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ORIGINAL ARTICLE

Eastern Beringian biogeography: historical and spatial genetic structure of singing voles in Alaska

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ABSTRACT

Aim Pleistocene climatic cycles have left marked signatures in the spatial and historical genetic structure of high-latitude organisms. We examine the mitochondrial (cytochrome *b*) genetic structure of the singing vole, *Microtus miurus* (Rodentia: Cricetidae: Arvicolinae), a member of the Pleistocene Beringian fauna, and of the insular vole, *Microtus abbreviatus*, its putative sister species found only on the St Matthew Archipelago. We reconstruct the phylogenetic and phylogeographical structure of these taxa, characterize their geographical partitioning and date coalescent and cladogenetic events in these species. Finally, we compare the recovered results with the phylogenetic, coalescent and spatial genetic patterns of other eastern Beringian mammals and high-latitude arvicoline rodents.

Location Continental Alaska (alpine and arctic tundra) and the St Matthew Archipelago (Bering Sea).

Methods We generated and analysed cytochrome *b* sequences of 97 singing and insular voles (*M. miurus* and *M. abbreviatus*) from Alaska. Deep evolutionary structure was inferred by phylogenetic analysis using parsimony, maximum likelihood and Bayesian approaches; the geographical structure of genetic diversity was assessed using analysis of molecular variance and network analysis; ages of cladogenetic and coalescent events were estimated using a relaxed molecular clock model with Bayesian approximation.

Results Regional nucleotide diversity in singing voles is higher than in other high-latitude arvicoline species, but intra-population diversity is within the observed range of values for arvicolines. *Microtus abbreviatus* specimens are phylogenetically nested within *M. miurus*. Molecular divergence date estimates indicate that current genetic diversity was formed in the last glacial (Wisconsinan) and previous interglacial (Sangamonian) periods, with the exception of a Middle Pleistocene split found between samples collected in the Wrangell Mountains region and all other singing vole samples.

Main conclusions High levels of phylogenetic and spatial structure are observed among analysed populations. This pattern is consistent with that expected for a taxon with a long history in Beringia. The spatial genetic structure of continental singing voles differs in its northern and southern ranges, possibly reflecting differences in habitat distribution between arctic and alpine tundra. Our phylogenetic results support the taxonomic inclusion of *M. miurus* in its senior synonym, *M. abbreviatus*.

Keywords

Alaska, Cricetidae, cytochrome *b*, microrefugia, *Microtus abbreviatus*, *Microtus miurus*, phylogeography, Pleistocene glaciation, Rodentia, social structure.

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INTRODUCTION

Most northern high-latitude species display marked historical (phylogenetic and coalescent) and spatial genetic structure that is correlated with Quaternary climatic oscillations (Hewitt, 1996, 2000; Abbott *et al.*, 2000; Abbott & Brochmann, 2003; Lessa *et al.*, 2003; Lister, 2004; Waltari *et al.*, 2007). Among mammals, genetic consequences of glacial–interglacial cycles include highly divergent lineages with deep phylogenetic splits dating to the mid or early Pleistocene (Conroy & Cook, 2000a; Jaarola & Searle, 2002; Arbogast, 2007); geographically isolated haplogroups in refugia or microrefugia from the last (Wisconsinan) glacial period (Bilton *et al.*, 1998; Haynes *et al.*, 2003); low genetic diversity in populations that underwent late glacial or post-glacial bottlenecks (Fedorov & Stenseth, 2001; Hundertmark *et al.*, 2002; Waltari & Cook, 2005); and population growth signatures indicative of recent colonization of previously ice-covered or isolated habitats (Galbreath & Cook, 2004; Piertney *et al.*, 2005). These genetic signatures permit us to test a priori predictions of biogeographical models of evolution.

For the mammals of eastern Beringia – the region that stretches from the Bering Sea to the mountain systems of north-western Canada (St Elias to the south and Mackenzie to the east) and encompasses Alaska, the Yukon Territory and north-western British Columbia (Fig. 1) – genetic patterns, together with ample fossil material (Kurtén & Anderson, 1980; Harrington, 1989; Matheus *et al.*, 2003), indicate a dynamic biogeographical history throughout the Pleistocene (Waltari *et al.*, 2007). Most of eastern Beringia was ice free during periods of glacial maxima; the constant change in ice sheet coverage created different vicariance and dispersal conditions with regard to eastern Siberia and south-western and central Canada (Fig. 1a). Beringia is considered a 'crossroads of the northern continents' (Cook *et al.*, 2004, p. 767) and the cyclical changes in the landscape and ecology created a complex network of biogeographical patterns (MacPherson, 1965; Youngman, 1975; Waltari *et al.*, 2007). At least six such patterns (Fig. 2, Table 1) have been recognized and related to geomorphological and ecological changes during Pleistocene climatic cycles. These patterns helped create the unique mammalian fauna of the region (see Youngman, 1975, and MacDonald & Cook, 2009, for systematic treatments of the mammalian fauna of the Yukon Territory and Alaska, respectively).

Mammals restricted to forests and woodlands of eastern Beringia (E Beringia), especially in southern Alaska and the Yukon Territory and westernmost British Columbia, are thought to be recent immigrants from areas to the south of the Laurentide and Cordilleran ice fields (referred to here as sub-Laurentidean North America). Species such as the northern flying squirrel (*Glaucomys sabrinus*; Arbogast, 1999, 2007), the wolverine (*Gulo gulo*; Kyle & Strobeck, 2001), the long-tailed vole (*Microtus longicaudus*; Youngman, 1975), the southern red-backed vole (*Myodes gapperi*; Runck & Cook, 2005), the north-western deermouse (*Peromyscus keeni*; Zheng

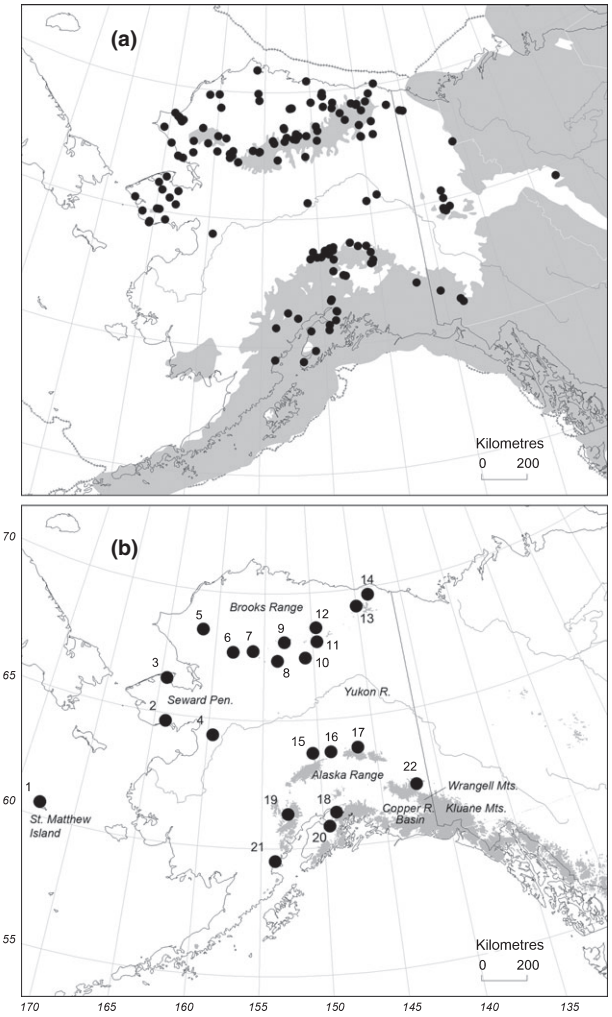

Figure 1 Map of eastern Beringia showing the distribution and sampling localities of singing voles and geographical features mentioned in the text. (a) Present distribution of singing voles overlaid with the maximum extent of glacial ice (grey) during the Last Glacial Maximum *c.* 18 ka (Manley & Kaufman, 2002). Empirical locality points are from all known specimens of *Microtus miurus* from the Arctos (http://arctos.database.museum) and MaNIS (http://manisnet.org) databases (accessed May 2008) and from specimens at the Canadian Museum of Nature. Voucher specimens from outlier localities (outside the core distribution of singing voles or in taiga-dominated habitats) were inspected for taxonomic identification (e.g. exemplars from the Plains of Abraham in the Northwestern Territories, the westernmost point on the map). (b) Sampled populations of *M. abbreviatus* (locality 1) and *M. miurus* (localities 2–22) used in the present study. The grey shading indicates the current extent of glaciers and ice fields. See Appendix S1 for additional locality and specimen information.

et al., 2003; Lucid & Cook, 2004) and the red squirrel (*Tamiasciurus hudsonicus*; Arbogast *et al.*, 2001) are not found in deposits from glacial intervals in E Beringia, have a shallow (i.e. coalescent) genetic structure within the region, and have low genetic (e.g. haplotype) variation. Eastern Beringian samples form monophyletic or paraphyletic groups, because

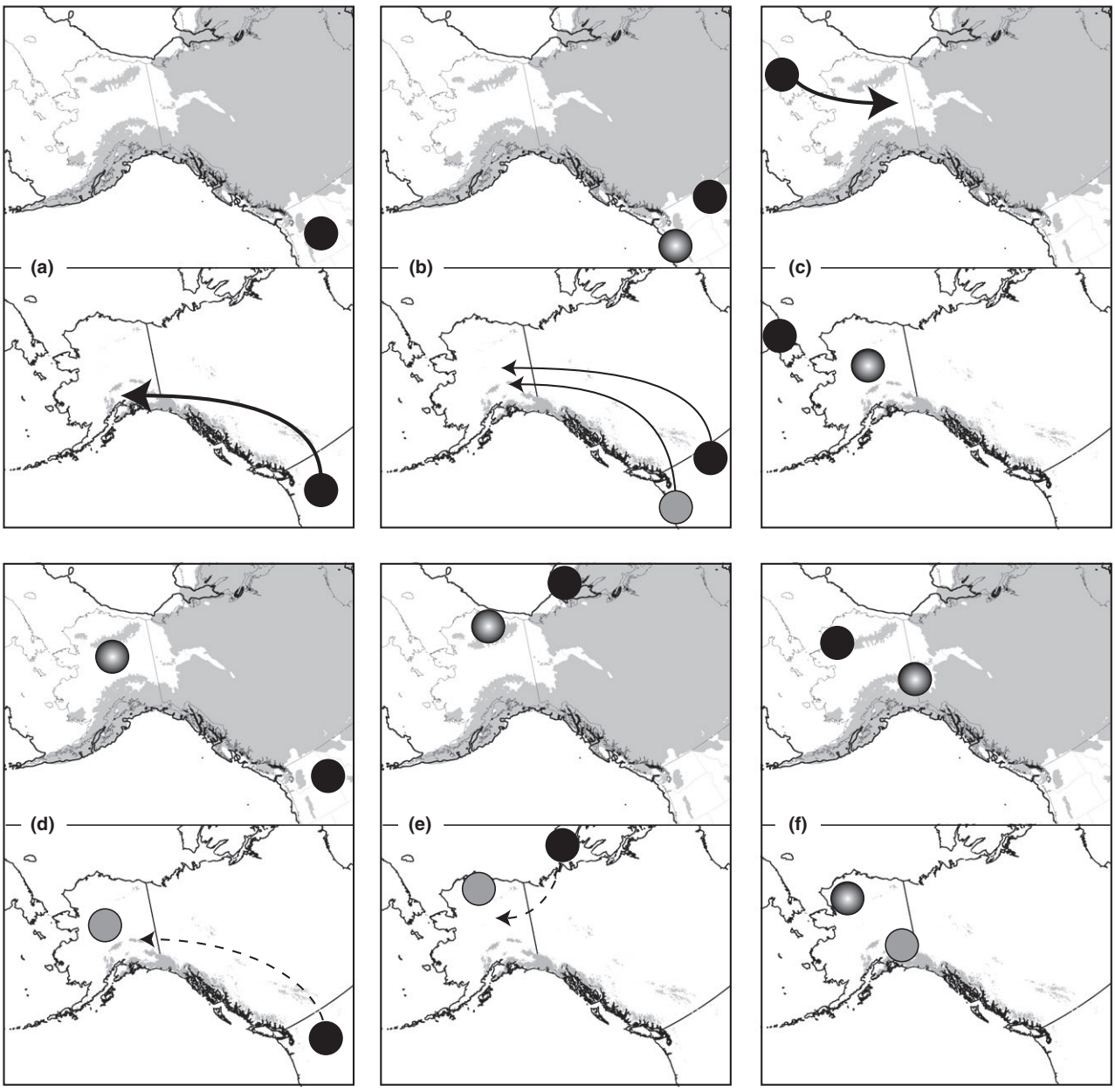

Figure 2 Biogeographical patterns observed among eastern Beringian mammals. Each pair of maps represents glacial (upper) and interglacial (lower; e.g. present times) periods. Grey areas represent ice-covered areas (Manley & Kaufman, 2002); the maximum glacial coastline of the Beringian Sea is approximated using the –100 m bathymetric line. (a, b) Single (a) or double (b) Holocene migration from sub-Laurentidean species (i.e. taxon not present in Alaska during last interglacial). (c) Mid or late Pleistocene migration from western Beringia (Siberia), when sea levels were *c.* 100 m below current levels. Western and eastern Beringian populations are currently isolated, creating allopatric conditions. (d, e) Pleistocene vicariance during previous glacial maximum conditions, when Beringian populations were isolated from sub-Laurentidean (d) or high-arctic (e) populations. Dispersion of formerly isolated populations could potentially lead to secondary contact zones. (f) Eastern Beringian *in situ* differentiation due to local processes, such as microrefugia, habitat fragmentation or isolation-by-distance, caused during glacial or interglacial (or both) periods. Different circle shadings correspond to different species or lineages; the concentric gradient circles refer to putative speciation events.

in some cases there was no time for complete lineage sorting (Fig. 2a, Table 1).

Alternatively, some recent immigrant taxa, such as the dusky shrew (*Sorex monticolus*; Demboski *et al.*, 1999; Demboski & Cook, 2001), the American marten (*Martes americana*; Stone *et al.*, 2002) and the American black bear (*Ursus americanus*; Byun *et al.*, 1997; Stone & Cook, 2000), display deeper phylogenetic structure with regard to Alaskan samples, suggesting that more than one sub-Laurentidean group migrated north (Fig. 2b, Table 1). For these taxa, two monophyletic groups are generally recognized and genetically associated with coastal and continental clades from sub-Laur-

Table 1 Biogeographical patterns of Alaskan mammals (see Fig. 2) and predictions for phylogeographical and population genetic estimates. Unless otherwise stated, predicted values are for eastern Beringian samples (Alaska, Yukon Territory and north-westernmost British Columbia; see Fig. 1). Some of the observed patterns are not mutually exclusive (e.g. ermine and caribou).

Pattern	Taxa	Habitat and distribution	*h*	TMRCA	Evolutionary structure (E Beringia)	Speciation*	References
Recent sub-Laurentidean colonization	*Glaucomys* spp. *Gulo gulo* *Marmota caligata* *Microtus longicaudus* *Mustela erminea* *Myodes gapperi* *Peromyscus keeni* *Tamiasciurus hudsonicus*	Forest, woodland; Nearctic	Low	Holocene	Coalescent; monophyletic or paraphyletic	No	Youngman (1975), Arbogast (1999), Steppan *et al.* (1999), Arbogast *et al.* (2001), Kyle & Strobeck (2001), Zheng *et al.* (2003), Lucid & Cook (2004), Runck & Cook (2005), Arbogast (2007)
Recent double colonization (coastal/continental clades)	*Martes americana* *Sorex monticolus* *Ursus americanus*	Forest, woodland; Nearctic	Low	Holocene†	Phylogenetic overall; polyphyletic	Yes (sub-Laurentidean)	Byun *et al.* (1997), Demboski *et al.* (1999), Stone & Cook (2000), Demboski & Cook (2001), Stone *et al.* (2002)
E Asia colonization (W Beringia)	*Alces americanus* *Microtus oeconomus* *Myodes rutilus* *Mustela erminea*	Forest, woodland, tundra; Holarctic	Low	Late Pleistocene	Coalescent; monophyletic or paraphyletic	No	Fleming & Cook (2002), Hundertmark *et al.* (2002), Iwasa *et al.* (2002), Brunhoff *et al.* (2003), Hundertmark *et al.* (2003), Cook *et al.* (2004), Galbreath & Cook (2004), Kurose *et al.* (2005), Brunhoff *et al.* (2006)
Vicariance between Beringian and sub-Laurentidean populations	*Canis lupus* *Bison bison athabascae* *Lemmus trimucronatus* *Mustela erminea*‡ *Peromyscus* spp. *Rangifer tarandus* *Sorex cinereus*	Woodland, tundra; Nearctic or Holarctic	High	Pleistocene	Phylogenetic overall; monophyletic or paraphyletic§	Only in pre-Wisconsinan vicariance events	Gravlund *et al.* (1998), Fedorov & Goropashnaya (1999), Vila *et al.* (1999), Fleming & Cook (2002), Demboski & Cook (2003), Fedorov *et al.* (2003), Flagstad & Røed (2003), Shapiro *et al.* (2004), Kurose *et al.* (2005), Weckworth *et al.* (2005), Lucid & Cook (2007)
Vicariance between Beringia and high-arctic populations	*Dicrostonyx groenlandicus* *Lepus* spp.	Tundra, arctic desert; Circumpolar	Low to high	Pleistocene	Phylogenetic; monophyletic	Possible but not necessary	Ehrich *et al.* (2000), Fedorov & Stenseth (2002), Waltari & Cook (2005)

Table 1 Continued

Pattern	Taxa	Habitat and distribution	*h*	TMRCA	Evolutionary structure (E Beringia)	Speciation*	References
Intra-Beringian differentiation	*Mammuthus primigenius* *Ovis dalli* *Rangifer tarandus* *Spermophilus parryii* *Ursus arctos* *Ursus maritimus*	Tundra, woodland; E Beringia endemic or Holarctic	High	Pleistocene	Phylogenetic and coalescent; monophyletic	Possible but not necessary	Talbot & Shields (1996), Gravlund *et al.* (1998), Barnes *et al.* (2002), Flagstad & Røed (2003), Eddingsaas *et al.* (2004), Worley *et al.* (2004), Barnes *et al.* (2007)

TMRCA, time to the most recent common ancestor; *h*, haplotype diversity.
*Speciation is defined here as the cladogenetic event leading to two or more contemporary clades recognizable as separate species (e.g. Sites & Marshall, 2004; Templeton, 1989).
†For each Alaskan haplogroup.
‡Vicariance of isolated population in putative Haida Gwaii refugium.
§In cases of reinvasion (secondary contact) into Beringia (e.g. brown lemming, ermine).

entidean North America; overall haplotype diversity is low, and the coalescent for each monophyletic group is estimated to date to the Holocene.

A second source of mammalian immigrants, during glacial periods, is from Asia (western Beringia). Several mammals found in tundra, woodland and forest areas, such as the root vole (*Microtus oeconomus*; Brunhoff *et al.*, 2003, 2006; Galbreath & Cook, 2004), the northern red-backed vole (*Myodes rutilus*; Iwasa *et al.*, 2002; Cook *et al.*, 2004) and the moose (*Alces americanus*; Hundertmark *et al.*, 2002, 2003), migrated eastward from Siberia and northernmost East Asia during the last glacial period of open Russia–Alaska connectivity (Fig. 2c, Table 1). These taxa have medium levels of overall genetic diversity and shallow phylogenetic structure within E Beringia, but the estimate of the time to the most recent common ancestor (TMRCA) for each taxon in E Beringia is for the Pleistocene, and their inter- and intra-population nucleotide diversity is higher than those of Holocene migrants. Some of these taxa also reveal marked levels of population growth, occupying areas that were previously glaciated. Waltari *et al.* (2007) can be consulted for a review of mammals and parasites that exhibit this pattern.

A third general pattern is found in cases where eastern Beringian populations form a deep and independent lineage from other North American populations, either from south of the Cordilleran and Laurentidean ice sheets or from high-arctic refugia. The first case (Fig. 2d) is exemplified by several mammals of the interior woodlands and tundra, such as the masked shrew (*Sorex cinereus*; Demboski & Cook, 2003), the ermine (*Mustela erminea*; Fleming & Cook, 2002; Kurose *et al.*, 2005), the extinct Alaskan steppe bison (*Bison priscus*; Shapiro *et al.*, 2004), the wolf (*Canis lupus*; Vila *et al.*, 1999; Weckworth *et al.*, 2005), the brown lemming (*Lemmus trimucronatus*; Fedorov *et al.*, 2003) and the caribou (*Rangifer tarandus*; Gravlund *et al.*, 1998; Flagstad & Røed, 2003). The second case (Fig. 2e) is observed among mammals of the northern tundra and arctic desert, such as the collared lemming (*Dicrostonyx groenlandicus*; Fedorov & Goropashnaya, 1999; Ehrich *et al.*, 2000; Fedorov & Stenseth, 2002) and the arctic hare (*Lepus arcticus*; Waltari & Cook, 2005). In some cases, isolation during past glacial maxima resulted in allopatric speciation conditions during the mid or early Pleistocene (e.g. Demboski & Cook, 2003; Lucid & Cook, 2007); in other cases, introgression or secondary contact of previously isolated populations followed retreat of the ice sheet after the Last Glacial Maximum (Fedorov *et al.*, 2003).

A final major pattern is that of *in situ* diversification (Fig. 2f), in which deeply divergent lineages and high levels of phylogeographical structure are observed within E Beringia. This pattern is observed in the phylogenetic and spatial genetic structure of several arctic and sub-arctic species in E Beringia, such as the arctic ground squirrel (*Spermophilus parryii*; Eddingsaas *et al.*, 2004), Dall's sheep (*Ovis dalli*; Worley *et al.*, 2004), the extinct woolly mammoth (*Mammuthus primigenius*; Barnes *et al.*, 2007), the caribou (Gravlund *et al.*, 1998; Flagstad & Røed, 2003) and both brown (*Ursus arctos*) and

polar (*Ursus maritimus*) bears (Talbot & Shields, 1996; Barnes *et al.*, 2002). The genetic structure of these species, members of the Pleistocene Beringian fossil fauna (Kurtén & Anderson, 1980; Harrington, 1989; Matheus *et al.*, 2003), was shaped *in situ* through the putative processes of microrefugial vicariance, isolation-by-distance and distributional shifts during the Pleistocene (and even earlier).

We present here an assessment of the genetic structure of a typical member of this discrete biogeographical area, the singing vole, *Microtus miurus* Osgood, 1901. One of the most abundant species in Pleistocene eastern Beringian fossil assemblages (Repenning *et al.*, 1964; Kurtén & Anderson, 1980; Jopling *et al.*, 1981; Harrington, 1989; Matheus *et al.*, 2003), this species currently inhabits alpine and arctic tundra landscapes of Alaska and north-western Canada which were extensively covered with ice fields and glaciers during past glacial periods (Fig. 1a). Cranial fragments of *M. miurus* (or forms undifferentiated from it) are found in Pleistocene fossil sites throughout Alaska and the Yukon Territory (Guthrie, 1968; Youngman, 1975) and in late Wisconsinan sites in the midwestern United States (Baker *et al.*, 1986; Semken & Falk, 1987; Woodman *et al.*, 1996), indicating that singing voles survived both in E Beringia and in glacial enclaves south of the Laurentidean ice sheet during the Last Glacial Maximum. We examine the geographical and historical structure of the genetic variation of singing voles in Alaska using the mitochondrial cytochrome *b* gene, and employ a series of estimation techniques and statistical tests from the phylogenetic–phylogeographic–population genetics continuum (Emerson *et al.*, 2001; Arbogast *et al.*, 2002; Carstens *et al.*, 2004). We include samples of the insular St Matthew vole, *Microtus abbreviatus* Miller, 1899, the presumed sister species of *M. miurus* (see Conroy & Cook, 2000b; Jaarola *et al.*, 2004; Triant & DeWoody, 2007), which is highly similar in external morphology, karyotype and singing and social behaviour (Rausch & Rausch, 1968). These similarities and low levels of mitochondrial DNA (mtDNA) sequence divergence have led some authors to suggest that only one species be recognized (e.g. Conroy & Cook, 2000b). Finally, we compare the recovered estimates of genetic diversity with that of other arvicoline rodents in order to assess the effects of the singing vole's unique ecological and social characteristics on its genetic structure (Slatkin, 1994; Storz, 1999). Singing voles are habitat specialists living in semi-colonial demes with patchy distribution and limited movement among demes (Bee & Hall, 1956; Douglass, 1984; Wolff, 1985; Batzli & Henttonen, 1990, 1993; Batzli & Lesieutre, 1995). At the southern extent of its range, *M. miurus* exists on 'islands' of alpine tundra, above the tree line (around 300–500 m) and below glaciers and ice fields (Heusser, 1983; Pojar & MacKinnon, 1994), in the mountain systems of southern Alaska and the Yukon Territory (e.g. the Alaska and Aleutian ranges, Wrangell–St Elias system). These ecosystems harbour unique communities of small mammals that appear highly adapted to changing climatological and environmental conditions. Understanding their evolutionary patterns might give us clues to the biological consequences of current global warming trends.

MATERIALS AND METHODS

Specimen sampling

Specimens were chosen to maximize geographical coverage of the current distribution of the species (Fig. 1b). The mitochondrial cytochrome *b* gene was sequenced for 95 specimens of *M. miurus* and *M. abbreviatus* collected by University of Alaska Museum (UAM) expeditions from 1992 to 2006. The two available sequences of *M. miurus* and *M. abbreviatus* generated by Conroy & Cook (2000a) were added to the dataset. All specimens are housed in the UAM Mammal Collection. Twenty-two localities in Alaska were included, with sample sizes varying from 1 to 14 per locality (see Appendix S1 in Supporting Information for locality information, catalogue numbers and GenBank accessions). Complete voucher information can be found on the University of Alaska Museum's online database (http://arctos.database.museum). Species identification was confirmed by morphological examination of voucher material conducted by the first author.

Molecular techniques

Genomic DNA was isolated from tissue samples preserved in ethanol using the DNeasy (Qiagen, Valencia, CA, USA) or Puregene (Gentra Systems, Minneapolis, MN, USA) extraction kits. A fragment containing the complete cytochrome *b* gene (1143 bp) was amplified with the primers Mus14095 and Mus15398, both from Anderson & Yates (2000), using standard polymerase chain reaction (PCR) procedures. Amplifications were performed in 20-μL reactions using AmpliTaq Gold polymerase Master Mix (Perkin-Elmer) and recommended concentrations of primers and templates. Reactions were subjected to 30–35 PCR cycles of denaturation at 94 °C for 20 s, annealing at 61 °C for 15 s and extension at 72 °C for 60 s. After purification, PCR products were directly sequenced with the same primers used in the PCR amplification and additional internal primers: L14648 and Cytb.LS (Irwin *et al.*, 1991) and miu-f1 (5′–GACTAATACGAGGGCGGTGA-3′; this study). Nucleotide sequences were determined using ABI 3100 or ABI 3130xl automated sequencers (Applied Biosystems, Foster City, CA, USA). At least four reads (with the two external and two inversely oriented internal primers) were obtained for each specimen. All resulting sequences have been deposited in GenBank (accession numbers GU809077–GU809171) and linked to their individual voucher records in UAM's database.

The resulting cytochrome *b* sequences were aligned with reference to the translated amino acid sequence. No indels, internal stop codons or contamination signals were detected (unorthodox sequences were double-checked with additional extractions and/or amplifications). Final sequences were subjected to Blast searches, and all matched *M. miurus*

and/or *M. abbreviatus* sequences deposited in GenBank. The terminal stop codon was removed for the analyses. The resulting alignment (1140 bp) is available in Appendix S2.

Phylogenetic analyses

Sequences were subjected to phylogenetic analysis using parsimony, maximum likelihood (ML) and Bayesian approaches. The GTR model (Rodríguez *et al.*, 1990), corrected for site-specific rate heterogeneity using a gamma distribution with four classes (Yang, 1994) and for invariant sites (Hasegawa *et al.*, 1985), was used in all likelihood-based analyses, based on Akaike information criterion (AIC) contrasts (Δ_i = 38 for the second-best model, HKY85; likelihoods were estimated on an uncorrected neighbour-joining tree). Adding parameters for rate variability and frequency of invariants steeply increased the likelihood by 353 (Δ_i = 708 for G+I). Initial base frequencies were computed from the data.

Phylogenetic trees were rooted with six outgroup taxa based on the available phylogenies of the genus *Microtus* (Conroy & Cook, 2000b; Jaarola *et al.*, 2004; Robovský *et al.*, 2008). Five species variously recovered as sister species to the singing vole are included here: *Microtus socialis* (GenBank accession AY513829), *Microtus pinetorum* (AF163904), *Microtus ochrogaster* (AF163901), *Microtus multiplex* (AY513815) and *Microtus xanthognathus* (AF163907); *Microtus oeconomus* (AY305186) was included as a more distant outgroup. The analyses were conducted with unconstrained ingroup and outgroup designations (Nixon & Carpenter, 1993), and trees were subsequently rooted with *M. oeconomus*.

Parsimony analysis was implemented using PAUP* 4.0b10 (Swofford, 2001) with informative characters treated as unordered and equally weighted. The heuristic search option was implemented, with 1000 random addition replicates and tree bisection–reconnection (TBR) branch swapping. Only nodes with at least one unambiguous synapomorphy were retained (options PSET COLLAPSE = MINIMUM, and FILTER BEST in PAUP*). Bootstrap values (Felsenstein, 1985) for the parsimony analysis were calculated using 1000 pseudoreplicates with heuristic searches employed within each replicate (five random addition replicates, TBR branch swapping, no more than 500 trees saved per replicate).

In the ML approach implemented in PAUP*, a neighbour-joining tree calculated from uncorrected nucleotide distances was used to estimate initial parameters for the base substitution matrix, the rate heterogeneity parameters (α) and the proportion of invariant sites (p_{inv}). ML tree searches were conducted using the heuristic search option in PAUP*, in which the initial parameter values were fixed and a random tree was used as a starting point for TBR branch swapping. The resulting parameter estimates from the ML tree were used in a subsequent round of branch swapping to provide another ML tree estimate; this stepwise cycle was repeated until likelihood values for the best trees did not change at all, indicating that likelihood estimation reached a plateau for both tree and substitution parameters. Final parameter values were estimated on this tree. Bootstrap support values for nodes in the ML tree were estimated based on 200 pseudoreplicates using the subtree pruning–regrafting (SPR) algorithm for branch swapping on starting trees obtained by neighbour joining and with parameters recovered from the full ML analysis.

Bayesian analyses were performed using Markov chain Monte Carlo (MCMC) sampling as implemented in MrBayes 3.1 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). Uniform interval priors were assumed for all parameters except base composition, for which we assumed a Dirichlet prior. We performed four independent runs of 10,000,000 generations each with 10 heated chains, sampling for trees and parameters every 10,000 generations. The first 2,500,000 generations were discarded as burn-in, and the remaining trees were used to estimate posterior probabilities for each node. All analyses were checked for convergence by plotting the log-likelihood values against generation time for each run using Tracer 1.4 (Rambaut & Drummond, 2007). All parameters have effective sample sizes over 200.

Date estimation

Divergence dates were estimated through MCMC sampling using BEAST 1.4.3 (Drummond & Rambaut, 2007). Analyses incorporated previous information on substitution rates, molecular divergence rates and fossil dates as priors (see below). Posterior likelihoods were estimated for trees under two sets of conditions (tree models): a birth–death model (Alfaro & Holder, 2006; Gernhard, 2008), which estimates dates of cladogenetic events (phylogenetic nodes), and a constant-population coalescent model (Drummond *et al.*, 2002), which estimates dates of coalescence events (population nodes). The coalescent model used the same set of specimens as the population-level analyses (core singing voles; see below). Taxon sampling for the birth–death model encompassed all species of *Microtus* available in GenBank (one sequence per species; alignment available in Appendix S2) and a sample of singing voles from each deeper clade of singing voles (see below). Both speciation and coalescent analyses employed the GTR+Γ(4) nucleotide substitution model and a relaxed molecular-clock model (Thorne *et al.*, 1998) that assumed uncorrelated (lognormal) distribution of rates among branches. Uniform or Jeffreys priors were assumed for accessory parameters, with initial values and range determined by the program; settings were optimized in successive runs using suggested values. MCMC sampling was performed using multiple runs of 20,000,000 generations, sampling each 5000th state, after a pre-burn-in of 2,000,000 generations and a burn-in of 10%. Marginal distributions of the TMRCA were estimated using Tracer.

Priors for the substitution rate (μ) were based on previous estimates of substitution or divergence rates for three arvicoline genera: *Microtus*, *Myodes* and *Lemmus* (Conroy & Cook, 2000b; Fedorov & Stenseth, 2001, 2002; Fedorov *et al.*, 2003; Galbreath & Cook, 2004; Luo *et al.*, 2004). These estimates were all based on corrected nucleotide divergence divided

by fossil ages, and average 0.066 substitutions site^{-1} Myr^{-1} (divergence rate Myr^{-1} = 13.1%), ranging from 0.038 (*Lemmus*; Galbreath & Cook, 2004) to 0.093 (*Microtus*; Triant & DeWoody, 2007) substitutions site^{-1} Myr^{-1}. We approximated these values using a gamma distribution with shape parameter (a) of 10 and scale parameter (b) of 0.006 for the substitution rate prior (=ucld.mean). The 95% prior interval (0.029–0.103 substitutions site^{-1} Myr^{-1}) encompasses the mitochondrial silent substitution rate estimated for mammals (Brown *et al.*, 1979, 1982; Irwin *et al.*, 1991; Li *et al.*, 1996) and is in accordance with values used in studies of other rodents (Arbogast, 1999; Arbogast *et al.*, 2001; Haynes *et al.*, 2003; but see the much lower values used in Smith & Patton, 1993; Lessa & Cook, 1998; Martin *et al.*, 2000; Eddingsaas *et al.*, 2004; and Deffontaine *et al.*, 2005).

Direct fossil information was also incorporated in the birth–death model. In addition to the prior on substitution rates, hard bounds for the tree root prior (uniform) were set to 1.2 million years ago (Ma), which is the age of the earliest fossil record of a member of crown (modern) *Microtus* (Repenning *et al.*, 1990; Maul & Markova, 2007), and 2.5 Ma, the age of the earliest fossils of the closely related *Allophiaomys*, which is the putative sister group (or most closely related paraphyletic assemblage) of modern *Microtus* (Martin *et al.*, 2000; Zheng & Zhang, 2000; Maul & Markova, 2007).

Population genetics parameters

Numbers of haplotypes, segregating sites and nucleotide diversity were calculated using Arlequin 2.0 (Schneider *et al.*, 2000). We assessed the genetic variation at different geographical and phylogenetic levels: (1) within populations from single localities with a sample size > 5; (2) within major geographical regions (south and north of the Yukon River); (3) within haplogroups recovered in the phylogenetic and network analyses; and (4) for the whole species (excluding the distant Wrangell Mountains sample; see below). The K2P-corrected (Kimura, 1980) nucleotide diversity estimates (π) were compared with values recovered in samples from similar geographical ranges of other high-latitude arvicoline rodents: *Microtus agrestis*, *Microtus arvalis*, *M. longicaudus*, *M. oeconomus*, *Myodes gapperi* and *Myodes glareolus* (Conroy & Cook, 2000a; Brunhoff *et al.*, 2003; Haynes *et al.*, 2003; Galbreath & Cook, 2004; Jaarola *et al.*, 2004; Deffontaine *et al.*, 2005; Runck & Cook, 2005). These temperate or subarctic species have less structured social systems than *Microtus miurus* (see Wolff, 1985), and their analysis may shed light on the potential effects of social structure on population genetic parameters. Differences between *M. miurus* and other arvicolines were tested using ANOVA and a one-tailed Dunnett test (Zar, 1999). We use π as an estimator of θ (Gillespie, 2004) because it is estimated in the majority of published analyses.

Spatial distribution of genetic variation was assessed by fixation indices (Wright, 1965, 1977) and analysis of molecular variance (AMOVA; Weir & Cockerham, 1984). The partition of genetic variation was computed among localities with $n > 5$ and similar geodesic distances in the Brooks Range (localities 5, 9, 13) and south-central Alaska (18–20). Significance levels of the recovered variance components were calculated in Arlequin using permutations of 10,000 pseudoreplicates (Excoffier *et al.*, 1992).

Mantel permutation tests (Mantel, 1967) were employed to test for significant correlation between geographical and genetic distances, as predicted by isolation-by-distance models (Sokal *et al.*, 1997). Average nucleotide divergence and geographical distance were calculated for all pairs of localities with five or more samples. The null hypothesis of no correlation between geography and genetic matrices was tested with 10,000 permutations.

To infer the presence of a population growth signal, the number of pairwise nucleotide differences among haplotypes was calculated, and the expected mismatch distribution under a model of recent population expansion (Rogers & Harpending, 1992; Schneider & Excoffier, 1999) was estimated with 10,000 bootstrap pseudoreplicates.

RESULTS

Fifty-seven haplotypes were identified among the 97 sequenced specimens, with 137 segregating sites and 96 parsimony-informative characters. Most haplotypes are restricted to a single collecting locality; only two haplotypes are shared among localities in the central Brooks Range (localities 9 and 10 in Fig. 1b) and Arctic National Wildlife Refuge (ANWR; localities 13 and 14). The maximum number of individuals per haplotype is seven (from St Matthew Island, 1), but 63% of haplotypes are singletons. Average K2P genetic divergence among localities was 1.5% and varied from 0.1% (Philip and Koyukuk, 10 and 11) to 5.3% (Anchorage and Wrangell Mountains, 18 and 22). Specimens from the Wrangell Mountains have a minimum of 4.2% sequence divergence from all other specimens.

Phylogenetic analyses

Results of parsimony, maximum likelihood and Bayesian analyses are very similar in topology and nodal support: insular (*M. abbreviatus*) and continental singing voles (*M. miurus*) are not reciprocally monophyletic, but instead a deep split (Fig. 3) among singing voles is recovered, with the two specimens from the Wrangell Mountains (locality 22) being basal to all other haplotypes, including those of *M. abbreviatus*. The ingroup (*M. miurus* and *M. abbreviatus*) is recovered as monophyletic with strong support, but sister-group relationships between singing voles and other *Microtus* species could not be determined. The root of core singing voles (i.e. excluding the Wrangell Mountains sample) is placed within samples of the Brooks Range, which are paraphyletic at the base. Support for this placement, however, is very low, and branch lengths are very short in this region of the tree.

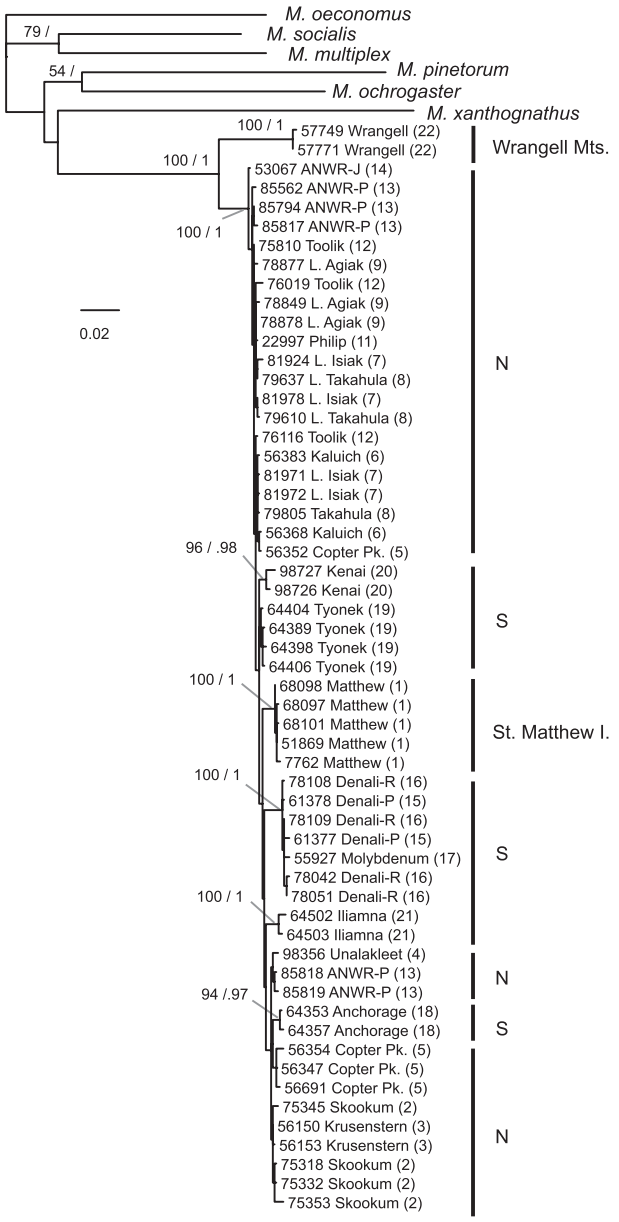

Figure 3 Phylogenetic tree of singing voles (*Microtus miurus* and *M. abbreviatus*) recovered under the maximum likelihood (ML) criterion. Nodal support is indicated for likelihood bootstrap (>50%) and posterior probability (>0.95). Parameters for the model are: substitution matrix = (A ↔ C = 0.58, A ↔ G = 6.21, A ↔ T = 0.43, C ↔ G = 1.9×10^{-7}, C ↔ T = 5.79, G ↔ T = 1), nucleotide frequencies = (0.32, 0.33, 0.11, 0.24), PInv = 0.57, alpha = 0.76. Branch lengths are proportional, and the scale is on ML distance units. Terminals are one representative per haplotype, identified by University of Alaska Museum (UAM) catalogue number, locality code (see Appendix S1), and locality number (see map in Fig. 1). Samples from north and south of the Yukon River are indicated by N and S, respectively.

Four major clades with long branches and strong support are recovered within the remaining singing voles. Two clades contain haplotypes from single populations, St Matthew Island (locality 1) and Iliamna (21), while a third clade is also geographically restricted, containing specimens from the central Alaska Range (15–17); in contrast, the last clade is composed of haplotypes from the Seward Peninsula (2 and 3), Unalakleet (4) and south-central Alaska (Anchorage, 18), as well as two haplotypes from the northernmost populations of the Brooks Range (Copter Mountain, 5; ANWR, 13 and 14). Although nodal support is low for relationships among clades, the support is very high for each of these clades (Fig. 3).

Maximum likelihood estimation of branch lengths indicates that the distance between the Wrangell Mountains specimens and remaining singing voles is much higher than between any other pairwise singing vole contrast (Fig. 3). The branch length leading to any individual haplogroup is always much longer than branches connecting any other pair of nodes, and very long branches are observed in the clades with restricted geographical range (Iliamna, St Matthew, Alaska Range). The branches connecting the haplogroups are very short.

Network

The haplotype network of core singing voles (Fig. 4) contains five groups with between one and nine putative unique fixed nucleotide substitutions; four of the five haplogroups correspond to clades recovered in the phylogenetic analyses: St Matthews Island, Alaska Range, Iliamna and Seward clades (Fig. 3). The last group comprises the paraphyletic assemblage at the base of the phylogenetic tree and is composed primarily of specimens from the Brooks Range, plus south-central samples of Tyonek and Kenai.

TMRCA dating

Median age estimates (Fig. 5) for coalescence of the haplogroups recovered in the network analysis ranged from 84,000 years ago [84 ka; 95% highest posterior density (HPD) = 33–169; Seward clade] and 93 ka (41–185; Brooks clade) for the large haplogroups, to 23 ka (5–53; St Matthew Island), 36 ka (8–86; Iliamna) and 41 ka (12–90; Alaska Range) for the smaller clades. The age of divergence between the Wrangell Mountains sample and the remaining singing voles, as estimated using the speciation model, is 435 ka (95% HPD = 254–651).

Population genetic parameters

Levels of genetic diversity within singing vole populations (Table 2) are in the range observed for other arvicoline species (Fig. 6), and are not statistically different from them ($F = 1.2$, $P = 0.3$). The samples from St Matthew Island and the Alaska Range (Denali) show lower levels of genetic diversity than populations from the Brooks Range (L. Isiak) and Seward Peninsula (Skookum). Intra-haplogroup nucleotide variation in singing voles also falls within the range observed for other arvicoline species; only *Microtus longicaudus* has significantly lower genetic diversity when compared with other species ($F = 5.8$, $P = 0.002$). In contrast, the diversity within the

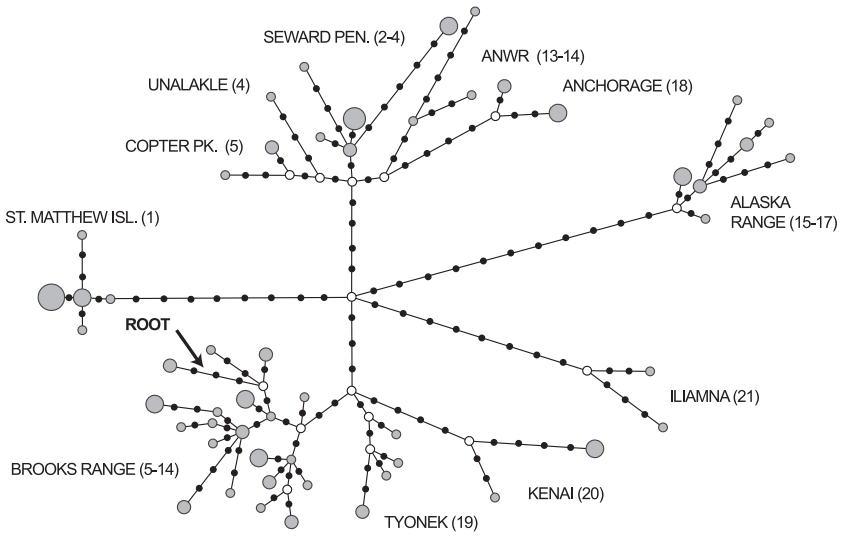

Figure 4 Network diagram for core singing vole (*Microtus miurus* and *M. abbreviatus*) haplotypes (excluding the Wrangell Mountains sample) as recovered by parsimony analysis (consensus of 18 cladograms, tree length = 712, consistency index = 0.51; retention index = 0.69). Grey circles represent haplotypes and are proportional to their frequency, while black circles denote substitutions. The position of the subtree root recovered in the phylogenetic analyses is indicated. See Appendix S1 and the map in Fig. 1 for additional locality information.

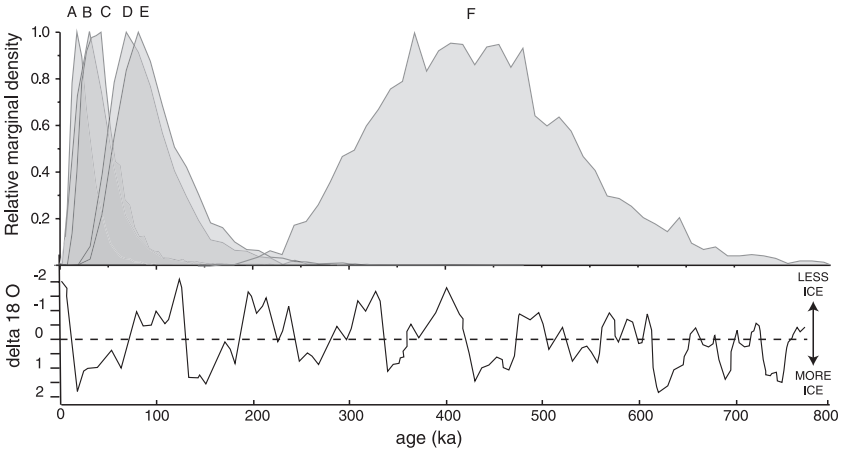

Figure 5 Pleistocene ice distribution (bottom) and Bayesian estimation (top) of the time to the most recent common ancestor (TMRCA) of singing voles (*Microtus miurus* and *M. abbreviatus*) haplogroups (A–E) and of the cladogenetic event between the Wrangell Mountains sample and remaining singing voles (F). Most recent common ancestors of haplotype groups were estimated using coalescent tree priors (constant population size), while the deeper bifurcation was estimated using a birth–death model; both analyses used a relaxed (lognormal) molecular clock. The TMRCA marginal distributions are for St Matthew Island (A), Iliamna (B), Alaska Range populations (C), Seward clade (D) and Brooks clade (E). The oxygen isotopic curve (δ^{18}O) is plotted as proxy for global ice distribution (data from the SPECMAP project: http://www.ngdc.noaa.gov/mgg/geology/specmap.html). Positive values indicate more extensive ice coverage; negative values indicate less ice.

northern and southern regions of the species' range is significantly higher than of other arvicolines (ANOVA $F = 6.18$, $P = 0.02$; Dunnett's test $P < 0.05$). Southern populations of singing voles, in particular, display an extremely high level of regional genetic diversity ($\pi = 1.4\%$), almost twice that found in similar geographical ranges of other species.

Fixation indices also reveal higher levels of genetic structure for southern populations ($F_{ST} = 0.87$) than for northern populations ($F_{ST} = 0.58$); both distributional groups have high levels of genetic structure as revealed by the AMOVA (permutation $P < 0.0001$ for both groups). The correlation coefficient (r) between cells in the genetic and patristic (geographical) distances matrix was 0.40. The Mantel test rejected the null hypothesis of random covariation ($P = 0.0012$), indicating putative isolation-by-distance processes. Correlations are even higher when contrasts are made

Table 2 Estimates of genetic parameters at various geographical levels in singing voles (*Microtus miurus* and *M. abbreviatus*). Estimates for all singing voles exclude Wrangell Mountains (sample 22) and include St Matthew Island (1). Northern and southern geographical areas are separated by the Yukon River (and also exclude the Wrangell Mountains). Locality numbers are given in parentheses.

Geographical sample	*N*	*H*	*S*	π
Populations				
St Matthew Is. (1)	14	5	5	0.10 ± 0.08
Skookum (2)	10	4	11	0.35 ± 0.21
L. Isiak (7)	10	4	10	0.39 ± 0.24
L. Agiak (9)	5	3	5	0.23 ± 0.17
ANWR-P (13)	5	5	19	0.88 ± 0.57
Denali-R (16)	8	4	5	0.18 ± 0.13
Anchorage (18)	5	2	3	0.16 ± 0.13
Tyonek (19)	5	4	6	0.25 ± 0.18
Kenai (20)	5	2	7	0.25 ± 0.18
Haplogroups				
Denali	11	7	12	0.26 ± 0.17
Seward	25	14	33	0.62 ± 0.34
Brooks	43	27	49	0.65 ± 0.34
Geographical areas				
Northern (2–14)	53	33	60	0.81 ± 0.42
Southern (15–21)	28	17	59	1.41 ± 0.71
Singing voles	95	55	109	1.21 ± 0.61

N, sample size; *H*, number of haplotypes; *S*, segregating sites; π, nucleotide diversity (%, average ± SD).

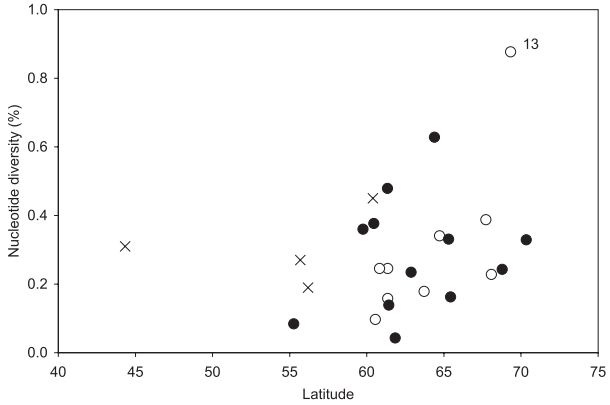

Figure 6 Genetic diversity in arvicoline rodents: nucleotide diversity (π) estimates for populations of singing voles (*Microtus miurus* and *M. abbreviatus*) (white circles; Table 2), southern red-backed voles (*Myodes gapperi*) (crosses; Runck & Cook, 2005) and root voles (*Microtus oeconomus*) (black circles; Galbreath & Cook, 2004) plotted against latitude. The nucleotide diversity of most populations of singing voles is in the range observed for populations of the two other species of voles, which are also found in tundra habitats but display less colonial social structures. The only outlier point is the population from Lake Peters (ANWR-P; locality 13 in Fig. 1), in which haplotypes from both Brooks and Seward haplogroups are found.

only within southern ($r = 0.67$, $P = 0.0097$) or northern ($r = 0.70$, $P < 0.0001$) populations. Pairwise nucleotide differences between sequences did not follow a unimodal distribution, as revealed by the mismatch permutation test (sum of square deviations = 0.0015, $P = 0.84$), suggesting the lack of recent population expansion. Results are the same if performed within each major geographical group, or including only core continental samples (i.e. excluding the Wrangell and St Matthew samples).

DISCUSSION

Eastern Beringian biogeography

The genetic structure of singing voles fits the pattern expected for long-term Pleistocene inhabitants of Beringia: overall high haplotype and nucleotide diversity, marked phylogenetic structure with (at least) one deep cladogenetic split, high levels of geographical structure in the genetic variation and no strong signal of recent exponential growth (Table 1). The massive ecological and topographical changes in the eastern Beringian landscape during glacial/interglacial cycles greatly influenced the genetic structure of this tundra specialist, a pattern that is likely to be found in other species distributed in arctic and alpine tundra.

During glacial intervals, most of E Beringia was characterized by extensive steppe-tundra and glacial barrens in the north, with perhaps a few pockets of spruce woodland in areas where the tree line was lowered to around 400–600 m (Heusser, 1983; Zazula *et al.*, 2003, 2007). Hemlock-coastal forests were not found in south-east Alaska and north-west Canada, as the Cordilleran glacier covered most of the region (Hamilton & Thorson, 1983). Glaciers and ice coverage were more extensive in the coastal mountain ranges, but many of the current habitats of singing voles in the Alaska Range and in the Brooks Range were also under ice (Fig. 1a). Nevertheless, unglaciated microrefugia have been proposed within the Cordilleran ice sheet and corroborated by depositional data for the Wisconsinan glaciation (Porsild, 1966; Youngman, 1975; Hamilton & Thorson, 1983; Fulton *et al.*, 1986; Hamilton *et al.*, 1986). Two of the putative microrefugia are the Copper River Basin (Hamilton & Thorson, 1983; Fulton *et al.*, 1986; Hamilton *et al.*, 1986) and the Kluane region (Porsild, 1966; Youngman, 1975), regions close to the sampling sites of the highly divergent Wrangell Mountains haplotype clade (Fig. 1b). Although the precise distribution of this clade is not known due to limited sampling of singing voles in the Wrangell, St Elias and Kluane ranges, at least one other rodent species appears to be endemic to the mountains of eastern Alaska and south-western Yukon Territory, an undescribed deer mouse (the 'Haines Junction clade' from the *Peromyscus keeni* species group; Lucid & Cook, 2007).

Fossil remains of *M. miurus* have been recovered from a few sites in the northern central plains of the United States that are dated as Wisconsinan (latest Pleistocene) in age and contain typical subarctic faunas (Baker *et al.*, 1986; Semken & Falk,

1987; Woodman *et al.*, 1996). Therefore, differentiation south of the Laurentidean ice sheet followed by northward migration (Fig. 2d) is another putative hypothesis for the observed deep divergence within singing voles. Further sampling of *M. miurus* from Canada will allow us to determine if the divergent group is an isolated endemic species or the result of Laurentidean vicariance. The estimated date for the split between this sample and remaining singing voles, however, pre-dates the Wisconsinan glaciation (Fig. 5, F). In this case, this would point to a possible long-term permanence of this subarctic species in temperate North America followed by recent sub-Laurentidean extinction, a pattern similar to that proposed by Fedorov *et al.* (2003) for the brown lemming (*Lemmus trimucronatus*).

In contrast to the Wrangell Mountains sample, the two large clades of singing voles – concentrated in the Brooks Range and the Seward Peninsula – appear to have coalesced at the same time during the Sangamon interglacial period around 100 ka (Fig. 5, D, E, respectively). Due to the geographical structure in this genetic partitioning, we interpret this date as the age of a probable geographical split within singing voles in conditions similar to the present, i.e. when tundra conditions were less widespread than in glacial times. The remaining haplogroups of singing voles (Fig. 5, A, B, C), with much more restricted distributions (found in St Matthew Island, Iliamna and the Alaska Range, respectively), have coalescent dates for the Last Glacial Period (LGP). These shallow estimates probably reflect a small number of colonizing individuals in the case of Iliamna (covered with ice during the LGP; Fig. 1a) or of bottleneck survivors in the case of St Matthew Island (after loss of land connection; see below). The central Alaska Range haplogroup, on the other hand, is found close to the ice-free areas during the LGP, on the border of the Cordilleran ice sheet (Fig. 1a), which could have served as an isolated microrefugium for singing vole populations.

The star pattern recovered in the network analysis (Fig. 4) of core singing voles contrasts with the insertion of the root within the Brooks Range haplogroup, rendering it polyphyletic in the phylogenetic analyses (Fig. 3). This root problem has been observed in several studies in which a long branch (e.g. distant outgroup) is attached to a clade with a putative hard polytomy (Blouin *et al.*, 1998; Halanych *et al.*, 1999). This can result in a flat likelihood surface for this area of the tree, and the node is attracted to the slightly better (or more parsimonious) position, usually to a longer branch within a densely sampled, but otherwise monophyletic, group. Alternatively, the Brooks Range may have functioned as a source for all other haplotypes found in the sink regions (Pulliam, 1988). In this scenario, the Brooks Range populations have failed to coalesce.

Effects of habitat heterogeneity and social structure

Microtus miurus is a habitat specialist, living in patches of mesic tundra often associated with willows and rocky areas (Bee & Hall, 1956; Douglass, 1984; Batzli & Henttonen, 1990; Batzli & Lesieutre, 1995). Singing voles are also gregarious, living in quasi-colonial demes (Wolff, 1985), with marked home ranges (Batzli & Henttonen, 1993) and limited movement among demes (Galindo & Krebs, 1985). The habitat of the species usually forms less than 25% of the Arctic tundra landscape in the northern portion of the species' distribution. The nucleotide diversity within populations of singing voles, however, is similar to that of other arvicoline species (Fig. 6). This result suggests that the social organization of these voles is not decreasing the genetic diversity within populations, as expected for a species with marked social structures (Wright, 1965; Storz, 1999). In contrast, the regional levels of nucleotide diversity are higher than those observed in other arvicoline species.

The southern range of the species' distribution has particularly high levels of nucleotide, haplotype and phylogenetic diversity. Two factors are likely to contribute to the high genetic diversity of these southern populations: (1) post-glacial colonization of stocks from different haplogroups; and (2) the disjunct distribution of populations in isolated patches of alpine tundra between an unsuitable matrix of forest (taiga and hemlock-spruce), ice fields and glaciers, in a 'sky-island' pattern (Demboski & Cook, 2001). Conversely, the population structure of this species in arctic tundra better fits a metapopulation model (Pannell & Charlesworth, 1999) with panmictic (promiscuous) mating within colonies (Batzli & Henttonen, 1990) and rare, but constant, movement between isolated colonies through the tundra matrix (Galindo & Krebs, 1985). Additional work on the finer genetic and demographic structure of the species is necessary to test these hypotheses and better describe the population model.

Taxonomic and conservation implications

Our results do not support the current taxonomy of singing voles, which recognizes two species (*M. abbreviatus* and *M. miurus*; Musser & Carleton, 2005). This is in agreement with previous analyses of cytochrome *b* from two individuals, one from the Brooks Range (nominally *M. miurus*), and one from St Matthew Island (*M. abbreviatus*; Conroy & Cook, 2000b). Cytochrome *b* data indicate that the population from St Matthew Island, currently referred to *Microtus abbreviatus*, is not a distinct species from *M. miurus*; rather, it is a recently isolated population of singing voles dating from a late Pleistocene (Fig. 5, C) expansion of singing voles, as first suggested by Rausch & Rausch (1968). The St Matthew Archipelago was last connected to the North American continent as recently as 13 ka (Hopkins, 1984; Elias *et al.*, 1996; Manley, 2002). The cranial morphological differences found by Rausch & Rausch (1968) between *M. miurus* and *M. abbreviatus* could be due to a sampling artefact, as their analyses included a single mainland sample from the Brooks Range. If, however, the differences are in fact real, additional morphological and morphometric analyses should be undertaken to evaluate whether the St Matthew's populations underwent (or are under) a phenotypic bottleneck or any other insular syndrome process (Van Valen, 1973; Raia & Meiri, 2006). The St Matthew Archipelago represents a unique

habitat for singing voles, and its protection may be essential to maintaining the phenotypic diversity of the species.

The synonymization of the two species presents a nomenclatural nuisance: *M. abbreviatus* Miller, 1899 has priority over *M. miurus* Osgood, 1901. Thus, the appropriate name for the common singing vole from both mainland Alaska and the St Matthew Archipelago is *M. abbreviatus*, with *M. miurus* representing a junior synonym (*M. abbreviatus* is an apt descriptor of the extremely short tail of the species). In turn, the name *Microtus cantator* Anderson, 1947, currently considered a junior synonym of *M. miurus* but whose type locality is less than 50 km from the Wrangell Mountains samples analysed here, is a candidate name for the divergent haplotype lineage sister to all other singing voles (Fig. 3). Testing this hypothesis, which we consider tentative, will require the rigorous analysis of additional specimens from this region of Alaska and north-western Canada as well as relevant type material. To date we have been unable to obtain Canadian samples of *M. miurus*; the eastern distributional range of singing voles is poorly known, and the status of the isolated population from the Plains of Abraham in the Northwestern Territories (Fig. 1a) is still unknown.

Finally, we also stress that additional genetic markers are necessary for a complete resolution of the genetic partitioning and more accurate estimate of temporal and phylogenetic parameters for the species. Cytochrome *b*, the marker of choice in phylogeographical studies of rodents (and mammals in general), provides a strong phylogenetic signal and a useful comparative benchmark across species, but it is nonetheless a single marker. Additional loci, and a larger sample size of key areas, will enable us to characterize with more precision the geographical and historical particulars of the evolution of singing voles. Our evolutionary knowledge of this species, and of the other members of small mammal communities from arctic and alpine tundra habitats, will be particularly important in our attempts to predict the biological consequences of global climatological changes in the Arctic and sub-Arctic, and for planning for conservation actions in areas that are already being affected by the current warming temperatures (Usher *et al.*, 2004).

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article:

Appendix S1 Locality and voucher information for sequenced specimens.

Appendix S2 Data matrices used in phylogenetic analyses.

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