and 1.5). Downstream of that location voles exhibited Central Asian clade haplotypes and upstream they represented the Beringian clade.

The nuclear marker ALDH1 (Lyons et al. 1997) further elucidated phylogeographic structure and interactions between the two clades in the region of contact zone (Fig. 1.5). As in the mtDNA, the nuclear marker exhibited evidence of distinct clades in western and eastern Siberia. A 17 bp deletion was revealed in 15 of 19 voles with Central Asian clade mtDNA, but not once in 39 individuals from North America or Siberia east of the Omolon River (i.e. Beringian clade voles). Central Asian clade voles that lacked the deletion shared a single haplotype that was not observed elsewhere in eastern Siberia, though it did appear in Alaska. The presence 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, indicating that introgression has occurred between members of the two mtDNA clades (Fig. 1.5).

 Estimates of divergence times

The ML 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. Likewise, the two cluster test indicated that relationships among branch lengths did not differ from expectations under a model of rate homogeneity ($Q = 109.9; p = 0.256$). However, Takezaki et al’s (1995) branch length test showed that
although most lineages exhibited clock-like behavior some were evolving at different
rates (Fig. 1.4). Therefore, divergence estimates used in establishing timing of historical
events were calculated based on a pruned data set in which rate heterogeneous lineages
were excluded.

As noted earlier, the percentage of variable sites differed little between cyt-b and
control region, and little between cyt-b and the entirety of the mtDNA fragment of
interest. Therefore, estimation of separate divergence rates for control region and cyt-b
is not necessary since the rate of evolution averaged across all 1638 base pairs should be
comparable to the rate for cyt-b. The two rate estimates (7.5% My⁻¹ and 13% My⁻¹)
were therefore applied to distances that were calculated from complete sequences. Net
distance between the Beringian and Central Asian clades (0.022 ± 0.0032 substitutions
per site) divided by the rate yielded a divergence time of 166 ± 25.5 Kya for the faster
divergence rate, and 289 ± 44.1 Kya for the slower. Although poor resolution of basal
relationships in the Beringian clade makes it difficult to identify the sister clade to the
Montague and Hinchinbrook group (Fig. 1.4), the long basal branch of the group
suggests a deep history of isolation from the rest of the Beringian populations.
Therefore, the entire Beringian clade was considered to be the sister group and used for
calculating net divergence (0.0068 ± 0.0019 substitutions per site), which resulted in
divergence time estimates of 52.4 ± 14.5 Kya and 90.9 ± 25.1 Kya for the 13% My⁻¹ and
7.5% My⁻¹ divergence rates, respectively.
Genetic diversity and historical bottlenecks

Comparison of genetic diversity between glaciated and non-glaciated regions (Table 1.2) did not indicate that populations undergo a reduction in genetic diversity during post-glacial colonization. The Wilcoxon two-sample test showed that neither haplotype (U = 15; one-tailed $P > 0.10$) nor nucleotide (U = 15; one-tailed $P > 0.10$) diversity was significantly greater in non-glaciated regions.

Results of the tests of population expansion fit predictions based on glacial history. The populations from the Omolon River, which has not been glaciated recently, did not possess the genetic signature of a recent population bottleneck and expansion ($F_s = 1.392, p = 0.726$; mismatch distribution test of goodness-of-fit, $p = 0.046$). Conversely, the populations from the upper Kolyma River and Magadan area matched the models of recent expansion well ($F_s = -7.97, p = 0.009$; mismatch distribution, $p = 0.890$). The only equivocal result came from the analysis of populations from recently glaciated areas of southcoastal Alaska. In this case, the mismatch distribution indicated a recent population bottleneck ($p = 0.802$), but the $F_s$ test did not ($F_s = -2.28, p = 0.060$). For the $F_s$ test, $p \leq 0.02$ is considered to be significant at the 0.05 level (Fu 1997). The effect of population subdivision on the $F_s$ test has not been studied, and it is possible that the high degree of genetic structuring among the populations of interest confounded the test. Because the effect of subdivision is better understood for mismatch distributions (Marjoram & Donnelly 1994; Rogers 1995), and steps were taken to account for it, the results of the mismatch distribution analysis were considered more reliable than the $F_s$ test. Application of the tests to the entire Beringian clade ($F_s = -7.972, p = 0.009$;
mismatch distribution, \( p = 0.878 \) and Central Asian clade (\( F_s = -10.345, p = 0.007 \); mismatch distribution, \( p = 0.424 \)) indicated that, as a whole, both possess the genetic signature of recent bottlenecks.

For sets of populations that exhibited recent expansion, the two methods of measuring post-bottleneck expansion time produced estimates that were broadly consistent across localities and methods (Table 1.1). Populations in the upper Kolyma and Magadan region were estimated to have undergone a bottleneck approximately 32 to 65 Kya depending on mutation rate, whereas the southcoastal Alaskan populations yielded a range of times between 45 and 83 Kya. Similar values were calculated for the entire Beringian clade, though they had a wider range (44 – 112 Kya).

DISCUSSION

Pleistocene glaciations and genetic differentiation

This study addresses two questions regarding the impact of Pleistocene glaciations on structuring genetic variation in northern species. The first question focuses on the role that ice ages may have played in either driving or inhibiting genetic differentiation. If glacial advances isolated populations and caused them to diverge in allopatry, spatial congruence between phylogeographic structure and historical glaciations would be expected (though not necessarily required). In addition, timing estimates for genetic breaks should place divergence events during glacial periods, rather than during interglacials. Conversely, if glaciations had an inhibitory effect on
differentiation by promoting population admixture, populations would be expected to be genetically homogeneous across regions of historical glacial activity.

These alternative hypotheses can be tested by examining the phylogeographic structure of *M. oeconomus*, but some discussion of the complex glacial history of Beringia is first necessary to provide a context for understanding historical processes of differentiation. Stadial and interstadial cycles in the Beringian region roughly correlate with other Northern Hemisphere glaciations in timing (Bowen *et al.* 1986; Arkhipov *et al.* 1986b), but not in magnitude (Arkhipov *et al.* 1986a). In general, though, glacial systems repeatedly originated in the same mountain ranges (Fig. 1.1). 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 (ca. 80 – 55 and ca. 25 – 10 Kya, respectively; Arkhipov *et al.* 1986b; Bowen *et al.* 1986). Glacial ice was not a permanent feature of the Beringian landscape during the Pleistocene, and the cyclical glacial advances differed in magnitude, particularly in Siberia (Fig. 1.1). During the Zyryanka, glaciers covered approximately 40% of northeast Siberia (Bespalyy 1984; Arkhipov *et al.* 1986a), forming a nearly 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 due to lowered sea levels and remained ice-free. In contrast, low precipitation during the Sartan glaciation 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 cross the boundary between Eurasia and Beringia. The Middle Pleistocene glaciations in Siberia (roughly coincident with the Illinoian in North America, ca. 270 – 130 Kya; Arkhipov et al. 1986b) are not as well known as those that came later, but deposition of boulders from the Chukotsk Peninsula on St. Lawrence Island during this time suggests that the glaciations were at least as extensive as the Zyryanka, if not more so (Arkhipov et al. 1986a). Climate warming between glacial advances produced interstadials or full interglacials, some of which had higher temperatures than the present day (Arkhipov et al. 1986b). Glaciers retreated during these periods, sometimes disappearing entirely, and opportunities for post-glacial expansion and gene flow among glacially isolated populations probably increased.

Within Beringia, *M. oeconomus* does not exhibit exceptional phylogeographic structure. Despite being separated for at least 10 thousand years (Elias et al. 1996), populations on either side of the Bering Strait are not significantly differentiated (Figs. 1.3 and 1.4), providing a qualitative indicator of relative timing of divergence events. Therefore, strong genetic discontinuities probably arose from isolation events that predated the most recent flooding of the Bering Land Bridge. The close genetic relationship between Siberia and Alaska corroborates karyotypic (Nadler et al. 1976), allozymic (Nadler et al. 1978; Lance & Cook 1998), and morphological (Paradiso & Manville 1961) data, and further supports the conclusion that tundra voles are recent colonizers of North America. Close amphiberingian relationships in other taxa are rare, but they do exist. Of the three other rodent species that have Holarctic distributions
(Spermophilus parryii, Clethrionomys rutilus, Lemmus trimucronatus), only the phylogeography of L. trimucronatus has been examined, and it was found to be genetically undifferentiated across the Bering Strait (Fedorov et al. 1999b). Surprisingly little genetic divergence across the strait is also observed in the cestode parasite, Andrya arctica, despite the fact that the strait separates its two lemming hosts, Dicrostonyx torquatus and D. groenlandicus (Wickström et al. 2001).

The largest genetic break for tundra voles in the Beringian region is located along the Omolon River in the Kolyma uplands (Fig. 1.2). Strong differentiation there despite the region’s complex glacial history suggests that the repeated advances and withdrawals of glaciers did not promote population admixture (proposed for insects, Coope 1979), because populations of the Beringian and Central Asian clades were largely allopatric. If glacial fluctuations had a mixing effect then the clades would be expected to overlap over a large range, but representatives from the two mitochondrial clades occurred together at only one locality. The nuclear marker 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 an eastern non-deletion haplotype in western voles could have resulted from mixing of ancestral populations during glacial periods, but such a scenario suggests that the deletion should therefore be found east of the Omolon River contact zone. Alternatively, the mixture of deletion and non-deletion haplotypes in the Central Asian clade may simply be the result of incomplete lineage sorting.
The geographical association between the genetic break and past glaciations is indicative of an historical glacial barrier that divided the ancestors of the Beringian and Central Asian clades and provided the impetus for their divergence. Congruence between the distribution of the Beringian clade and the traditional boundaries of Beringia (Fig. 1.2) further reinforces this conclusion by implying that the clade originated in the Beringian refugium. This fits the established model of the refugium as a center of evolution in which populations became isolated and diverged onto unique evolutionary trajectories (Guthrie & Matthews 1971; Sher 1986). However, spatial congruence between phylogeographic structure and glacial history is insufficient to unequivocally demonstrate that glaciers caused the differentiation of the Beringian clade. Temporal congruence would create a much stronger case for glacially driven divergence.

Unfortunately, molecular clocks do not provide an unambiguous estimate of the timing of the divergence. The faster of the two rates used in this study placed the divergence between the Beringian and Central Asian clades at approximately 166 Kya, which coincides with the Middle Pleistocene glaciations in Siberia (Arkhipov et al. 1986b; Bowen et al. 1986). Such a date is consistent with the hypothesis that glacial vicariance initiated differentiation. However, the slower rate estimate produced a divergence time of roughly 289 Kya, which falls during the Bolsheretsian interglacial prior to the Middle Pleistocene glaciations (Arkhipov et al. 1986b; Bowen et al. 1986). If the slower rate is correct, then alternative causes of differentiation must be considered. A possible example could be isolation across the Bering Strait, which was flooded at
that time, followed by a range shift that produced the current distribution of the clades. A similar scenario has been proposed to explain the current biogeographic distribution of species in the genus *Lemmus* (Fedorov et al. 1999b). However, additional lines of evidence contradict this “range shift” hypothesis for *M. oeconomus*, and strengthen the inference that the observed differentiation was caused by isolation across a barrier in the region of the Kolyma and Omolon Rivers.

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. It is also notable that this region roughly coincides with the subdivision between two vegetatively distinct subarctic climatic zones (Lozhkin & Anderson 1995), which may have maintained 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 (e.g. Bering Strait; Fedorov et al. 1999b) indicates that the barrier has 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 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) and a recent taxonomic revision upheld this conclusion (Kostenko 2000). The distributions of the eastern and western subspecies are congruent with those of the Beringian and Central Asian molecular clades, respectively. Though correlation does not prove causation, the evidence for an historical barrier in this region of repeated glacial advances 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" and permitted dispersal is contradicted (Hoffmann 1981). However, glaciers alone could not have produced the observed differentiation, even if they were responsible for its initiation. Given that the divergence was dated to ≥ 166 Kya, the barrier to gene flow that produced it apparently persisted through repeated cycles of climate warming and cooling. Ice sheets were present in Siberia during some of this period, but large glaciers were not always a major part of the landscape. Glaciers probably played a role in separating the two clades, but other factors, such as ecological discontinuities, must have acted to maintain the isolation when glaciers were insubstantial. For example, even though glaciers did not cover large portions of Siberia during the Sartan glaciation, it has been suggested that the Kolyma uplands were 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 paleoenvironments suggest that the center 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.

The timing estimates for the genetic break permit an additional inference. Both estimates suggest that tundra voles entered western Beringia no later than the Illinoian period, which placed them in a position to colonize the Nearctic well before the Wisconsin glaciations. This is significant because *M. oeconomus* has traditionally been considered to be a Wisconsin period invader of North America (Rausch 1963; MacPherson 1965). To date, the only evidence for an earlier invasion came from a single fossil assemblage (Jopling et al. 1981; Zakrzewski 1985), but if tundra voles were in western Beringia during the Illinoian glaciation then they may have entered the Nearctic at that time. Once in Beringia, no substantial physical or ecological barriers stood in the way of eastward expansion. Indeed, if central Beringia was particularly well suited for colonization by mesic-adapted species (Elias et al. 2000; Guthrie 2001), tundra voles probably would have moved eastward during glacial periods. A population centered in Beringia would have been divided when ocean levels rose following the ice ages, thereby establishing the first population endemic to North America.

*Did glaciers drive differentiation? The case of the Prince William Sound refugium*

A combination of glacial and ecological factors probably were responsible for promoting differentiation between the Central Asian and Beringian clades, but glacially driven vicariant isolation was almost certainly the most important force behind the
divergence of tundra voles on the Prince William Sound islands of Montague and Hinchinbrook. These populations form a strongly supported monophyletic group that is genetically distant from the rest of the Beringian clade (Figs. 1.3 and 1.4). The apparently deep shared history of the group contrasts with other populations in the clade, which generally exhibit low levels of differentiation and poorly resolved relationships.

Molecular clock estimates indicate that the populations from Montague and Hinchinbrook diverged from the rest of the Beringian clade earlier than 50 Kya. Slower, more conventional mutation rates push their separation farther back in time, indicating that the populations survived in isolation through at least the 2nd Wisconsin glaciation and perhaps the 1st Wisconsin glaciation as well. The implication is that during the ice ages, tundra voles became isolated in one or more glacial refugia south of the Cordilleran ice sheet (Fig. 1.1). Separate populations of voles were then partitioned onto the two Prince William Sound islands after glaciers retreated and sea levels rose.

The extent of glaciers on the continental shelf in southcentral Alaska remains a controversial issue (Mann & Hamilton 1995). However, parts of both Hinchinbrook and Montague may have been non-glaciated (Tarr & Martin 1914) during the last glacial maximum. Furthermore, though it is likely that ice did reach the edge of the continental shelf in the Gulf of Alaska (Mann & Hamilton 1995), there is some evidence that it did not cover the entire shelf (Tarr & Martin 1914). If it did not, one model suggests that a glacial lobe that excavated Hinchinbrook Entrance, the deep channel that runs between the two islands, may have separated ice-free areas to the east and west (Molnia 1986). After glacial retreat, Hinchinbrook Entrance probably immediately filled with water.
(von Huene et al. 1967) so refugial populations on either side would not have had the opportunity to intermix as rising ocean levels pushed them back to the only dry land available. If the glacially sculpted channels that separate Montague and Hinchinbrook Island from the mainland flooded as quickly as Hinchinbrook Entrance, which seems likely (von Huene et al. 1967), the retreating voles would have been restricted to the islands. This model suggests that tundra vole populations could have persisted in adjacent, but isolated, refugia on the continental shelf during the last glacial maximum.

Isolation in separate refugia may be responsible for differentiation that is apparent between the two island populations. Voles on Montague Island have long been known to be distinct based on morphological characteristics (Osgood 1906; Zimmermann 1942; Paradiso & Manville 1961; Lance & Cook 1998), and they currently are classified as an endemic subspecies (*M. o. elymocetes*). In contrast, Hinchinbrook Island voles are considered to be morphologically more similar to the mainland (Klein 1965), though no study has explicitly tested that assertion. The island populations also differ from each other biochemically, each possessing unique allozymic alleles (Lance & Cook 1998), and they do not share any mitochondrial haplotypes (this study).

Differentiation between the island populations may not have arisen from isolation in separate refugia during the last glacial maximum, but rather via rapid evolution after the populations were established post-glacially from a single southcoastal refugium. Insular populations can undergo exceptionally rapid evolution (e.g. Losos 1997). Small population sizes increase the random effects of genetic drift and speed up lineage sorting that might lead allopatric populations onto divergent evolutionary
pathways. A dearth of predators on the islands also could have promoted rapid morphological changes (Adler & Levins 1994). Finally, local differentiation of other tundra vole populations that were established after the last glacial maximum (Galbreath & Cook in prep) suggests that distinct evolutionary lineages can arise quickly.

Though rapid evolution could explain inter-population differences between Montague and Hinchinbrook, it does not adequately account for the deeper differentiation between the Prince William Sound island populations and the rest of the Beringian clade. No other insular populations that were examined (St. Lawrence, Kodiak, Baranof, Chichagof) showed an equivalent depth of differentiation. Also, post-glacial colonization of the islands and subsequent differentiation would predict independent lineages arising from the Beringian clade. In contrast, the island populations exhibit a strong relationship that is indicative of a deep, shared history. Such a relationship is not exhibited by any other set of post-glacially established populations, and it implies the existence of a refugial population.

Evidence for a refugium in Prince William Sound is significant for at least two reasons. First, it shows that glacial isolation in small refugia did promote differentiation during the ice ages, and second, there has hitherto been little biological support for a southcentral Alaskan refugium, despite much speculation and debate by physical scientists (Tarr & Martin 1914; Molnia 1986; Mann & Hamilton 1995). Not only did the refugium probably exist, it also supported an ecosystem that was complex enough to maintain a population of small mammals throughout the last glacial maximum. Another southcoastal ice age refugium, Kodiak Island, was apparently only capable of supporting
plants and arthropods (Lindroth 1969, 1971). Though six mammalian species now
inhabit Kodiak Island, there is no strong evidence that any of them persisted there
through the last ice age (Rausch 1969). A glacial refugium in the Alexander
Archipelago in southeast Alaska may have maintained a more diverse flora and fauna
than those of Kodiak or Prince William Sound. Genetic and fossil data suggest that
brown bears inhabited the Alexander Archipelago continuously through the last
glaciation (Heaton et al. 1996), and divergent genetic lineages in shrew, vole, and
mustelid species may indicate that these diverse groups also survived in coastal refugia
(Byun et al. 1999; Cook et al. 2001; but see Demboski et al. 1999). The Prince William
Sound, Kodiak Island, and Alexander Archipelago refugia provide three contrasting
perspectives on ice age survival in small glacial refugia. Future work in these areas
should focus on identifying other potentially paleoendemic taxa (i.e. species that
persisted through the last glacial maximum) in order to develop a comparative
framework for examining the causative factors that permitted certain species to survive
through glacial advances, while others were driven to local extinction.

Post-glacial colonization and historical bottlenecks

The second major objective of this study is to examine the genetic consequences
of post-glacial colonization at the population level. Do populations of *M. oeconomus*
that inhabit recently deglaciated areas exhibit low genetic diversity due to founder effect
bottlenecking (Hewitt 1996), or do they follow the pattern observed in some other
northern rodents, in which populations from glaciated and non-glaciated regions possess
equivalent levels of diversity (Fedorov et al. 1999b)?
Superficially, results from the tests of diversity and recent bottlenecks provide contradictory answers to this question. Comparisons of haplotype and nucleotide diversity show that populations from the recently deglaciated southcoastal Alaska region do not have less genetic diversity than populations from the Omolon and upper Kolyma/Magadan region, which were ice-free during the last glaciation (though the latter region had been glaciated at an earlier time). A result of no difference is consistent with the hypothesis that post-glacial expansion by northern species is not associated with a reduction of genetic diversity (Fedorov et al. 1999b). However, in the tests for recent bottlenecks, the two sets of populations from areas that are known to have been glaciated during the last major glacial advances (southcoastal Alaska and upper Kolyma/Magadan area) possess the genetic signature of bottlenecks. Those from the Omolon River, a region that has remained free of glaciers for >130 Kya, do not. In contrast to the results of the diversity comparisons, this pattern is consistent with post-glacial colonization through a series of founder events in which genetic diversity was lost as new populations were established (Hewitt 1996).

Post-bottleneck expansion time estimates provide a key to reconciling the conflicting inferences. In all cases where populations matched the models of recent bottlenecks, expansion times were roughly equivalent (Table 1.1). A separate analysis of the entire Central Asian clade (identical to the "recently non-glaciated" region of the diversity comparisons) produced similar results, though including the Omolon River populations diminished the goodness-of-fit to the expansion model. Similar expansion time estimates suggest that the different populations examined here underwent
bottlenecks simultaneously, causing genetic diversity levels to be equilibrated at a single point in time historically. Extant genetic diversity is the result of the accumulation of mutations since that time, and since cyt-b and control region appear to be evolving in a clock-like manner, no significant differences in genetic diversity between the regions should be expected.

Congruent post-bottleneck expansion times regardless of glacial history reject the idea that bottlenecks occurred during post-glacial colonization. If bottlenecks accompanied glacial events, timing estimates should coincide with the end of the most recent major glacial advance for each area, but they clearly do not in all cases. In the Central Asian clade, the last glaciation (Sartan) was not very extensive (Fig. 1.1), so the bottleneck should predate that glaciation and coincide with the previous one (Zyryanka). Because of uncertainty in the mutation rate, expansion times should be interpreted cautiously, but the fact that all estimates of post-bottleneck time for the upper Kolyma and Magadan fall closer to the Zyryanka glaciation than the Sartan (Table 1.1) appears to match expectations derived from the post-glacial bottlenecking hypothesis. However, expansion times for the Alaskan populations do not match the same prediction. During the last glacial maximum in North America (2nd Wisconsin), southcoastal Alaska was almost entirely buried under the Cordilleran ice sheet, much as it was during the preceding glacial advances (Hamilton et al. 1986; Mann & Hamilton 1995; Fig. 1.1). With the exception of the putative refugial population in Prince William Sound, the ice sheet presumably eradicated all local populations of tundra voles. Despite this, the post-bottleneck expansion times solidly predate the 2nd Wisconsin. Although it could be
argued that the observed bottleneck was in response to the 1st Wisconsin and the later glaciation simply did not have the same impact, such an explanation seems unsatisfactory. A species should respond similarly to roughly equivalent glacial advances in the same geographic region. This casts doubt on the conclusion that post-glacial colonization caused the observed bottlenecks. Furthermore, when the tests for recent bottlenecks were applied to the entire Beringian clade, the goodness-of-fit to the models of expansion was even better than when the southcoastal Alaskan populations were tested alone. Apparently the progenitors of the Beringian clade underwent a major bottleneck prior to the last glacial advance, and the genetic signature of that event was strong enough to permit it to be detected when a fraction of the descendent populations were sampled (i.e. southcoastal Alaska only).

The comparisons of genetic diversity and reconstructed demographic history of *M. oeconomus* is consistent with the predictions of Fedorov et al.'s (1999b) hypothesis that expansion of periglacial populations of northern species into newly deglaciated areas did not result in decreased genetic diversity, but some caveats are worth noting. The historical bottleneck that was observed in the Beringian clade could have masked a weaker post-glacial bottleneck in southcoastal Alaska. The tundra vole populations that colonized southcoastal Alaska after the last glacial retreat arose from ancestral stock that had recently been impacted by a strong and significant bottleneck event. At the time that founder populations were becoming established along the Gulf of Alaska coast, they had already suffered a significant reduction in genetic diversity. These populations may have had little genetic variation remaining, and even if the diversity that had
accumulated since the earlier event was lost, it may have been an insignificant reduction compared to the prior bottleneck. Also, the seemingly substantial genetic diversity that is observed in southcoastal Alaska alone (38 distinct haplotypes) may not reflect diversity that was retained during post-glacial colonization. The lack of resolution of relationships among populations in the Beringian clade and no shared haplotypes among populations hints at rapid local differentiation of populations once they became established (Figs. 1.3 and 1.4). Conceivably, all extant diversity could have arisen from a single ancestral haplotype in the time since re-colonization took place.

*Changing paleoenvironments and population bottlenecks*

Though it is tempting to imagine that harsh ice age environments produced the observed bottleneck in Beringian clade *M. oeconomus*, the fact that it probably predates the last glacial maximum implies that it may not have been triggered by a glacial period at all. Tundra voles are adapted to high-latitude tundra environments, so a glacial period may not have reduced their populations significantly. The species is the most northerly distributed of all *Microtus*, occurring above 71° N latitude in North America (Hoffmann & Koeppl 1985) and Eurasia (Kostenko 2000), and thriving in arctic tundra ecosystems (Getz 1985). Among rodents, only lemmings, which inhabit the high arctic, may have been better equipped to survive unhindered through the glaciations of the Pleistocene.

Interglacial periods may have posed a greater problem for tundra vole populations than stadials. The post-bottleneck expansion time for the Beringian clade using the 7.5% divergence rate, which is probably the more reliable of the two rates for reasons given earlier, was consistent with the start of the major glacial advance that
marked the 1st Wisconsin (Bowen et al. 1986). This suggests that the bottleneck actually occurred during the preceding interglacial (the Sangamon in North America, Kazantsevo in Siberia). Interglacials are associated with warm climates that are favorable to many species, potentially increasing interspecific competition in the north as climatological constraints on temperate species relaxed and permitted them to expand their ranges northward. It is unclear if a warmer climate could have directly impacted tundra vole populations since they are known to inhabit a broad latitudinal range in central Asia, living as far south as 45° N (Kostenko 2000). However, the voles of the Beringian clade may represent a lineage that, through isolation in the Beringian refugium during repeated stadial cycles, has become specialized for life in colder climates. Despite having no obvious geographic barriers to southward expansion into North America, representatives of the Beringian clade are not found below 56° N in Canada and southeast Alaska, and their post-glacial expansion has been most extensive along the northern coast of the Canadian mainland (66° - 70° N; Hoffmann & Koeppl 1985).

The last interglacial had warmer temperatures than the Holocene (Arkhipov et al. 1986b; Brigham-Grette & Hopkins 1995), producing a major ecological shift in Siberia and Alaska. Pollen records and fossil data indicate that arctic tundra was largely replaced by larch, birch, and pine forests in Siberia (Grichuk 1984; Lozhkin & Anderson 1995; but see Sher 1991). Spruce forests also expanded far into the north and west of Alaska, possibly even north of the Brooks Range (Ager & Brubaker 1985; Brigham-Grette & Hopkins 1995; Muhs et al. 2001). The change in ecological communities probably would have benefited forest-dwelling rodents such as M. xanthognathus and C.
*rutilus*, but the forest expansion of the last interglacial could have reduced and fragmented tundra vole populations, potentially producing a genetic bottleneck. Low genetic diversity in Siberian populations of *Dicrostonyx* may be the result of similar environmental changes that occurred during the post-Pleistocene (Fedorov 1999; Fedorov et al. 1999a). Mesic environments along river corridors might have served as ecological refugia, which could explain the fact that no bottleneck was observed in the populations from the Omolon River.

The end of the last interglacial accompanied climate cooling and a return of tundra ecosystems to Beringia (Ager 1983; Grichuk 1984; Ager & Brubaker 1985). Expansion of tundra vole habitat probably was associated with expansion of tundra vole populations, yielding the genetic signature of a population expansion coincident with the start of the 1st Wisconsin/Zyryanka glaciation. Since that time, forests have not replaced tundra as they did during the Sangamon/Kazentsevo interglacial. Even during the warm Wisconsin interstadial, spruce forests in eastern Beringia were largely restricted to the interior of Alaska, and herbaceous tundra communities predominated (Ager & Brubaker 1985). The last glacial maximum forced most of the forests out of Alaska (Ager 1983; Ager & Brubaker 1985), and though forest communities have since recolonized northwestern North America and eastern Siberia, large expanses of mesic tundra have persisted throughout the Holocene to the present (Ager 1983; Khotinskiy 1984). None of the climate fluctuations since the Sangamon/Kazentsevo interglacial have had an equivalent ecological impact in the Beringian region, so a lack of evidence for later population bottlenecks is not unexpected.
Conclusions

Pleistocene glaciations influenced the genetic structure of *M. oeconomus* at the deep phylogeographic level, but not at shallower population genetic levels. Glacial events probably played a role in promoting intraspecific differentiation during the ice ages, although the signature of isolation events was not erased during interglacial periods, indicating that ecological factors may have reinforced population divergence. At the scale of populations, however, there is little evidence that stadials had a significant impact on genetic diversity. Instead, ecological shifts during interglacial periods may have contributed to structuring extant patterns of diversity.

Current models of Beringian paleoenvironments should be further examined by testing predictions for a diverse set of high latitude taxa based on knowledge of specific ecological requirements. If the center of the Beringian refugium provided a moist zone that was ideal for mesic-adapted species (Elias *et al.* 2000; Guthrie 2001), such taxa should exhibit a central core of relatively undifferentiated populations that spans the Bering Strait. Populations from the eastern and western borders of the refugium should show evidence of longer separation. Conversely, species that prefer xeric conditions might be more likely to be genetically differentiated across the Bering Strait. If river corridors acted as ecological refugia for tundra voles during the last interglacial, patterns of genetic diversity similar to those found on the Omolon River might be expected from other major Siberian and Alaskan rivers. Finally, if climate warming during the last interglacial period was severe enough to cause a population decline in arctic-adapted species, bottlenecks may be observed in many high latitude taxa. In contrast, taxa with
more temperate ecological preferences may exhibit signatures of population expansions that are coincident with the bottlenecks observed in the high latitude species. Continuing development of sophisticated analytical methods for estimating demographic histories (reviewed in Emerson et al. 2001) will undoubtedly prove useful in examining these predictions. Furthermore, the ever-growing body of knowledge of Pleistocene paleoenvironments provides an excellent framework for developing hypotheses that can be tested using molecular phylogeographic techniques. Such a synthesis of paleoecology, climatology, geology, and evolutionary biology holds great promise for elucidating the historical processes that determined extant patterns of differentiation and population genetic structure of northern species.

ACKNOWLEDGMENTS

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LITERATURE CITED


Cook JA, Runck AM, Conroy CJ (In prep) Historical biogeography at the crossroads of the northern continents: molecular phylogenetics of red-backed voles (Rodentia: Arvicolidae).


Fedorov VB, Fredga K, Jarrell GH (1999a) Mitochondrial DNA variation and the evolutionary history of chromosome races of collared lemmings (Dicrostonyx) in the Eurasian Arctic. Journal of Evolutionary Biology, 12, 134-145.


Galbreath KE, Cook JA (In prep) Phylogeography and post-glacial colonization of the tundra vole (Microtus oeconomus) in Beringia.


Alaska Geological Society, Anchorage.


Nadler CF, Zhurkevich NM, Hoffmann RS, Kozlovskii AI, Deutsch L, Nadler CF, Jr (1978) Biochemical relationships of the Holarctic vole genera (*Clethrionomys, *


Tarr RS, Martin L (1914) *Alaskan Glacier Studies*. National Geographic Society, Washington, DC.


Figure 1.1. Maximal extent of the Late Pleistocene glaciations in Beringia and the Bering Land Bridge (modified from Arkhipov et al. 1986b; Hamilton et al. 1986). Black dots indicate sampling localities (identified in Fig. 2).
Figure 1.2. Distribution of *M. oeconomus*, the Central Asian and Beringian clades, and sampling localities in the Beringian region. Numbers designate sampling localities.
Figure 1.3. Maximum-likelihood test of monophyly for *M. oeconomus* based on the GTR + I + Γ model of evolution. Numbers on branches are bootstrap values based on 100 replicates.
Figure 1.4. Neighbor-joining phylogeny of all 102 observed haplotypes using the Jukes Cantor model of evolution. Numbers next to branches are bootstrap values based on 5000 replicates. Asterisks (*) indicate lineages that were rate heterogeneous according to Takezaki et al.’s (1995) branch length test.
Figure 1.5. Distribution of Beringian (B) and Central Asian (CA) clade mtDNA and nuclear (ALDH1) lineages along the contact zone on the Omolon River. Each vole at Labaznaya and Bol'shaya is listed separately to show relationships between the nuclear and mtDNA lineages and illustrate the presence of introgression. Question marks indicate voles that lacked the Central Asian clade nuclear deletion since uncertainty remains as to whether or not they inherited their ALDH1 DNA from the Beringian or Central Asian clade.
Table 1.1. Post-bottleneck expansion times and effective population size estimates for expanding populations.¹

<table>
<thead>
<tr>
<th></th>
<th>East Clade</th>
<th>95% CI²</th>
<th>East Clade: Southcoastal Alaska</th>
<th>95% CI²</th>
<th>West Clade</th>
<th>95% CI²</th>
<th>West Clade: upper Kolyma and Magadan</th>
<th>95% CI²</th>
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<tr>
<td>M</td>
<td>13.8</td>
<td>9.60 - 20.3</td>
<td>9.54</td>
<td>6.75 - 13.5</td>
<td>8.77</td>
<td>6.42 - 11.7</td>
<td>6.80</td>
<td>3.80 - 11.8</td>
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<tr>
<td>θ₀</td>
<td>4.42</td>
<td>0 - 15.9</td>
<td>0</td>
<td>0 - 4.76</td>
<td>0.871</td>
<td>0 - 3.56</td>
<td>2.01</td>
<td>0 - 6.75</td>
</tr>
<tr>
<td>θ₁</td>
<td>8911</td>
<td>196 - 9510</td>
<td>6660</td>
<td>167 - 10 100</td>
<td>34.80</td>
<td>20.8 - 4680</td>
<td>13.5</td>
<td>6.45 - 101</td>
</tr>
</tbody>
</table>

Expansion time estimates in thousands of years:

7.5% My⁻¹ divergence rate
Using τ | 76.5 | 50.0 - 120 | 83.3 | 46.2 - 109 | 78.2 | 50.0 - 113 | 65.3 | 27.3 - 149 |
Using m | 112 | 78.1 - 165 | 77.6 | 54.9 - 110 | 71.4 | 52.2 - 95.5 | 55.3 | 30.9 - 95.8 |

13% My⁻¹ divergence rate
Using τ | 44.1 | 28.8 - 69.6 | 48.0 | 26.6 - 63.2 | 45.1 | 28.8 - 65.4 | 37.7 | 15.7 - 85.8 |
Using m | 64.8 | 45.1 - 95.5 | 44.8 | 31.7 - 63.4 | 41.2 | 30.1 - 55.1 | 31.9 | 17.8 - 55.2 |

Effective population size estimates in thousands of individuals:

7.5% My⁻¹ divergence rate
N₀ | 89.9 | 0 - 324 | 0 | 0 - 38.7 | 17.7 | 0 - 72.3 | 41.0 | 0 - 137 |
N₁ | 181 000 | 3 990 - 194 000 | 54 200 | 1360 - 82 500 | 708 | 423 - 95 200 | 275 | 131 - 2060 |

13% My⁻¹ divergence rate
N₀ | 51.9 | 0 - 187 | 0 | 0 - 22.3 | 10.2 | 0 - 41.7 | 23.6 | 0 - 79.3 |
N₁ | 105 000 | 2 300 - 112 000 | 31 200 | 784 - 47 600 | 409 | 244 - 54 900 | 159 | 75.7 - 1190 |

¹ See text for formulas and definitions of τ, m, θ₀ and θ₁. Calculations assume 2.5 generations per year.
² Intervals for expansion time and effective population size estimates are not true 95% confidence intervals due to uncertainty in the divergence rate estimate.
Table 1.2. Genetic diversity indices for populations from regions that were glaciated and non-glaciated during the last glacial maximum (2nd Wisconsin/Sartan). Number of individuals ($n$), number of haplotypes ($Nh$), haplotype ($h$) and nucleotide ($\pi$) diversities, and their respective standard errors (SE) are given.

<table>
<thead>
<tr>
<th>Geographical Locality</th>
<th>$n$</th>
<th>$Nh$</th>
<th>$h$</th>
<th>SE</th>
<th>$\pi$(%)</th>
<th>SE(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glaciated</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cold Bay</td>
<td>10</td>
<td>6</td>
<td>0.778</td>
<td>0.137</td>
<td>0.083</td>
<td>0.063</td>
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<tr>
<td>Anchorage</td>
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<td>4</td>
<td>0.821</td>
<td>0.101</td>
<td>0.440</td>
<td>0.263</td>
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<tr>
<td>Cordova</td>
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<td>4</td>
<td>0.711</td>
<td>0.118</td>
<td>0.357</td>
<td>0.211</td>
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<tr>
<td>McCarthy</td>
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<td>0.556</td>
<td>0.090</td>
<td>0.136</td>
<td>0.094</td>
</tr>
<tr>
<td>SE Alaska</td>
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<td>0.467</td>
<td>0.132</td>
<td>0.028</td>
<td>0.031</td>
</tr>
<tr>
<td><strong>Non-glaciated</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Magadan</td>
<td>10</td>
<td>9</td>
<td>0.978</td>
<td>0.054</td>
<td>0.345</td>
<td>0.204</td>
</tr>
<tr>
<td>Elegan River</td>
<td>5</td>
<td>4</td>
<td>0.900</td>
<td>0.161</td>
<td>0.220</td>
<td>0.156</td>
</tr>
<tr>
<td>Kontakt Creek</td>
<td>11</td>
<td>3</td>
<td>0.473</td>
<td>0.162</td>
<td>0.042</td>
<td>0.039</td>
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<tr>
<td>Omolon 1</td>
<td>11</td>
<td>5</td>
<td>0.709</td>
<td>0.137</td>
<td>0.506</td>
<td>0.287</td>
</tr>
<tr>
<td>Omolon 2</td>
<td>15</td>
<td>6</td>
<td>0.648</td>
<td>0.134</td>
<td>0.314</td>
<td>0.180</td>
</tr>
</tbody>
</table>
Appendix I. Specimens listed by locality and University of Alaska Museum AF number. Locality numbers correspond to those used in Figure 1.1.

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, AF41261, AF41258, 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,
10. Omolon River 2: AF38290, AF38291, AF38349, AF38350, AF38351, AF38356, AF38366, AF38371, AF38376, AF38391, AF38396, AF38397, AF38402, AF38403, AF38405
11. Kamchatka: AF32747
12. Ust Chaun: AF3762, AF3758, AF3759, AF3760, AF3761, AF3763, AF3771, AF3772, AF3773, AF3774
13. Providenya: AF7468, AF7470, AF7472

Alaska

14. St. Lawrence Island: AF20801, AF20802, AF20808, AF20805, AF20807, AF20812, AF20817, AF20818, AF20819
15. Seward Peninsula: AF7370, AF7462, AF7463, AF7464, AF36721, AF36722, AF36752, AF36753, AF39706, AF39707
16. Colville River: AF22101, AF22104, AF22103, 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: AF801, AF794, AF795, AF796, AF797, AF798, AF835, AF838, AF839, AF840
22. Anchorage: AF8819, AF8828, AF8831, AF8843, AF11600, AF11320, AF11373, AF11380
23. Montague Island: AF535, AF510, AF513, AF514, AF515, AF516, AF517, AF1951, AF1952, AF1953
Appendix I (continued). Specimens listed by locality using their University of Alaska Museum AF number. Locality numbers correspond to those used in Figure 1.1.

**Alaska**

24. Hinchinbrook Island: AF461, AF462, AF470, AF476, AF458, AF460, AF494, AF495, AF496, AF498
25. Cordova: AF452, AF453, AF454, AF455, AF456, AF505, AF506, AF507, AF1978, AF1979
26. McCarthy: AF3289, AF3277, AF3278, AF3279, AF3280, AF3284, AF3287, AF3288, AF3294
27. Southeast Alaska mainland: AF2032, AF2033, AF2034, AF2054, AF2055, AF7820, AF7821, AF7822, AF7836, AF7837
28. Chichagof Island: AF16083, AF16082
29. Baranof Island: AF7601, AF7610, AF7613, AF7657, AF7658, AF7721, AF17071, AF17133, AF17085, AF17082

**Finland**

Kilpisjärvi: AF1944, AF1948, AF1949
Phylogeography and Post-glacial Colonization of the Tundra Vole  
(Microtus oeconomus) in Beringia

ABSTRACT

By repeatedly modifying the ecological and geophysical landscape of Beringia, Pleistocene climatic fluctuations played a major role in structuring routes of gene flow and post-glacial colonization, as well as patterns of local differentiation and endemism. Populations of the northern rodent Microtus oeconomus (tundra vole) probably tracked climate-driven environmental changes closely, and therefore the vole provides a good model for exploring the effects of the changing Beringian landscape on northern faunas. Variation in mtDNA (cytochrome b and control region) was examined for 211 voles distributed across Beringia. A minimum spanning network of haplotypes indicated that transberingian movement of tundra voles into North America may have been partitioned into separate northern and southern bands, which may have implications for recently developed models of Beringian paleoecology. Post-glacial colonization of southcoastal Alaska probably proceeded via coastal routes from the west, though direct routes through coastal mountain ranges may have been used to a lesser degree. Rapid post-Pleistocene local differentiation suggests a high incidence of neoendemism.

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INTRODUCTION

The last major glacial period came to a close along with the Pleistocene approximately 10 thousand years ago (Kya; Bowen et al., 1986). This was accompanied by complex climatological and biogeographic changes that impacted species’ distributions and rearranged ecological communities on a global scale (Webb and Bartlein, 1992). As the climate warmed, massive ice sheets in North America and Eurasia receded, opening dispersal routes to colonizing organisms, making previously glaciated land available for habitation, and easing constraints on populations that had been restricted to isolated refugia during the glacial maximum. Species’ responses to this relatively sudden climatological change have been the subject of much discussion (e.g. Coope, 1979; Davis, 1983; Hewitt, 1999; Huntley and Webb, 1989; Jacobsen et al., 1987; MacPherson, 1965; and others).

The retreat of the glaciers particularly affected organisms dwelling in eastern Beringia, the North American portion of the large refugium that spanned northeastern Siberia, northwestern North America, and the intervening continental shelf during the ice ages. The Cordilleran and Laurentide ice sheets bounded the region to the south and east, respectively, at the time of the last glacial maximum. When the ice receded, species were able to greatly expand their ranges eastward across Canada and south along the Gulf of Alaska coast as suitable habitat became available (Klein, 1965; MacPherson, 1965).

Though relatively swift on a geological time scale (Ager, 1983; Mann and Hamilton, 1995), the establishment of post-glacial communities was a gradual process of
ecological succession that began at the local level. As glaciers receded, plants and animals that were adapted to early successional habitats would have quickly colonized newly exposed land. Such organisms probably tracked the glacial retreat closely, and the signature of routes of colonization may still be written in their genes. Reconstruction of a history of colonization can provide information on corridors of gene flow and genealogical relationships among populations, which has direct implications for issues of conservation and management by clarifying patterns of endemism and population interconnectedness (Cook et al., 2001; Cook and MacDonald, 2001).

The tundra vole, *Microtus oeconomus*, is an excellent candidate for examining colonization history and genetic structure of post-glacially established populations. The ecological requirements of this northern rodent indicate that it is likely to be one of the first mammalian colonizers of newly exposed land after glacial recession. As the northernmost (> 71° N latitude) representative of *Microtus* in North America (Hoffmann and Koepppl, 1985), tundra voles are well adapted to living in cold environments. They are generally found in wet meadow and tundra habitats (Getz, 1985; Peterson, 1967; Quay, 1951; Tast, 1966), though in the Palearctic they have broader ecological affinities, also inhabiting mesic environments in taiga, forest-steppe, and mixed forest (Hoffmann and Koepppl, 1985). Their primary food sources are usually herbaceous vegetation such as grasses and sedges (Getz, 1985). These habitat preferences describe the early successional communities that followed glacial recession in Beringia well (Ager, 1983; Mann and Hamilton, 1995).
As the only species of *Microtus* that spans all three northern continents (Hoffmann and Koeppl, 1985), and one of only four Holarctic rodents (Rausch, 1994), the tundra vole occupies a unique position on the Holarctic stage. Its relatives in North America probably originated from a single ancient invasion from the Palearctic (Conroy and Cook, 2000b), whereas tundra voles entered the Nearctic across the Bering Land Bridge relatively recently (Galbreath and Cook, in prep; Lance and Cook, 1998; Macpherson, 1965; Rausch, 1963). Their short history in Beringia suggests that any observed genetic structure should reflect recent events, simplifying interpretation of phylogeographic patterns. Also, because *M. oeconomus* has a nearly complete circumarctic range, it is a good model for examining evolutionary patterns and processes across the Holarctic.

Current subspecific taxonomy and studies of mitochondrial (Galbreath and Cook, in prep) and nuclear (Lance and Cook, 1998) DNA provide a phylogeographic framework for assessing patterns of differentiation and developing hypotheses. Ten subspecies of *M. oeconomus* in North America (Hall, 1981), and three (Chernyavski, 1984) or two (according to a recent revision; Kostenko, 2000) from mainland eastern Siberia have been described based on morphological criteria. The extent to which these classifications are reflected by genetic structure has not been fully examined. A strong phylogeographic break occurs on the Omolon River in Siberia, separating a Beringian clade that includes tundra voles from northeastern Siberia and all of North America, and a Central Asian clade that is found in the upper Kolyma River basin and further south and west (Galbreath and Cook, in prep; Fig. 2.1). Using allozyme electrophoresis,
Lance and Cook (1998) examined several Beringian populations and showed that there was generally little nuclear variation among individuals representing five North American and one Siberian subspecies. Intrapopulation variability was especially low in coastal and island populations from southcentral Alaska. Some southcoastal populations possessed rare unique alleles, suggesting that they are on distinct evolutionary trajectories. These populations are probably post-glacial neoendemics whose founders originated in the Beringian refugium and either followed the receding Cordilleran ice sheet along the coastline or colonized via corridors through the coastal mountain ranges (Cook et al., 2001; Heaton et al., 1996). Shared alleles between coastal and inland sites suggest a history of gene flow (Lance and Cook, 1998). Alternatively, coastal and island populations may have arisen from paleoendemic populations that survived the glacial maximum in small refugia south of the ice (e.g. Heaton et al., 1996; Heusser, 1960; Lindroth, 1969). Strongly differentiated populations of tundra voles on Hinchinbrook and Montague Island in Prince William Sound may be an example of the latter (Galbreath and Cook, in prep; Lance and Cook, 1998).

In this study, mtDNA sequence variation was examined from the same populations that were studied by Lance and Cook (1998) to gain a different molecular perspective on the history of post-glacial tundra vole expansion and colonization in Beringia. Additional populations in Alaska and many more in Siberia were included, permitting relationships within and among tundra vole populations to be assessed across the entire Beringian region. Four goals motivated the study: 1) Genetic affinities among inland and coastal populations were investigated for evidence of routes of post-glacial
colonization. Unless expansion was too rapid for mutations to accumulate between colonization events, genealogical relationships among populations should reflect a stepwise progression from first colonized to last. This could elucidate dispersal corridors that tundra voles used to reach newly deglaciated sites. 2) The relationship between Siberian populations and those from Interior and southcoastal Alaska was studied for evidence of a coastal route of gene flow across the Bering Land Bridge. Lance and Cook (1998) observed greater genetic affinities between Siberian and southcoastal Alaskan populations than between southcoastal and Interior Alaskan populations, implying that recent transberingian gene flow excluded Interior populations. 3) Population genetic structure was examined for patterns of neoendemism (Cook et al., 2001). Well-differentiated populations that possess shallow genetic lineages may be post-glacial neoendemics. 4) The degree to which genetic structure was congruent with current subspecies taxonomic designations was assessed.

MATERIALS AND METHODS

Tissue samples were obtained from the University of Alaska Museum for tundra vole specimens (N = 211) collected from 29 localities across eastern Siberia, Alaska, and Canada (Fig. 2.1). Between 1 and 11 voles were examined per locality (Table 2.1). Specimens represented six North American M. oeconomus subspecies (macfarlani, operarius, elymocetes, yakutatensis, sitkensis, innuitus; Hall, 1981) and three (tschuktschorum, kamtschaticus, koreni; Chernyavski, 1984) or two (koreni, tschuktschorum = kamtschaticus; Kostenko, 2000) subspecies from Siberia. Genomic
DNA was extracted and a mtDNA fragment (total length: 1637-8 bp; 2 indels), which included the complete cytochrome *b* gene, two tRNA sequences, and a portion of the 5' end of the control region, was amplified and sequenced. Detailed descriptions of molecular protocols are given elsewhere, as is a complete list of the specimens used in the study (Galbreath and Cook, in prep).

For all analyses that considered populations of tundra voles, a “population” was defined as a group of individuals that was collected from a single locality. Low amounts of gene flow have been demonstrated for another microtine rodent on a much smaller spatial scale than the distances between populations studied here (Stacy et al., 1997), so it was assumed that voles taken from different collecting sites do not share the same gene pool. As a test of this assumption, analysis of molecular variance (AMOVA; Excoffier et al., 1992) was used to examine partitioning of genetic variation among and within populations. The AMOVA was applied using the software package Arlequin 2.001 (Schneider et al., 2000), as were all calculations and tests described hereafter unless otherwise indicated.

Two indices of genetic diversity (Table 2.1) were determined for each population based on a matrix of pairwise nucleotide differences calculated from sequence data. Nucleotide diversity (π) is an average sequence divergence between haplotypes that is weighted by haplotype frequency (Grant and Bowen, 1998). Haplotype diversity (*h*) is an index ranging from 0 to 1 that incorporates the number and frequency of haplotypes (Grant and Bowen, 1998). A minimum spanning network (MSN; Excoffier and Smouse, 1994), which combines all equally parsimonious minimum spanning trees (MSTs;
Kruskal, 1956; Prim, 1957) was also generated for the Beringian and Central Asian clades. In phylogeographic studies, MSTs are useful for elucidating relationships among haplotypes because unlike other phylogenetic tree building methods, they do not assume ancestral haplotypes to be extinct and they permit multifurcations (Crandall and Templeton, 1996; Excoffier and Smouse, 1994). Therefore, it is possible to draw inferences regarding haplotype genealogy and relationships among extant lineages.

To explore patterns of neoendemism within the East and West clades and to assess congruence of genetic structure with subspecific taxonomy, PAUP* (Swofford, 2000) was used to generate separate unrooted neighbor-joining (NJ) trees for the East and West clades from Jukes-Cantor (JC) distances (Jukes and Cantor, 1969). Support for tree topologies was evaluated by bootstrapping (5000 replicates). The JC model was chosen based on recommendations by Nei and Kumar (2000), who state that when genetic distances within clades are very small, a simple model of evolution will yield accurate topologies without the high variances associated with models that have many parameters. This has been tested using the data set for this study. The JC model was compared to a number of more complex substitution models and shown to retrieve equivalent topologies and similar branch lengths (Galbreath and Cook, in prep).

RESULTS

Population genetic structure and diversity

The AMOVA demonstrated that populations were strongly subdivided genetically, and confirmed the assumption that members of geographically separated
populations do not interbreed freely. Approximately 68% \((P < 0.001)\) and 47% \((P < 0.001)\) of genetic variation was partitioned among populations in the Beringian and Central Asian clades, respectively. The greater value in the Beringian group may reflect the fact that the Alaskan populations are more widespread and therefore more isolated by distance than those in the Central Asian group.

A large number of haplotypes \((N = 101)\) were identified within the sample, which was reflected in high haplotype diversity values both across the entire data set \((h = 0.987)\) and within individual populations (Table 2.1). This result was surprising given the short history of tundra voles in Beringia and low genetic diversity observed in nuclear markers (Lance and Cook, 1998), though nucleotide diversity was quite low for most populations \((\pi < 0.5\% \text{ Table 2.1})\). These values are similar to those obtained for a related post-glacial colonizer, \(M. longicaudus\) \((0.12 - 0.44\%; \text{ Conroy and Cook, 2000a})\).

To place the low values into perspective, the nucleotide diversity of the complete data set, which included samples from the two well-differentiated clades (Beringian and Central Asian clades: \(~ 3\% \text{ divergence}\)), was 1.60%. The two clades overlap near the Bol’shaya River on the Omolon (Fig. 2.1), which is responsible for the high value of \(\pi\) from that location.

**Relationships among haplotypes**

The MSN was subdivided into sections for the two major clades (Fig. 2.2). Both sections exhibited some ambiguous relationships due to multiple, equally parsimonious connections among haplotypes. However, ambiguities were mostly localized within cohesive lineage clusters, permitting some resolution of relationships. The haplotypes
that are most common (Donnelly and Tavaré, 1986; Watterson and Guess, 1977), and that are found at the interior nodes of a minimum spanning tree (Castelloe and Templeton, 1994; Crandall and Templeton, 1993), are expected to represent the oldest lineages. Peripheral lineages are therefore expected to be more derived and, in the context of this study, represent more recently colonized populations.

Although haplotypes from different populations often differed by only a few substitutions, in only one case in each clade was a haplotype shared by more than one population. Furthermore, haplotypes from a single population were generally found together within the same cluster, suggesting that they stem from a common ancestral lineage. Notable exceptions to this were lineages from Anchorage and the North Slope in the Beringian clade, and Omolon 1, Omolon 2, and Ust Omchut in the Central Asian clade. Individuals from these populations were divided among multiple clusters.

In the Beringian clade, three distinct lineage clusters (Q, R, and S; Fig. 2.2) radiated out from a single group. Limits for this central cluster are somewhat arbitrary because there is no clear distinction between where it ends and peripheral lineages begin. Haplotypes that differed from the center-most Baranof haplotype by fewer than five mutational steps were considered to be part of the central cluster, which was reasonable given that interhaplotypic differences within other lineage clusters, and even within populations, were often at least five steps. Because almost no haplotypes were shared among populations, no single haplotype was widely spread throughout the entire set of population samples to suggest an ancestral lineage. However, to the extent that "most common haplotype" can be interpreted as "most common set of closely related
haplotypes", the central group of the MSN satisfies both criteria for ancestral lineages. Aside from its internal location in the MSN, this haplotype cluster had a widespread Alaskan distribution, including southeast (Baranof), Interior, northcentral (Colville), northwest (Seward Peninsula) and southwest (Kodiak and Cold Bay) Alaska. However, no haplotypes from Siberia were found within this group. Since populations across the Bering Strait were probably in contact up until the last flooding of the Bering Land Bridge (Galbreath and Cook, in prep; Lance and Cook, 1998), a widespread group of ancestral haplotypes would be expected to be present across the entire region. The haplotypes from Beringian clade Siberian populations all were on relatively long branches. These lineages were peripherally associated with cluster Q (particularly Kodiak Island), and either cluster R (St. Lawrence and Seward Peninsula) or the central group (Baranof). Though some ambiguity remains with respect to the relationship between the Siberian and Alaskan lineages, it is apparent that there are at least two distinct lineages in Siberia. Montague and Hinchinbrook Island (group S) were together on a long branch, as expected from evidence that these populations have a deep evolutionary history together (Galbreath and Cook, in prep; Lance and Cook, 1998). The fact that this group is unambiguously connected to the central Baranof haplotype provides some support for interpreting the central group to be ancestral. Populations from Montague and Hinchinbrook may have diverged from other Alaskan populations more than 50 Kya (Galbreath and Cook, in prep), so it is likely that their closest relatives would have ancestral haplotypes.
The MSN for the Central Asian clade (Fig. 2.2) had two terminal groups (X and Y) connected to a single interior cluster (Z). The central position of haplotypes from Magadan and the upper Kolyma (Z) suggests that they represent ancestral lineages that gave rise to the two terminal clusters (Castelloe and Templeton, 1994; Crandall and Templeton, 1993). However, this group is localized in a single region, which indicates that it does not meet the criterion of representing a widespread ancestral type (Donnelly and Tavaré, 1986; Watterson and Guess, 1977). In contrast, one of the terminal clusters (Y) includes haplotypes from nearly the entire range of sampled locations. The discrepancy may be due to the fact that this MSN represents only one end of the entire distribution of the Central Asian clade *M. oeconomus* (Brunhoff et al., in prep). It is likely that more complete sampling across the entire range of the clade would yield additional haplotype clusters that would shift the center of the tree to a different group. Both lineage clusters X and Y include individuals from the Omolon River. Cluster X, which is found only among Omolon River populations, includes individuals from farther upstream than cluster Y.

The NJ trees showed that despite poor resolution at the base of both the Central Asian and Beringian clade, there are numerous well-supported nested subclades within each major clade (Fig. 2.3). In nearly all cases in the Beringian clade, nested clades consist of individuals from a single location, illustrating the high degree of population genetic subdivision detected using analysis of molecular variance. A similar pattern was apparent in the Central Asian clade, though individuals from different, usually adjacent, populations were sometimes found together in nested subclades.
DISCUSSION

Post-glacial colonization

Southcoastal Alaska is bounded by the Alaska, Chugach, Wrangell, and St. Elias mountain ranges (Fig. 2.4), which undoubtedly were formidable barriers to tundra voles as they expanded from Beringia into coastal areas after the last glacial recession. Recolonization of the region could have occurred via two different paths. Voles either circumnavigated the coastal ranges from the west and then traveled east and south along newly opened shorelines, or they invaded coastal sites directly by crossing the mountain ranges via ice-free corridors. Colonization of coastal regions probably commenced after 15 Kya, as glaciers receded and primary succession herb tundra communities became established (Mann and Hamilton, 1995). Oceans may not have risen to current levels immediately after glacial recession (Vrba, 1995), so early coastal routes for tundra vole expansion were probably relatively broad. Also, glaciers might have persisted in parts of Prince William Sound and along the coast of southeastern Alaska until approximately 10 Kya (Mann and Hamilton, 1995; Molnia, 1986), so it is likely that colonization routes for most of southcoastal Alaska were first available from the west. Direct routes of colonization through the mountain ranges are currently only speculative, but likely candidates are the Copper River, which is the primary corridor through the Chugach Mountains, and Rainy Pass, which cuts through the western portion of the Alaska Range.

Lineage cluster Q in the MSN for the Beringian clade (Fig. 2.2) includes haplotypes from each of the southcoastal populations between Kodiak and McCarthy.
The Kodiak haplotypes are situated toward the center of the tree, suggesting that they are older lineages (Castelloe and Templeton, 1994; Crandall and Templeton, 1993). Anchorage, Cordova, and McCarthy are on terminal branches and may therefore be more derived. This pattern is consistent with colonization from the west that first established the Kodiak population (Fig. 2.4), followed by expansion along the coast to the north (Anchorage) and east (Cordova). The position of McCarthy as a terminal branch in this cluster suggests that it was colonized from the coast via the Copper River, indicating that the river corridor was important for colonization, but in the reverse direction to that previously suggested. This is consistent with the suggestion that a rare allozymic allele shared between Interior and coastal voles may indicate a history of gene flow through the Copper River corridor (Lance and Cook, 1998).

Haplotypes from Colville, a locality on Alaska’s North Slope, were also found in the predominantly southcoastal lineage cluster Q, as well as the central group. Given the high level of genetic subdivision observed among tundra vole populations, it seems unlikely that gene flow since colonization could have carried representatives of a lineage from southwest Alaska to north of the Brooks Range. However, during the last glacial maximum, ancestral haplotypes of the different extant lineages probably were intermixed within the Beringian refugium. Species like *M. oeconomus*, which prefer mesic environments, might have concentrated in the center of the refugium during the glacial maximum (Guthrie, 2001), which would have further homogenized the gene pool. When vole populations expanded after the ice age ended, some individuals with
haplotypes similar to those of the original Kodiak colonizers may have colonized the North Slope along with individuals representing the ancestral lineage.

Like Colville, Anchorage has representatives from two distinct lineages (lineage groups Q and R, Fig. 2.2), though in this case both sets of haplotypes are on terminal branches in the MSN, implying a derived state. This could be the result of assortment during post-glacial expansion, but if lineage R entered southcoastal Alaska with Q post-glacially, then evidence of it would be expected from other southcoastal locations besides Anchorage. In this case, colonization from different sources might be a reasonable alternative explanation for multiple lineages in Anchorage. As mentioned earlier, haplotypes in group Q may have been established in a colonization event from the southwest. Haplotypes in group R, however, are more similar to individuals from western Alaska (e.g. McGrath), which is close to Anchorage, but separated from it by the Alaska Range. More detailed studies of genetic relationships across likely colonization corridors through the Alaska Range (e.g. Rainy Pass) should clarify post-glacial connections between coastal and central Alaskan populations.

The distinct geographic pattern of three well defined groups of lineages in the Central Asian clade MSN (Fig. 2.2) may be the result of overlapping colonization events interspersed with periods of isolation and divergence. Reconstruction of this history is complicated by the fact that the most ancestral of the three lineage clusters is unclear. Cluster X is found exclusively on the Omolon, and it extends farther upstream than cluster Y, which overlaps its distribution and also occurs in the upper Kolyma River basin. If colonization of the river corridor began at low elevations and moved upstream,
which seems likely, this biogeographic pattern suggests that lineage X colonized the river first and subsequently differentiated. A later invasion by lineage Y could have produced the current overlapping distribution. The complex glacial and ecological history of the region (Arkhipov et al., 1986a,b; Lozhkin and Anderson, 1995) may suggest explanations for these patterns, but more thorough sampling of the entire Central Asian clade (Brunhoff et al., in prep) is required to provide better resolution of phylogeographic structure and ancestral lineages.

*Transberingian connections among populations*

Relationships between Alaskan and Siberian populations were not well resolved by either the MSN (Fig. 2.2) or the NJ tree (Fig. 2.3), though both trees showed evidence of at least two distinct Siberian lineages. An unambiguous relationship between two Siberian haplotypes (Labaznaya/Bol’shaya and Kamchatka) and the southcoastal Alaskan populations in lineage cluster Q (Fig. 2.2) was consistent with allozymic affinities across the Bering Strait that was distinct from interior Alaskan populations (Lance and Cook, 1998). This may have arisen from an exclusively southern Beringian connection between Siberia and Alaska. Conversely, Ust Chaun, which is on the north coast of Siberia, formed a clade with Providenya and other Labaznaya/Bol’shaya individuals (Fig. 2.3), and the MSN suggested that they were associated with either northwest Alaska (St Lawrence, Seward Peninsula) or the basal ancestral group (Fig. 2.2). This implies a separate, more northerly connection across Beringia. The possibility that faunal exchange across the land bridge was partitioned latitudinally is a new concept that should be explored within the context of models of Beringian
paleoecology (e.g. Elias et al., 2000; Guthrie, 2001). For example, coastal ecosystems along the Arctic Ocean to the north and Bering Sea to the south may have supported similar faunal assemblages, but distance and different ecological zones in the interior of the land bridge could have created a barrier between them. The latitudinal breadth of the land bridge (>1500 km) was substantial, indicating that from a geographic perspective such partitioning was plausible.

**Patterns of endemism**

Recently established populations that through isolation and differentiation have embarked upon unique evolutionary trajectories are considered to be neoendemics (Cook et al., 2001). Such populations are expected to form well defined monophyletic clades, but exhibit low levels of nucleotide diversity. In general, populations of *M. oeconomus* match these criteria closely, showing both low values of \( \pi \) (Table 2.1) and significant genetic subdivision (AMOVA, Fig. 2.3). The genetic structure of the tundra vole populations is indicative of a rapid expansion followed by a substantial reduction in female mediated gene flow. Low levels of gene flow in small rodent populations are not unexpected (Stacy et al., 1997). Subsequent genetic drift or accumulation of mutations has apparently acted within local populations to initiate differentiation.

Evidence of neoendemic populations of diverse mammalian taxa in southcoastal Alaska points to post-glacial colonization as a factor in producing these unique lineages (e.g. Bidlack and Cook, 2001; Cook et al., 2001; Demboski et al., 1998). Lance and Cook (1998) observed unique alleles in populations from Cordova and mainland southeast Alaska and suggested that they were indicative of a history of strong isolation.
These are good candidates for classification as neoendemics. Indeed, individuals from the southeast Alaska mainland formed a monophyletic clade with good bootstrap support (Fig. 2.3), though the population had the second lowest nucleotide diversity (0.028) of the study. Support for a single Cordova clade was not evident, however. This group had higher nucleotide diversity due to the presence of separate distinct lineages (Figs. 2.2 and 2.3). The position of Cordova at the intersection of the coastal colonization route and the Copper River may have produced more opportunities for gene flow from different source populations.

The mtDNA data suggest that other populations may be neoendemics. There is very strong bootstrap support for a monophyletic Cold Bay clade and moderate support for an Interior clade. The former population is isolated at the tip of the Alaska Peninsula, so it is logical that genetic differentiation has begun there. Strong differentiation of Interior voles is more unexpected because there are no major physiographic barriers isolating them from other Alaskan populations (e.g. Seward Peninsula, McGrath). The observed differentiation is probably due to isolation by distance. Further studies of populations that are at increasing, evenly-spaced distances across Alaska will help elucidate the geographic breadth of the Interior group, and whether or not it represents a sharply delineated lineage or part of a continual gradient of genetic variation.

**Taxonomic congruence**

In North America, *M. oeconomus* subspecies taxonomy and genetic structure are partially congruent (Fig. 2.3). Of the six subspecies examined, the mtDNA data suggest
that three may consist of single monophyletic lineages. Notably, these are the three subspecies from which a single population was sampled. The strong evidence for local differentiation in tundra voles suggests that if further populations are sampled, monophyly for these subspecies will no longer be supported. *Microtus oeconomus innuitus* from St. Lawrence Island is monophyletic in the NJ tree (Fig. 2.3), but bootstrap support for the basal bifurcation in the clade is weak (56%). Still, these voles are clearly isolated from other populations, and it is undoubtedly only a matter of time before they are strongly differentiated. Furthermore, since this subspecies is an island endemic, gene flow across its whole range may be sufficient to suggest that samples presented here are representative of the total diversity on the island. *Microtus oeconomus yakutatensis* from the southeast Alaska mainland formed a well supported monophyletic clade. Having sampled only one population it is impossible to fully assess congruence of this subspecies with molecular structure, but these data suggest that subspecies level morphological variation and mtDNA differentiation may be consistent. Sampling of additional *M. o. yakutatensis* populations will further clarify this issue and may yield information regarding the isolation and divergence of southeast Alaskan populations after colonization. The Montague Island vole, *M. o. elymocetes*, is the most genetically divergent of all of the North American subspecies, which implies that in this case taxonomy reflects phylogeny. However, poor resolution of the relationship between the Montague and Hinchinbrook populations (Fig. 2.3) raises the possibility that *M. o. elymocetes* may be paraphyletic with respect to *M. o. operarius* from Hinchinbrook Island. This is almost certainly due to incomplete lineage sorting since
the time that the two populations became isolated. Ongoing gene flow between the populations is unlikely. Klein (1965) noted that though the red-backed vole (*Clethrionomys rutilus*) has been able to reach Hinchinbrook Island from the mainland, it has not crossed Hinchinbrook Entrance to Montague Island. The water barrier, which is over 10 km wide and 300 m deep, is probably equally formidable to tundra voles. Therefore, as with *M. o. innuitus*, the Montague Island vole will eventually become unequivocally monophyletic if its current state of isolation is maintained.

The three remaining North American subspecies, *operarius*, *macfarlani*, and *sitkensis*, are not supported by mtDNA structure at all. Local differentiation within each of these subspecies has formed distinct lineages within individual populations, but the lack of resolution at the base of the East clade (Fig. 2.3) prevents proper assessment of monophyly for the subspecies. Though this does not necessarily preclude the possibility that different subspecies represent distinct, reciprocally monophyletic lineages, it does indicate that affinities among populations within the subspecies are weaker than relationships within individual populations. This calls into question the validity of the current taxonomy as a reflection of cohesive evolutionary lineages.

The subspecific status of the voles of Hinchinbrook Island may be questioned based on molecular analyses. Both allozymic (Lance and Cook, 1998) and mitochondrial (this study) data show that this population is strongly differentiated from other populations of *M. o. operarius*. Mitochondrial affinities are closest to *M. o. elymocetes*, though both the nuclear markers and mtDNA show that Hinchinbrook is differentiated from Montague. Morphological assessments of North American
subspecies have not addressed the possibility that the Hinchinbrook Island vole is unique. Such studies have either excluded specimens from Hinchinbrook (Paradiso and Manville, 1961), or simply assigned them *a priori* to *M. o. operarius* in comparisons with other subspecies (Lance and Cook, 1998). Klein (1965) stated that voles from Hinchinbrook were more similar to mainland forms than to Montague, but gave no support for the assertion. To better understand the relationship of the Hinchinbrook vole population to other tundra voles, and to clarify their taxonomic position, a morphological assessment is necessary.

In northeastern Siberia, subspecific designations for *M. oeconomus* follow deeper genetic breaks than in North America. Chernyavski (1984) considered voles from Kamchatka, Chukotka, and the upper Kolyma River basin to represent different subspecies (*kamtschaticus*, *tschuktschorum*, and *koreni*, respectively). The line that separates *M. o. koreni* and *M. o. tschuktschorum* is highly congruent with the deep phylogenetic break between the Beringian and Central Asian tundra vole clades. If, therefore, Beringian clade voles from Chukotka can be considered to represent *M. o. tschuktschorum*, then there is evidence that they form an almost perfectly monophyletic lineage. Only two Siberian voles within the Beringian clade did not fall within this group (Fig. 2.3). The single specimen from Kamchatka prevents an adequate assessment of the classification of *M. o. kamtschaticus*, though the fact that it showed no strong affinities to the rest of the Siberian lineages (Fig. 2.3) implies some differentiation. Kostenko (2000) revised *tschuktschorum* to be a synonym of *kamtschaticus*, while retaining *koreni*. The mtDNA data support this classification in that *tschuktschorum* and
*kamtschaticus* are both found within the East clade. If this criterion for combining subspecies is applied across Beringia, nomenclatorial priority dictates that all of the subspecies in North America would become synonyms of *M. o. kamtschaticus*, with the possible exception of the well differentiated *M. o. elymocetes*. Though such an extreme revision is unlikely to occur, the idea of combining subspecies across Beringia is not a new one. In the last review of subspecies designations, Paradiso and Manville (1961) suggested that morphological differentiation across the Bering Strait may be insufficient to justify *M. o. operarius* and *M. o. tschuktschorum* as separate subspecies. The genetic similarities among subspecies in the Beringian region suggest that re-examination of the current taxonomy is warranted, particularly since taxonomic categories play a critical role in legal issues surrounding conservation and management priorities (Cook and MacDonald, 2001).

**Conclusions**

This study builds upon the phylogeographic foundation established by Lance and Cook (1998) for Beringian tundra voles and provides a framework for examining routes of post-Wisconsin dispersal, transberingian faunal connections, and local differentiation of populations during the Holocene. Colonization of southcoastal Alaska following the breakup of the Cordilleran ice sheet was probably accomplished by a single expansion along the coast from west to east. Direct colonization through the coastal mountain ranges most likely was not a major factor, although some tundra voles may have entered the Anchorage area by crossing the Alaska Range. In contrast, the Copper River corridor may have permitted colonization of inland sites by coastal voles.
Genetic affinities between Siberian and Alaskan haplotypes hint at separate northern and southern connections among populations across the Bering Land Bridge, which may have relevance for understanding heterogeneity in Beringian paleoenvironments. Finally, a high degree of genetic subdivision among populations throughout Beringia is indicative of low levels of gene flow and rapid local differentiation (neoendemism).

Codistributed taxa and independent molecular markers should be examined to assess taxonomic and gene-tree concordance of the observed patterns (Riddle, 1996). Sampling efforts that are focused on possible post-glacial colonization corridors may clarify faunal responses to glacial recession, as well as provide information on patterns and rates of gene flow among populations. A potentially fruitful avenue of study includes the parasites of tundra voles and other taxa. Parasites have long been recognized as excellent biological indicators of contemporary and historical biogeographic and ecological associations (Brooks and McLennan, 1991; Hoberg, 1997; Manter, 1966). Because of tight associations with their hosts, host-specific parasites are likely to provide a useful tool for resolving relationships among host populations across Beringia and in areas where post-glacial colonization routes are of interest (e.g. southcoastal Alaska).

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LITERATURE CITED

Ager, T. A. 1983. Holocene vegetational history of Alaska. Pp. 128-141, in Late-
Quaternary Environments of the United States, vol. 2 (H. E. Wright, Jr., ed.).
University of Minneapolis Press, Minneapolis, Minnesota, 277 pp.

A. Velicko. 1986a. Ice-sheet reconstructions. Quaternary Science Reviews, 5:475-
483.

Siberia and north-east USSR. Quaternary Science Reviews, 5:463-474.

Bidlack, A. L., and J. A. Cook. 2001. Reduced genetic variation in insular northern
flying squirrels (Glaucomys sabrinus) along the North Pacific Coast. Animal
Conservation, 4:283-290.

Bowen, D. Q., G. M. Richmond, D. S. Fullerton, V. Šibrava, R. J. Fulton, and A. A.
Quaternary Science Reviews, 5:509-510.

research program in comparative biology. University of Chicago Press, Chicago,
Illinois, 434 pp.

phylogeography of the root vole (Microtus oeconomus) based on cytochrome b
sequence analysis.


Galbreath, K. E., and J. A. Cook. In prep. Genetic consequences of Pleistocene glaciations for the tundra vole (Microtus oeconomus) in Beringia.


Figure 2.1. Distribution of *M. oeconomus*, the Central Asian and Beringian clades, and sampling localities in the Beringian region.
Figure 2.2. Minimum spanning network showing relationships among Beringian clade (A) and Central Asian clade (B) haplotypes. Circles represent different haplotypes, which are numbered by specimen locality. Circle size is proportional to the number of specimens observed with a given haplotype (ranging from 1 to 10). Breaks in dashed lines represent one mutational step between two haplotypes. Dotted lines are equally parsimonious alternative connections. Letters in bold (Q, R, S, X, Y, Z) identify specific lineage clusters, which are shown within gray fields. The loop around haplotypes in the center of the Beringian clade MSN delineates haplotypes that differ from the center by fewer than five mutational steps.
Figure 2.2 (continued). Minimum spanning network showing relationships among Beringian clade (A) and Central Asian clade (B) haplotypes. Circles represent different haplotypes, which are numbered by specimen locality. Circle size is proportional to the number of specimens observed with a given haplotype (ranging from 1 to 10). Breaks in dashed lines represent one mutational step between two haplotypes. Dotted lines are equally parsimonious alternative connections. Letters in bold (Q, R, S, X, Y, Z) identify specific lineage clusters, which are shown within gray fields. The loop around haplotypes in the center of the Beringian clade MSN delineates haplotypes that differ from the center by fewer than five mutational steps.
Figure 2.3. Unrooted NJ trees of all Beringian clade (A) and Central Asian clade (B) individuals based on Jukes-Cantor distances. For the Beringian clade, the position of different subspecies of *M. oeconomus* is indicated. Numbers above branches are bootstrap values based on 5000 replicates.
Figure 2.3 (continued). Unrooted NJ trees of all Beringian clade (A) and Central Asian clade (B) individuals based on Jukes-Cantor distances. For the Beringian clade, the position of different subspecies of *M. oeconomus* is indicated. Numbers above branches are bootstrap values based on 5000 replicates.
Figure 2.4. Hypothesized colonization routes into southcoastal Alaska. The letters Q and R represent the different lineage clusters observed in the MSN (Fig. 2.2), and arrows represent proposed routes of post-glacial expansion. The dashed line indicates the edge of the continental shelf, which may have been exposed briefly following glacial retreat before sea levels rose (Vrba 1995).
Table 2.1. Haplotype \((h)\) and nucleotide \((\pi)\) diversity statistics by population. Column numbers correspond to locality numbers in Figures 2.1 and 2.2.

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<td>0.090</td>
<td>0.132</td>
<td>0.50</td>
<td>0.080</td>
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Cyclical climatic fluctuations during the Pleistocene exerted a strong influence on the historical biogeography of Microtus oeconomus in Beringia. The expansion of tundra voles into the Nearctic was facilitated by lowered sea levels that exposed the Bering Land Bridge during a recent stadial. Glacial advances in Siberia and Alaska periodically destroyed some populations while fragmenting and isolating others. Climate warming during interglacial periods produced a similar effect in central Beringia with the flooding of the Bering Land Bridge, and tundra vole populations elsewhere responded to receding glaciers by expanding into newly ice-free areas. The history of major distributional shifts and local population movements associated with these climate-driven events are recorded in a mosaic of overlapping and hierarchical genetic signatures.

At the deepest level of intraspecific genetic structure, M. oeconomus exhibits a single strong phylogeographic break in the Beringian region, which separates two widespread clades (Central Asian and Beringian). The division between the clades is congruent with probable glacial (Bespaly, 1984; Arkhipov et al., 1986a) and ecological (Grichuk, 1984; Lozhkin and Anderson, 1995) barriers, as well as genetic breaks in codistributed taxa (Fedorov et al., 1999a,b) and independent molecular markers. Timing estimates indicate that differentiation was initiated more than 160 Kya, which implies that isolation was maintained through repeated cycles of climate warming and cooling.
Both glacial and ecological barriers, alternating as the climate fluctuated, probably contributed to producing the observed differentiation.

Climate-induced range shifts have also been implicated in structuring broad-scale patterns of genetic diversity. Serial founder events during post-glacial expansion following stadials may account for a general trend of lower genetic diversity in high latitude populations of temperate species (Hewitt, 1996). Conversely, ecological characteristics of arctic-adapted species may help them to maintain genetic diversity during recolonization of deglaciated regions (Fedorov et al., 1999b). Tundra voles in Beringia, however, do not exhibit the genetic signature of either response because of a strong reduction in genetic diversity that occurred independently of recent glaciations. Timing estimates for this event predate the last glacial maximum, raising the possibility that environmental changes during the last interglacial were responsible for the observed reduction in genetic diversity. Indeed, climate warming during that period was associated with major forest expansions in Beringia that displaced much of the existing tundra (Grichuk, 1984; Ager and Brubaker, 1985; Brigham-Grette and Hopkins, 1995; Lozhkin and Anderson, 1995; Muhs et al., 2001). Such an ecological shift may have led to a reduction in effective population size of Beringian tundra voles, which are most abundant in mesic meadow and tundra ecosystems (Getz, 1985).

Just as Pleistocene climate changes had a significant influence on broad-scale phylogeographic patterns in *M. oeconomus*, localized climate-driven events structured genetic patterns at smaller scales. Population responses to specific events can be traced by examining genealogical relationships among haplotypes, which provide information
on historical patterns of gene flow. Southcoastal Alaska was almost completely engulfed by the Cordilleran ice sheet during the last glacial maximum (Hamilton and Thorson, 1983; Mann and Hamilton, 1995), and post-glacial colonizers could have used a variety of alternative pathways into the region. Genetic affinities among southcoastal populations show that only two routes of entry were probably used, and the major coastal expansion bypassed the coastal mountains to the west and followed the glacial retreat eastward. Similarly, tentative transberingian associations among haplotypes in Siberia and Alaska suggest that prior to the flooding of the land bridge, faunal connections across Beringia may have been partitioned latitudinally. This finding hints at an unexpected biogeographic pattern that may help to reveal the ecological landscape of the land bridge, particularly when examined within the context of recently developed models of Beringian paleoenvironments (Elias et al., 2000; Guthrie, 2001).

The genetic signatures revealed within *M. oeconomus* provide one perspective on organismal responses to Pleistocene climatic events in Beringia. Other phylogeographic studies of Beringian mammals (Fedorov et al., 1999a,b; Hundertmark et al., 2002; Brunhoff et al., in prep), birds (Wenink et al., 1996), and parasites (Hoberg et al., 1999; Wickström et al., 2001) offer additional viewpoints. By integrating these studies within a comparative biogeographic and phylogeographic framework, hypotheses may be articulated regarding the structure and history of Beringian biotic communities and the effects of historical and contemporary climate changes across the Holarctic. By examining specific climatological events and responses by species that represent a broad range of vagilities, reproductive strategies, ecological requirements, and other divergent
life histories, it may be possible to determine generalities that govern the ways in which
different groups of organisms interact with their environment. Beringia, with its varied
climatic history and biogeographic complexity, provides an excellent system for
continuing to explore the interface between species, climate, and geography.
XI. LITERATURE CITED


America and Adjacent Oceans During the Last Deglaciation (W. F. Ruddiman and H.

and faunal evidence for the occurrence of pre-Sangamonian artefacts in northern
Yukon. Arctic, 34:3-33.

Mammalian Protein Metabolism, vol. 3 (H. N. Munro, ed.). Academic Press, New
York, New York, 571 pp.

environments of the Soviet Union (A. A. Velichko, ed.). University of Minnesota
Press, Minneapolis, Minnesota, 327 pp.

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

Klein, D. R. 1965. Postglacial distribution patterns of mammals in the southern coastal

Klicka, J., and R. M. Zink. 1997. The importance of recent Ice Ages in speciation: a

Knowles, L. L. 2001. Did the Pleistocene glaciations promote divergence? Tests of
explicit refugial models in montane grasshoppers. Molecular Ecology, 10:691-701.


