



A climate for speciation: Rapid spatial diversification within the *Sorex cinereus* complex of shrews

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ABSTRACT

The cyclic climate regime of the late Quaternary caused dramatic environmental change at high latitudes. Although these events may have been brief in periodicity from an evolutionary standpoint, multiple episodes of allopatry and divergence have been implicated in rapid radiations of a number of organisms. Shrews of the *Sorex cinereus* complex have long challenged taxonomists due to similar morphology and parapatric geographic ranges. Here, multi-locus phylogenetic and demographic assessments using a coalescent framework were combined to investigate spatiotemporal evolution of 13 nominal species with a widespread distribution throughout North America and across Beringia into Siberia. For these species, we first test a hypothesis of recent differentiation in response to Pleistocene climate versus more ancient divergence that would coincide with pre-Pleistocene perturbations. We then investigate the processes driving diversification over multiple continents. Our genetic analyses highlight novel diversity within these morphologically conserved mammals and clarify relationships between geographic distribution and evolutionary history. Demography within and among species indicates both regional stability and rapid expansion. Ancestral ecological differentiation coincident with early cladogenesis within the complex enabled alternating and repeated episodes of allopatry and expansion where successive glacial and interglacial phases each promoted divergence. The *Sorex cinereus* complex constitutes a valuable model for future comparative assessments of evolution in response to cyclic environmental change.

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1. Introduction

The history of diversification among extant terrestrial biota has been strongly influenced by geologic and climate change over broad latitudinal and longitudinal gradients, coupled with intermittent transcontinental biotic exchange through the Neogene period (23–2.6 million years [My]). Examples of large-scale change include the Beringian Isthmus that repeatedly formed between Eurasia and North America, and the recent connection of North and South America across the Isthmus of Panama. As such, the relative influence of different drivers of evolution related to environmental change has been of interest when considering variable genetic responses. Independent phylogeographic studies have revealed substantial population structure and in some cases speciation as a consequence of Quaternary climate change (2.6 Ma – present; e.g., Brochmann and Brysting, 2008; Egan and Crandall, 2008;

Hewitt, 1996; Weir and Schluter, 2004), particularly at higher latitudes. Conversely, Pleistocene epoch (2.4 My) climate periodicity may have been too brief to promote speciation in many instances due to repeated reconnection and admixture of allopatric populations (e.g., Petit et al., 2003). Particularly at lower latitudes, geologic change as a consequence of tectonic movement has been invoked as the major cause of cladogenesis working over a deeper timeframe (Riddle, 1995, 1998). Within North America, a broad latitudinal range means that the relative severity and influence of climate versus geophysical change on patterns of diversification may vary considerably from north to south (Weir and Schluter, 2007). In addition, major geologic change during the Neogene has been substantially more pronounced in western North America than in the east (Finarelli and Badgley, 2010). Although tectonic movement is ongoing, major geologic features influencing the North American biota were in place by onset of the Pleistocene. Finally, advances in comparative phylogeographic approaches have improved our understanding of common geographic and climatic elements in both Eurasia and North America that have influenced diversification, including phylogeographic breaks, barriers to dispersal, areas of endemism, refugia, or zones of contact over multiple taxa (Burbrink et al., 2008; Hewitt, 2004; Stewart and Lister, 2001;

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Swenson and Howard, 2005; Taberlet et al., 1998). With a growing knowledge of the factors influencing evolutionary processes, we can now posit and test predictions of the sequence of evolution within a given taxonomic group. We use genetic information from multiple independent loci to investigate the history of speciation within the *Sorex cinereus* complex (*cinereus* complex), a widespread and diverse suite of closely related shrews occurring throughout North America and westward into Siberia.

The recent geologic history of North America sets the evolutionary stage for diversification. Beginning in the middle Miocene epoch (15 My), initial formation of the Basin and Range Province in western North America was followed by uplift of the Cascade and Sierra Nevada ranges around 10 My and with further development of the Rocky Mountains (first formed in the Eocene) and Colorado Plateau in the late Miocene (<10 Ma; Brunnsfeld et al., 2006; Ruddiman et al., 1989; Webb and Bartlein, 1992). Uplift in concert with aridification of western and central North America contributed to the formation of the grasslands of the Great Plains, shrublands of the Columbia Plateau/Basin, and the extensive deserts (Hafner and Riddle, 2005) as evidenced by molecular analyses of disparate floral and faunal groups (e.g., Jaeger et al., 2005; Houston et al., 2010; Neiswenter and Riddle, 2010; Riddle and Hafner, 2006). Highly heterogeneous physiography as a consequence of geologic change influenced diversification both independently and in combination with climatic variability. Mountain ranges, generally orientated north/south, fragment desert ecosystems while montane forest ecosystems are isolated by intervening arid-lands (Brunnsfeld et al., 2006). Eastern North America geologic history over this time frame was much less variable, perhaps contributing to lower diversity estimates compared with western North America (Badgley, 2010), and yet major biogeographic provinces in this region are associated with more ancient features such as the Appalachian Range, the Atlantic-Gulf of Mexico divide, and various riverine systems (Soltis et al., 2006). By the late Pliocene epoch (3 My), a cooling trend allowed formation of permafrost at the highest latitudes and the associated tundra biome (Matthews, 1979). Glacial cycling during the Pleistocene caused repeated climate fluctuations that increased in intensity due to onset of 100 ky cycles by 0.8 My (Lisiecki and Raymo, 2005). These glaciations shunted the biota dramatically and were major drivers of diversification. Connection of Eurasia and North America through Beringia provided multiple opportunities for transcontinental exchange and divergence (e.g., Waltari et al., 2007). Members of the *cinereus* complex exhibit broad ecological associations and a vast collective geographic distribution, providing an excellent and rare opportunity to investigate spatial and temporal evolution across the latitudinal and longitudinal extent of North America and eastern Siberia (e.g., Pigot et al., 2010).

Questions about the evolutionary history of the *cinereus* complex remain despite previous studies of variation in morphology, chromosomes, allozymes, and mitochondrial DNA (mtDNA; see Demboski and Cook (2003) for overview). The complex currently comprises 13 species in the subgenus *Otisorex* (Hutterer, 2005) including the recently allied *Sorex emarginatus* (Esteve et al., 2010). Members occupy most terrestrial ecosystems in North America, except southwestern deserts, and stretch across the Bering Strait into Siberia. The species complex is split into two major clades described as Beringian and Southern (van Zyll de Jong, 1991; Table 1). The Beringian clade contains eight species, six of which occupy the tundra and taiga biomes at northern high-latitudes (Fig. 1A and B). *Sorex ugyunak* occurs in tundra of northernmost Alaska and Canada, having expanded eastward out of the Beringian refugium following retreat of continental ice sheets since the Last Glacial Maximum (LGM) to occupy its current distribution. *Sorex jacksoni* (St. Lawrence Island) and *Sorex pribilofensis* (St. Paul Island) are island endemics of the Bering Sea that are found in

tundra habitat and represent remnants of a previously more widespread distribution across the Beringian Isthmus that existed (10,000–16,000 BP) before inundation (Hopkins et al., 1982). *Sorex portenkoi* occupies tundra within portions of Chukotka and northern Kamchatka in Siberia and *Sorex camtschatica* is found in tundra and taiga both on the Kamtchatka Peninsula and southern Magadanskaya Region, Russia. *Sorex leucogaster* is also an insular endemic known only from Paramushir Island within the Kurile group. Although these northernmost forms are described as separate species, their taxonomic status is still debated (Demboski and Cook, 2003). Divergence of mtDNA is minimal suggesting a recent common ancestor. Other members of the Beringian clade occur farther south in North America. *Sorex haydeni* occupies grasslands of the northern and central prairies and to a lesser extent deciduous forest. *Sorex preblei* occupies arid sagebrush shrubland centered on the Columbia Basin. An early dichotomy within the Beringian clade, as indicated from mtDNA, separates *S. preblei* from other members, followed by divergence of *S. haydeni* (Demboski and Cook, 2003).

The Southern clade is represented by five nominal species (Hutterer, 2005): *Sorex cinereus* (sensu stricto) is the most widespread shrew in North America occupying most available boreal coniferous forest in the west and north, and deciduous forests of the east (Whitaker, 2004; Fig. 1B). Occurrence of *S. cinereus* in the southwestern United States comprises mountaintop sky island populations within remnant patches of previously more widespread forest. Along the Pacific and Atlantic coasts, *S. cinereus* is represented on multiple land-bridge islands, although there are few systematic assessments of these populations (e.g., Stewart and Baker, 1992) and their evolutionary histories remain poorly understood. *Sorex cinereus* has also spread northward to treeline to occupy much of Canada and Alaska reflecting a rapid range expansion from the south following continental ice sheet retreat (Demboski and Cook, 2003). *Sorex cinereus* does not occur in Oregon or California, but *S. lyelli* has a limited distribution in eastern California including mid- to high-elevation arid habitats of the Sierra Nevada. Although *S. lyelli* has previously been considered a member of the Southern clade, this first genetic assessment places *S. lyelli* within the Beringian clade (Figs. 2 and 3). In the eastern United States, *S. longirostris* occurs in the southern Appalachian deciduous forests near bogs and marshes. Finally, both *S. milleri* and *S. emarginatus* form isolated populations in montane forests in central eastern and central western Mexico, respectively (Esteve et al., 2010).

Within the *cinereus* complex, low genetic divergence (~6% uncorrected sequence divergence in the cytochrome *b* [Cyt *b*] gene; Demboski and Cook, 2003) suggests a recent radiation and presents an excellent opportunity to investigate the evolutionary drivers of contemporary diversification (Egan and Crandall, 2008). We present (1) a multi-locus spatiotemporal assessment of evolution within the *cinereus* complex by examining explicit predictions; (2) a review of the major features in North America and Beringia that played a role in speciation within this group; and (3) evidence for a rapid contemporary radiation among this widely distributed suite of shrews.

2. Methods

2.1. Taxonomic and genetic sampling

Over all analyses, we included DNA sequence data from 374 specimens (59 from GenBank; Table S1) representing six outgroups, all recognized species within the *cinereus* complex (Table 1; Appendix A), and populations within the widespread *S. cinereus* (sensu stricto) that are coincident with distinct geographic regions (Fig. 1). *Sorex emarginatus* was excluded from final genetic analyses

Table 1Major clades, general habitat affinities, and distributions of species within the *cinereus* complex. References identifying ecological associations are included (N/A = not available).

Clade ^a /Species ^b	Distribution	Habitat	Ecological literature
Beringian clade		Generally xeric	
<i>S. camtschatica</i>	Paleartic: Russia, Magadanskaya and Kamchatka Pen.	Tundra/taiga	Okhotina (1977) and Van Zyll de Jong (1982)
<i>S. leucogaster</i>	Paleartic: Russia, Paramushir Is., Kuriles	Tundra	Okhotina (1977) and Van Zyll de Jong (1982)
<i>S. portenkoi</i>	Paleartic: Russia, Chukotka	Tundra	Okhotina (1977) and Van Zyll de Jong (1982)
<i>S. jacksoni</i>	Bering Strait: St. Lawrence Is.	Tundra	Van Zyll de Jong (1982)
<i>S. pribilofensis</i>	Bering Strait: St. Paul Is.	Tundra	Van Zyll de Jong (1982)
<i>S. ugyunak</i>	Nearctic: northernmost Alaska and Canada	Tundra	Van Zyll de Jong (1982, 1976)
<i>S. haydeni</i>	Nearctic: Great Plains of Canada and USA	Grassland	Brunet et al. (2002) and Van Zyll de Jong (1980)
<i>S. preblei</i>	Nearctic: west North America, Columbia Basin/Plateau	Sagebrush shrubland	Cornely et al. (1992), Hoffmann and Fisher (1978) ^c , Kirkland Findley (1996) ^d , Ports and George (1990) ^e , Shohfi et al. (2006) and Tomasi and Hoffmann (1984) ^c
Southern clade		Generally mesic	
<i>S. cinereus</i>	Nearctic: widespread	Coniferous forest	Brannon (2000), Kirkland et al. (1997), Moore (1949), Van Zyll de Jong (1976, 1980) and Whitaker (2004)
<i>S. lyelli</i> ^e	Nearctic: California, east Sierra Nevada Mtns.	Coniferous forest, shrubland	Epanchin and Engilis (2009)
<i>S. emarginatus</i>	Nearctic: Mexico, Sierra Madre Occidental	Coniferous forest	N/A
<i>S. milleri</i>	Nearctic: Mexico, Sierra Madre Oriental	Coniferous forest	Villalobos et al. (2005)
<i>S. longirostris</i>	Nearctic: southeast United States	Deciduous forest, marsh, bog	French (1980)

^a Clade relationships first described by van Zyll de Jong (1991).^b Assignment to clades based on van Zyll de Jong (1991), Demboski and Cook (2003), and Esteva et al. (2010).^c Likely refers to the taxon identified in the current study as *S. sp. RM*.^d Likely refers to the taxon identified in the current study as *S. sp. SW*.^e Inclusion of *S. lyelli* within the Southern clade reflects previously hypothesized relationships and not the current study.

as the single sequence available (M. Esteva, pers. comm.) appears problematic because it exhibited an abnormally long branch during preliminary phylogeny estimation. Attempts to resequence this sample from degraded DNA were minimally successful (Appendix A) but identified potential sequencing errors in the original experiment. Locality information for this species however was included in ecological analyses. Outgroups were *S. fumeus*, *S. monticolus*, *S. nanus*, *S. palustris*, *S. rohweri*, and *S. trowbridgii* based on close phylogenetic relationships with the *cinereus* complex (George, 1988) and availability of tissue samples. All belong to the subgenus *Otisororex* (Hutterer, 2005). For specimens sequenced, heart, liver, or skin was obtained from frozen (stored at -80°C), ethanol-preserved, or dried samples. Genomic DNA was obtained through standard salt extraction and sequences were obtained through PCR and cycle sequencing methods (Hope et al., 2010). We amplified variable lengths of the Cyt *b* gene (430–1140 bp) based on the quality of the tissue. A single primer pair for the entire Cyt *b* gene was used for frozen high quality samples (MSB05/MSB14; Hope et al., 2010). For degraded DNA samples, multiple internal primers were developed (Table S2). To improve DNA amplification from degraded DNA samples, we modified protocols of Hope et al. (2010) by substituting AmpliTaq Gold[®] DNA polymerase and 10 \times PCR buffer Gold. Automated sequencing of complementary strands was conducted using an Applied Biosystems 3110 DNA sequencer (Molecular Biological Facility, UNM). Complementary strands of DNA were compared and sequences were deposited in GenBank (Appendix A). We also sequenced 99 individuals of the *cinereus* complex and outgroup species (excluding *S. fumeus*; Appendix A) for 4 nuclear loci including 2 nuclear exons (500 bp of the apolipoprotein B gene [ApoB] and 397 bp of the breast cancer susceptibility 1 gene [BRCA1]) found to be phylogenetically informative for other *Sorex* species (Dubey et al., 2007; Hope et al., 2010) and 2 nuclear introns (345 bp of the interferon 1 gene [IFN1] and 326 bp of the myosin heavy chain 2 gene [MYH2]; Lyons et al., 1997). Primer

pairs ApoBF/ApoBR and BRCA1F/BRCA1R (Dubey et al., 2006, 2007) were used to amplify and sequence ApoB and BRCA1 genes, respectively, and IFN1F/IFN1R and MYH2F/MYH2R (Lyons et al., 1997) were used to sequence the IFN1 and MYH2 genes, respectively.

Sequences were edited and aligned in SEQUENCHER 4.8 (GeneCodes, Ann Arbor, Michigan) and checked by eye. Sequences for all coding regions were translated to amino acids and examined for internal stop codons, rates of transition/transversion changes, and relative first, second and third position changes in codons that might indicate a pseudogene. Alleles of nuclear heterozygotes were inferred using PHASE (Stephens et al., 2001; Stephens and Scheet, 2005). PHASE implements a Bayesian method for reconstructing haplotypes from nuclear sequences that include multiple heterozygous base sites within individuals. To estimate allele frequencies, PHASE was run five times. The run with best goodness-of-fit to an approximate coalescent model was retained, resulting in 2 nuclear haplotype sequences or alleles per individual for all independent nuclear genealogies using the same individuals ($n_{tot} = 220$).

2.2. Phylogeny reconstruction

We estimated a phylogeny using Bayesian methods for variable lengths of the Cyt *b* gene (430–1140 bp; $n = 164$) representing all species within the *cinereus* complex and outgroup species. We included partial sequences amplified from poor quality samples to maximize sequence diversity for the mitochondrial locus. The best model of DNA substitution was determined using MrModeltest v2.3 (Nylander, 2004) under the Akaike Information Criterion. Markov chain Monte Carlo (MCMC) searches of tree space were performed in MrBayes v3.1 (Ronquist and Huelsenbeck, 2003) to estimate phylogenies. Two separate runs were conducted with sequences partitioned by codon position. Each run computed 10 million generations, sampling every 100 generations, with five

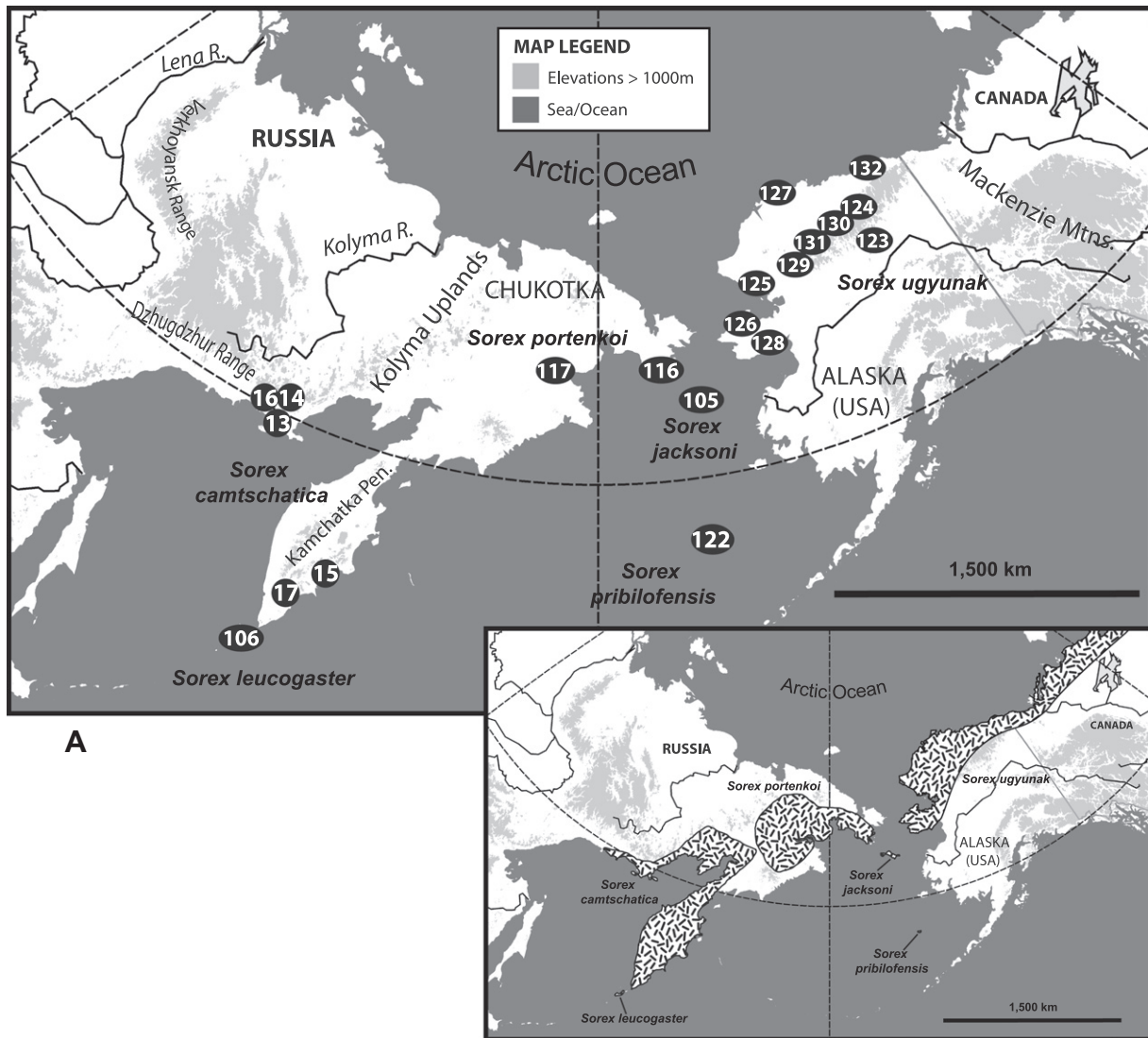


Fig. 1. Maps of the study area indicating localities by number coincident with Appendix A: (A) map of Beringia illustrating the distribution of sampled localities for high-latitude Beringian clade species including *S. camtschatica* (13–17), *S. jacksoni* (105), *S. leucogaster* (106), *S. portenkoi* (116, 117), *S. pribilofensis* (122), and *S. ugyunak* (123–132). Inset map depicts geographic range for each high-latitude Beringian clade species (capsule pattern) adapted from IUCN assessments (IUCN, 2010) and updated based upon additional records included in the present study; (B) map of North America illustrating the distribution of lower-latitude Beringian clade species including *S. sp. RM* (1–7), *S. sp. SW* (8–12), *S. haydeni* (101–103), *S. lyelli* (112, 113), *S. preblei*, (118–121), and distributions of Southern clade species including *S. cinereus* (east; 18–39), *S. cinereus* (midwest; 23, 40–51), *S. cinereus* (southwest; 52–64), *S. cinereus* (west; 2, 3, 6, 7, 65–99), *S. emarginatus* (100), *S. longirostris* (107–111), *S. milleri* (114, 115). Inset map depicts geographic range of Beringian clade species (capsule pattern), Southern clade species (horizontal stripe pattern), and *S. longirostris* (dot pattern) adapted from IUCN assessments (IUCN, 2010) and updated based upon additional records included in the present study and recognition of distinct taxa.

independent chains, and the first 10,000 trees discarded as burn in. To avoid arriving at a false optimum long-tree topology, we utilized the short-branch method employed by Marshall (2010). Convergence of MCMC runs was assessed in Tracer v1.4 (Rambaut and Drummond, 2007). The resulting phylogram with posterior probabilities was midpoint-rooted and visualized in FigTree v1.2.2 (Rambaut, 2009).

2.3. Species tree estimation from multiple loci

Evidence from multiple independent genes is critical to inferring species relationships (Edwards, 2009; Maddison, 1997) because gene tree/species tree discord can result from gene duplication, introgression, sorting of ancestral polymorphism or rapid fixation of linked loci through selective sweeps (Carstens and Knowles, 2007). For species tree estimation, we used a method involving coalescent coestimation of multiple gene trees embedded within

a corresponding species tree topology using *BEAST (Heled and Drummond, 2010) and implemented in the program BEAST v1.6.1 (Drummond and Rambaut, 2007). This method uses a Bayesian MCMC technique. Independent loci were partitioned (unlinked) according to substitution models estimated for each locus (Table S3), molecular clock model, and gene tree estimation. A strict molecular clock was employed and mutation rates for the 4 nuclear loci were estimated based on a Cyt *b* mutation rate for *Sorex* of 5.5% My⁻¹ (Hope et al., 2010). A Species Tree: Yule Process tree prior was assigned for all 5 loci with a Piecewise Linear & Constant Root population size model. Initial tree root height was estimated and default settings were used for all priors and operators. The MCMC chain was run for 100 million iterations. Resulting tree files were annotated in TreeAnnotator v1.6.1 (part of the BEAST software package) and the species tree topology visualized in Figtree v1.2.2. Independent genealogies for each locus were also estimated in MrBayes v3.1 utilizing methods identical to the expanded

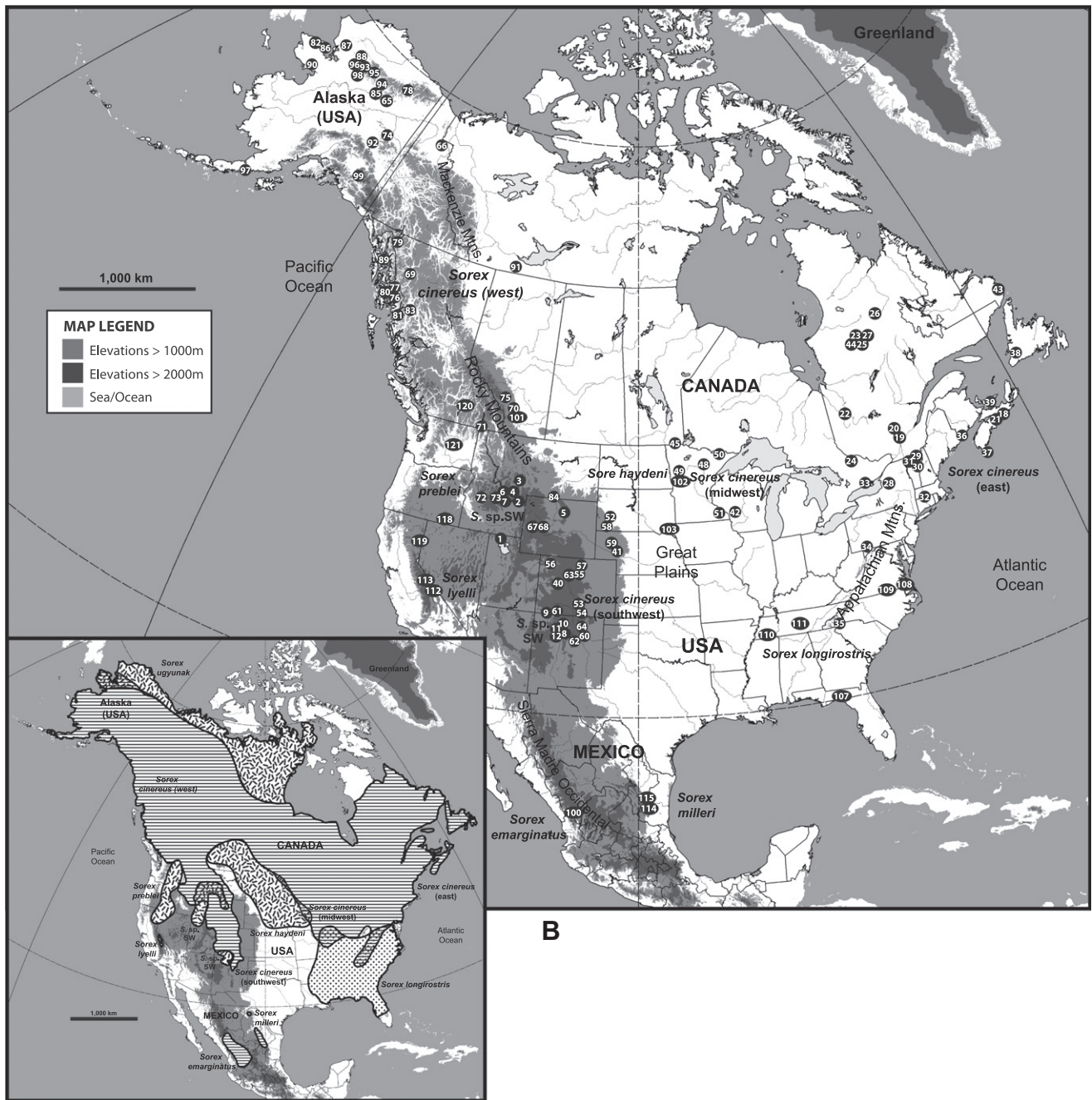


Fig. 1 (continued)

mitochondrial phylogeny but employing models of evolution specific to each locus (Table S3). Independent taxa for the species tree estimation included separate geographic clades within *S. cinereus* (sensu stricto). *Sorex milleri* and *S. leucogaster* were not included as sequencing of nuclear loci from degraded samples was not possible.

2.4. Population demographics

Populations for demographic analyses were expanded where possible to include additional *Cyt b* sequences ($n_{\text{tot}} = 361$; Table 2) and were assigned based not only on previous studies but also on the results of our *Cyt b* Bayesian phylogeny as a null estimate of relationships within the species complex (Table S5). Species designations within this complex have undergone numerous changes as

a result of limited geographic sampling, low genetic and/or morphological differentiation (Demboski and Cook, 2003), and instances of purported hybridization (Brunet et al., 2002; Stewarter and Baker, 1997). We therefore retained each nominal species (Hutterer, 2005) as a separate group, and also included several populations that may represent new taxa. Demboski and Cook (2003) recognized a population of shrews from the Jemez Mountains of New Mexico that were genetically similar to *S. haydeni* based on mtDNA. We subsequently expanded sampling from this area (*S. sp. SW*; Table S1). In addition, multiple samples collected from riparian and arid sagebrush habitats in the central Rocky Mountains form one or more seemingly distinct evolutionary lineages (*S. sp. RM*; Table S1). All species of the Beringian clade (Demboski and Cook, 2003) were closely allied so within this clade we designated

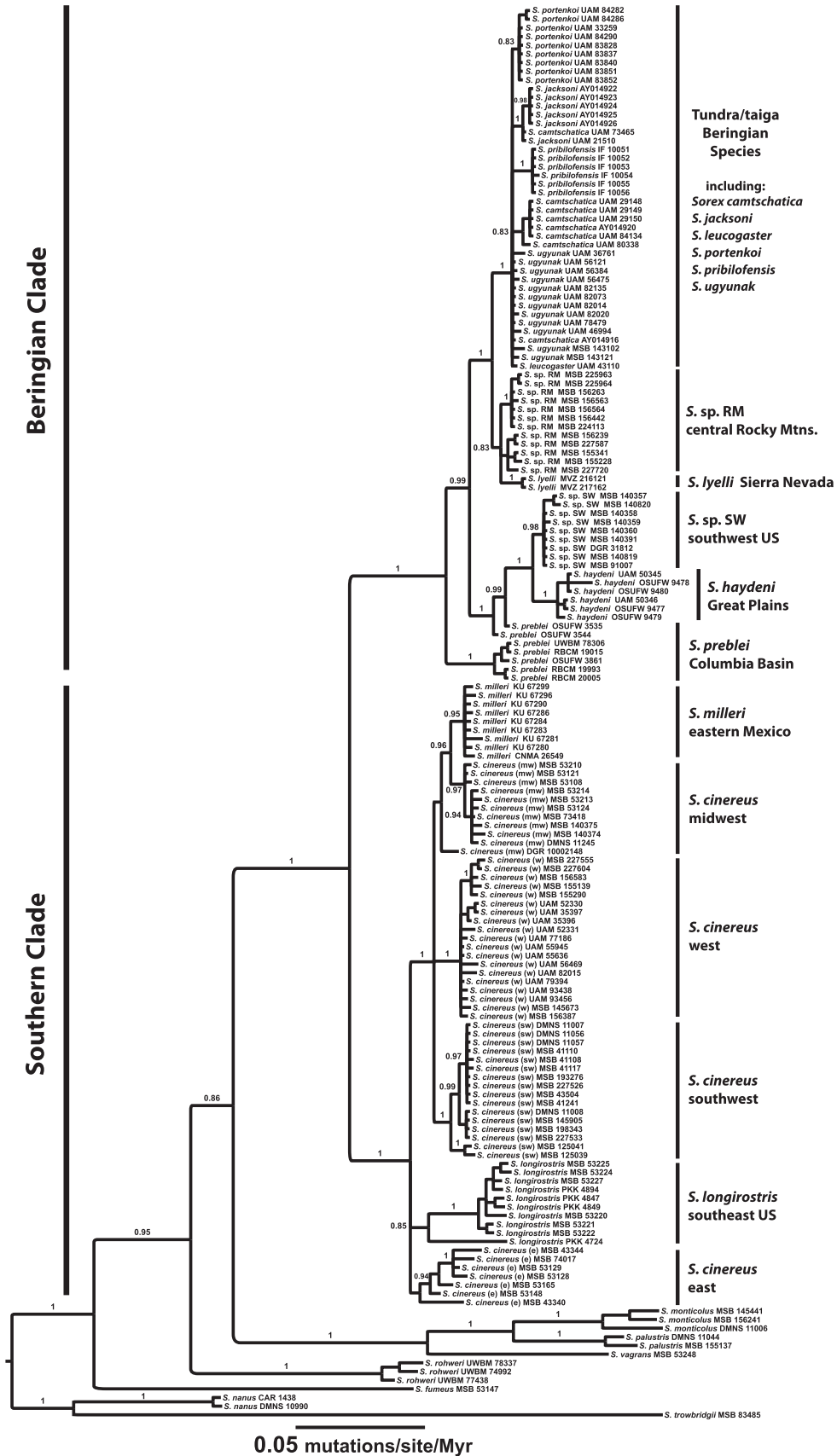


Fig. 2. Mitochondrial cytochrome *b* Bayesian phylogeny for the *cinereus* complex of shrews and associated outgroups. Specimen numbers are coincident with Appendix A. Posterior probability nodal support values <0.8 are not provided and major unlabeled nodes are only weakly supported.

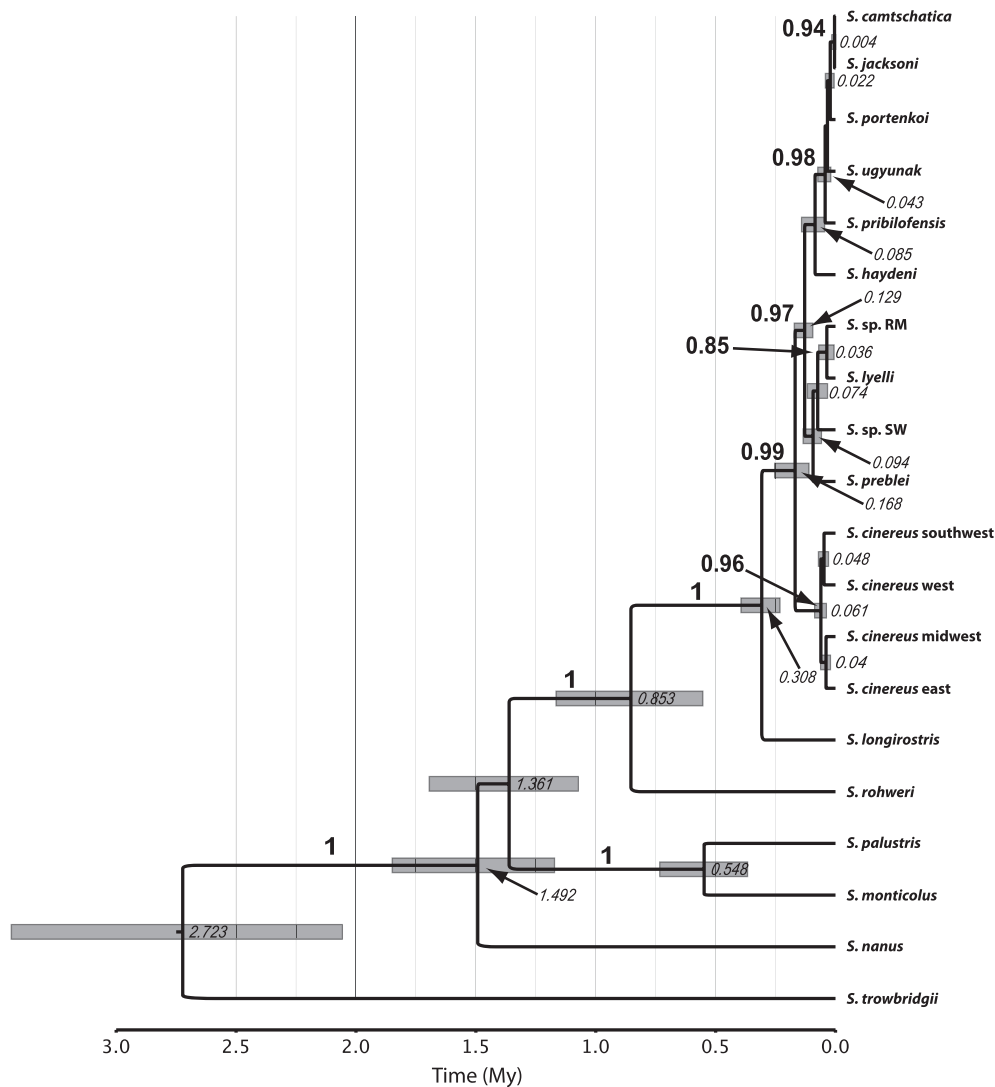


Fig. 3. Species tree estimation from 5 independent loci including mitochondrial cytochrome *b* gene, and regions from nuclear apolipoprotein B, breast cancer susceptibility 1, interferon 1, and myosin heavy chain 2 genes. Phylogeny estimation was performed in *BEAST, providing both posterior probability nodal support values (bold, left of node) and coalescence times (millions of years; italics, right of node). Shaded bars around nodes indicate 95% confidence intervals for coalescence estimates. The topology is presented as ultrametric and proportional to an evolutionary timeline (bottom) that extends from the present (right) to the past (left).

three subgroups to investigate demography independent of the recognized taxonomy. The first subgroup includes three arid-land forms that consistently are associated on the basis of the nuclear loci (*S. lyelli*, *S. sp. SW*, *S. sp. RM*). We also grouped all high-latitude tundra/taiga species (*S. ugyunak*, *S. pribilofensis*, *S. jacksoni*, *S. portenkoi*, *S. camtschatica*, and *S. leucogaster*) and all Siberian species (*S. portenkoi*, *S. camtschatica*, and *S. leucogaster*). We grouped all species within the Southern clade (Demboski and Cook, 2003), and additionally separated *S. cinereus* (sensu stricto) into four subgroups reflecting a western clade, a southwestern clade, a midwestern clade, and an eastern clade, with the latter equivalent to the nominal species *S. fontinalis* (George, 1988; Hollister, 1911). Summary statistics were calculated in DnaSP (Librado and Rozas, 2009) for each population to assess genetic diversity and included segregating sites (*S*), haplotype diversity (*Hd*), and nucleotide diversity (π). Uncorrected pairwise sequence divergence between populations was calculated as percentage divergence My^{-1} .

For tests of demographic expansion, we used DnaSP to calculate Tajima's *D* (Tajima, 1989), Fu's *F_s* (Fu, 1997), and *R₂* (Ramos-Onsins and Rozas, 2002) and assessed significance with 10,000 coalescent simulations. In addition we assessed pairwise mismatch distributions to

investigate potential demographic expansion of each major group (samples ≥ 7) from a small ancestral population. Assuming panmixia with an infinite-sites model of neutral nucleotide substitution (Rogers and Harpending, 1992; Slatkin and Hudson, 1991), a smooth unimodal distribution would indicate sudden population expansion whereas a ragged distribution would reflect stable or contracting populations. Multimodal distributions may indicate either multiple expansions or alternatively subdivision within the complex. All groups and loci were assessed for potential selection using the McDonald–Kreitman Test (McDonald and Kreitman, 1991). Summary statistics, genetic diversity indices, and tests of demographic expansion also were completed for each nuclear locus recognizing three groups: the entire *cinereus* complex, the Beringian clade, and the Southern clade.

Previous studies have recognized general ecological associations among species of the *cinereus* complex that distinguish members of the Beringian clade as xeric adapted and members of the Southern clade as mesic adapted (summarized in Table 1). We used annual precipitation values for all geographic specimen localities included in the present study (Appendix A) to quantify the extent of ecological separation between these major clades. Locality coordinates were compiled in DIVA-GIS v.1.4 (Hijmans et al., 2001) and current annual

Table 2

Demographic statistics for members of the *cinereus* complex. Values were calculated for the cytochrome *b* gene except where indicated otherwise (ApoB = apolipoprotein B gene; BRCA1 = breast cancer susceptibility 1 gene; IFN1 = interferon 1 gene; MYH2 = myosin heavy chain 2 gene). Summary statistics include *n* = number of sequences (Phased sequences for nuclear loci); Length = number of base pairs used in demographic analyses; *S* = segregating sites; *h* = number of haplotypes; *Hd* = haplotype diversity; π = nucleotide diversity. Population growth statistics include Tajima's *D*, Fu's *Fs*, and Ramos-Onsins and Rozas' *R2*. Significance of *D*, *Fs* and *R2* are indicated by asterisks.

Species/group	<i>N</i>	Length (bp)	<i>S</i>	<i>h</i>	<i>Hd</i>	π	<i>D</i>	<i>Fs</i>	<i>R2</i>
All	361	859	190	163	0.985	0.0315	−0.517	−34.076**	0.070
Beringian clade	129	947	85	46	0.960	0.0137	−0.731	−7.758	0.075
<i>S. preblei</i>	9	726	3	4	0.778	0.0014	−0.359	−1.039	0.167
<i>S. haydeni</i>	11	821	6	6	0.855	0.0031	0.890	−0.976	0.200
Arid-land species	49	957	35	18	0.893	0.0108	0.943	0.486	0.144
<i>S. lyelli</i>	2	1044	0	1	N/A	N/A	N/A	N/A	N/A
<i>S. sp. RM</i>	25	1030	15	13	0.890	0.0032	−0.622	−4.686*	0.099
<i>S. sp. SW</i>	22	957	9	7	0.645	0.0020	−0.695	−1.077	0.104
Tundra/taiga species	60	847	26	20	0.904	0.0040	−1.247	−7.415**	0.060
<i>S. ugyunak</i>	23	847	13	12	0.739	0.0017	−2.073**	−9.209***	0.050***
<i>S. pribilofensis</i>	9	981	1	2	0.222	0.0002	−1.088	−0.263	0.314
<i>S. jacksoni</i>	7	1044	5	3	0.524	0.0016	−1.024	0.904	0.284
Siberian species	21	941	7	6	0.779	0.0026	0.808	0.314	0.167
<i>S. portenkoi</i>	11	941	1	2	0.509	0.0005	1.186	1.023	0.255
<i>S. camtschatica</i>	9	1044	5	4	0.583	0.0017	−0.270	0.077	0.152
<i>S. leucogaster</i>	1	634	0	1	N/A	N/A	N/A	N/A	N/A
Southern clade	232	853	143	119	0.977	0.0133	−1.717**	−107.569***	0.038*
<i>S. emarginatus</i>	1	827	0	1	N/A	N/A	N/A	N/A	N/A
<i>S. milleri</i>	7	388	0	1	N/A	N/A	N/A	N/A	N/A
<i>S. cinereus</i> (All)	214	859	129	112	0.975	0.0122	−1.688*	−100.859***	0.038**
<i>S. cinereus</i> (west)	122	851	73	58	0.955	0.0039	−2.434**	−70.110***	0.021***
<i>S. cinereus</i> (southwest)	40	894	32	18	0.865	0.0057	−1.200	−4.397	0.071
<i>S. cinereus</i> (midwest)	21	1034	27	17	0.976	0.0038	−1.847*	−11.764***	0.067***
<i>S. cinereus</i> (east)	31	919	56	26	0.983	0.0122	−0.907	−10.796***	0.083
<i>S. longirostris</i>	10	755	16	10	1.000	0.0057	−1.137	−6.603***	0.083***
ApoB All	200	500	40	42	0.883	0.0057	−1.721*	−31.691***	0.035*
ApoB Beringian clade	102	500	18	19	0.710	0.0033	−1.585*	−11.251***	0.044
ApoB Southern clade	98	500	28	29	0.885	0.0070	−1.068	−14.557***	0.061
BRCA1 All	200	397	26	27	0.889	0.0082	−0.741	−8.565**	0.062
BRCA1 Beringian clade	102	397	19	17	0.747	0.0051	−1.280	−6.492*	0.052
BRCA1 Southern clade	98	397	12	13	0.833	0.0042	−0.749	−4.026*	0.069
IFN1 All	200	345	34	28	0.635	0.0071	−1.695*	−14.023***	0.036*
IFN1 Beringian clade	102	345	12	9	0.169	0.0010	−2.269***	−9.199***	0.033
IFN1 Southern clade	98	345	28	23	0.887	0.0110	−0.946	−6.313*	0.069
MYH2 All	200	326	47	51	0.920	0.0177	−0.826	−24.543***	0.060
MYH2 Beringian clade	102	326	27	18	0.798	0.0122	−0.706	−2.210	0.072
MYH2 Southern clade	98	326	33	38	0.944	0.0163	−0.522	−19.257***	0.078

* $P \leq 0.05$.** $P \leq 0.01$.*** $P \leq 0.001$.

precipitation values for each locality (spatial resolution 1 km²) were extracted from the WorldClim v.1.4 GIS database (Hijmans et al., 2005). Tests of significant differences between mean precipitation values for the combined Beringian clade ($n = 127$) and combined Southern clade ($n = 215$) were performed using both parametric (*Z*-test) and non-parametric (Mann–Whitney *U*) methods.

3. Results

3.1. Phylogenies

The Cyt *b* phylogeny is largely coincident with earlier estimates of the *cinereus* complex (Demboski and Cook, 2003), although new relationships are identified (Fig. 2). A well-supported (posterior probability [PP] = 1.00) group within the Beringian clade consists of high-latitude species including *S. ugyunak* in Alaska and Canada; Bering Sea island endemics *S. pribilofensis* and *S. jacksoni*; and *S. portenkoi*, *S. camtschatica*, and *S. leucogaster* in Far Eastern Russia. Within these tundra/taiga species, relationships are poorly resolved due to low levels of divergence. *Sorex leucogaster* is nested within *S. ugyunak* while *S. ugyunak* is paraphyletic with respect to other species and *S. camtschatica* is polyphyletic. Only *S. portenkoi* and *S. pribilofensis* are monophyletic. Lower-latitude members of the Beringian clade include *S. lyelli* (previously allied to the Southern clade) as sister-tax-

on to *S. sp. RM* (the latter which are here considered separately from *S. preblei*); both *S. lyelli* and *S. sp. RM* are closely allied to high-latitude species. Specimens from the southwestern United States (Jemez and San Juan mountains; *S. sp. SW*) are reciprocally monophyletic (Cyt *b*) with *S. haydeni* from the central Great Plains, a relationship first described by Demboski and Cook (2003). *Sorex preblei* is basal but paraphyletic with respect to the remainder of the Beringian clade. Within the Southern clade, there is ambiguity with respect to recognized taxonomic relationships. The Mexican endemic *S. milleri* is nested within *S. cinereus*. *Sorex longirostris* and *S. cinereus* (east) are divergent from other clades within *S. cinereus*, including well-supported west, midwest, and southwest clades (Fig. 2). An earlier phylogeny estimation allied *S. emarginatus* closely with *S. cinereus* (Esteve et al., 2010).

For the species tree phylogeny (Fig. 3), the 4 nuclear loci varied in estimated mutation rate (0.7–1.7%), models of evolution, and associated observed base frequencies (Table S3). Species tree relationships were broadly concordant with the mitochondrial topologies (expanded Cyt *b* dataset [Fig. 2]; multi-locus Cyt *b* dataset [(Supplementary Fig. S1)]), with a few notable differences (Fig. 3). The *cinereus* complex as a whole is well supported (PP = 1.00) with *S. longirostris* as sister taxon to the remainder of the complex and therefore not a member of the Southern clade as previously recognized (George, 1988). The Beringian clade and a sub-clade consisting of all the high-latitude tundra/taiga species are well

supported. *Sorex haydeni* is weakly allied with the tundra/taiga species. All the arid-land species (*S. lyelli*, *S. sp. RM*, and *S. sp. SW*), together with *S. preblei*, form a weakly supported clade. *Sorex cinereus* (sensu stricto) forms a distinct monophyletic clade but with little supported phylogeographic structure (Fig. 3). Among the outgroup taxa, *S. rohweri* is sister to the *cinereus* complex. *Sorex monticolus* and *S. palustris* are consistently grouped over all loci as previously reported (Demboski and Cook, 2001), and *S. trowbridgii* is consistently the most distant outgroup from midpoint-rooted genealogies (Supplementary Figs. S1–S5).

Coalescence times indicate time to most recent common ancestor (T_{MRCA}) and are estimated at roughly 60 ky for all *S. cinereus* and 43 ky for all the tundra/taiga species (Fig. 3). The Beringian clade is dated to ~130 ky. The *cinereus* complex excluding *S. longirostris* is dated to 170 ky and including *S. longirostris*, $T_{\text{MRCA}} \sim 310$ ky. The *cinereus* complex and *S. rohweri* coalesce at ~850 ky and the root of the tree is estimated at 2.7 My. Confidence intervals surrounding the most recent estimates in some instances overlap, although they are narrow compared with confidence surrounding basal tree relationships (Fig. 3; Table S4).

3.2. Diversity and demography

No locus exhibited evidence of selection based on the McDonald–Kreitman Test. Nucleotide and haplotype diversity for the *Cyt b* gene varied considerably among groups (Table 2). Nucleotide and haplotype diversity were consistently lowest among species of the Beringian clade, particularly the tundra/taiga species at highest latitudes. Within the Southern clade, each population exhibited high nucleotide diversity. Among nuclear loci, MYH2 exhibited the highest nucleotide diversity. Diversity indices were lower for the Beringian clade than the Southern clade over all loci except *BRCA1* (Table 2).

A range of 0.1–6.8% uncorrected pairwise divergence was found for *Cyt b* for the entire *cinereus* complex (excluding *S. emarginatus*; Table S5). Divergence within both the Beringian and Southern clades ranged from ~0.5–3%, and between clades from 4.6–6.8%. Within the Beringian clade, the tundra/taiga species exhibited divergence of <1%. Among populations of *S. cinereus*, divergence ranged from 1.1% to 2.7%, values comparable with distances between arid-land species (Table S5).

Demographic expansion was generally not significant for species within the Beringian clade (Table 2), with the exception of *S. ugyunak*. Within the Southern clade, *S. cinereus* (west) and *S. cinereus* (midwest) exhibited significant signals of expansion. Mismatch distributions for *S. ugyunak* and west and midwest clades of *S. cinereus* illustrate a unimodal distribution also indicating population expansion, whereas other species exhibit ragged distributions apart from *S. pribilofensis* and *S. portenkoi*, species with extremely low nucleotide diversity (Supplementary Figs. 6 and 7). Multimodal (ragged) distributions for all subgroups are indicative of population subdivision or multiple expansion events (Supplementary Figs. 6 and 7).

Species within the Beringian clade were generally associated with more xeric areas than members of the Southern clade as represented by annual precipitation at geographic specimen localities (Fig. 4). The range of precipitation values was highest for *S. cinereus* (west) reflecting a wide latitudinal distribution. Mean precipitation was significantly different between Beringian (more xeric) and Southern (more mesic) clades (Z -test: $Z = 3.814$, $P < 0.001$ two-tailed; Mann–Whitney U : $n_1 = 215$, $n_2 = 127$, $U = 17022.0$, $P < 0.001$ two-tailed).

4. Discussion

There are few instances where complexes of closely related mammal species occupy such a broad range of geography and climate as shrews of the *cinereus* complex, whose largely parapatric distribu-

tions reflect a high degree of ecological diversification. The fundamental split between xeric (Beringian) and mesic (Southern) associations that characterizes the species of these two clades indicates ecological divergence early in the evolution of the complex. *Sorex longirostris*, the sister taxon to all other species, is associated with the highest mean precipitation values (Fig. 4) consistent with a mesic ancestral association. It has been hypothesized that initial divergence in this shrew complex may have been pre-Pleistocene, possibly associated with Miocene/Pliocene geologic change and followed by differentiation during the Pleistocene glacial regime associated with multiple episodes of allopatric differentiation (Demboski and Cook, 2003). Riddle (1995) asserts that diversification in response to climate cycling generally should follow onset of the more extreme 100 ky periodicity of the mid- to late-Pleistocene (about 700 ka). Alternatively, van Zyll de Jong (1982, 1991) proposed that diversification within the entire complex was as recent as the latest Wisconsinan glaciation. Our results strongly indicate that diversification of the entire complex is well within the boundaries of the Pleistocene because an eightfold decrease of mutation rate for the *Cyt b* gene ($\sim 0.7\% \text{ My}^{-1}$) would be necessary to allow a coalescence time for the entire complex that would be coincident with onset of the Pleistocene. The mutation rate of $5.5\% \text{ My}^{-1}$ we use is a point estimate that is based on vicariance of two sister species in the subgenus *Sorex* under the assumption that our focal taxa share similar mutation rates (Kumar and Subramanian, 2002). Identification and dating of *Sorex* fossils has been problematic (e.g., Harris, 1998; Kurtén and Anderson, 1980; Rzebik-Kowalska, 2005) and only applied to questions related to deeper structure within soricomorphs (e.g., Dubey et al., 2007; Esselstyn and Brown, 2009).

4.1. Rapid speciation

The history of the *cinereus* complex spans only the last 3 glacial cycles of the Quaternary (Fig. 3; $T_{\text{MRCA}} = 0.308 \pm 0.1$ Ma) and represents a rapid mammalian radiation. Considering that the probability of speciation is dependent on the range of the species (Rosenzweig, 1975) a high speciation rate among the *cinereus* complex is reasonable with such a vast combined geographic range. Though it is recognized that successive allopatric splitting of the geographic ranges of sister species often results in multiple smaller ranges, decreasing speciation rate through time (Waldron, 2007; Weir, 2006), evolution of the *cinereus* complex is best characterized by episodes of allopatric differentiation followed by range expansion, therefore maintaining relatively large ranges for sibling species and overall high speciation rate (Pigot et al., 2010; Fig. 5).

An initial transcontinental invasion of *Sorex* shrews into the Nearctic across the Beringian Isthmus occurred during the Miocene (13.9 Ma) from which species of the subgenus *Otisorex* differentiated (Dubey et al., 2007). Among the outgroup taxa represented here, coalescence of *S. trowbridgii* and other species is estimated at ~2.7 Ma, with a confidence interval that encompasses the onset of the Pleistocene (Fig. 3; Table S4). *Sorex trowbridgii* has recently been closely allied to a large clade mostly occurring in Mexico (Esteva et al., 2010). Remaining species within the subgenus *Otisorex* comprise the other major clade with a Nearctic origin. Onset of Pleistocene glaciations (~2.6 Ma) may be the primary driver of this early dichotomy in North America. Similarly, diversification among many Eurasian shrews in the subgenus *Sorex* have been dated to within the Pleistocene (Hope et al., 2011) suggesting that high diversity within the entire genus (77 extant species; Hutterer, 2005) was driven largely by major Pleistocene climate episodes and not geophysical change during the Miocene and Pliocene. We estimated a date of 850 ka for the T_{MRCA} of *S. rohweri* and the *cinereus* complex (Fig. 3), nearly coincident with onset of 100 kyr glacial periodicity. The entire *cinereus* complex coalesces at ~310 ka, coincident with the interglacial during Marine Isotope Stage 10

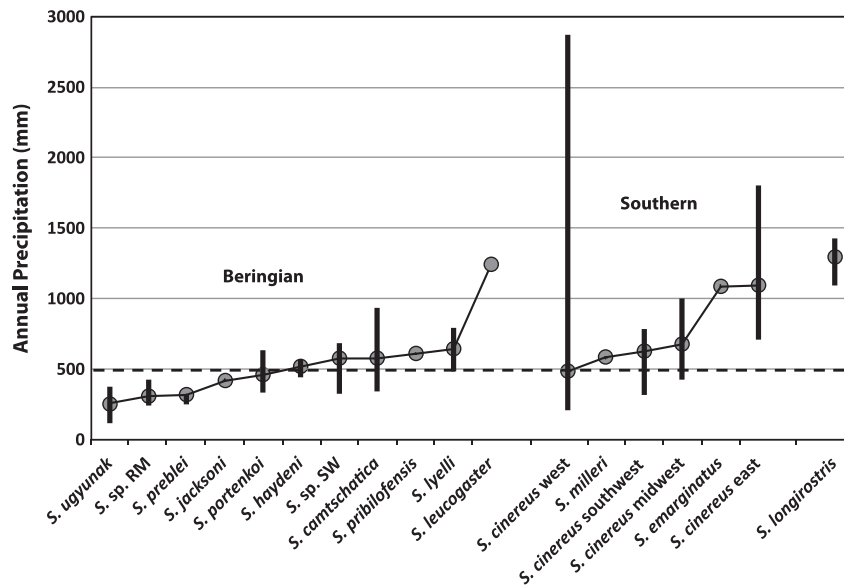


Fig. 4. Graph illustrating annual precipitation values by taxon. Annual precipitation for all specimen localities in Appendix A were compiled from the WorldClim database. Circles indicate mean annual precipitation and vertical bars indicate the range for each taxon. Taxa from each major clade are ordered by increasing mean precipitation and joined by a trend line through mean values. The horizontal dashed bar at 500 mm precipitation per year is provided as a rough transition from semi-arid desert (below line) to more temperate areas (above line).

at which time *S. longirostris* diverges, likely in eastern North America. Well-differentiated eastern clades also characterize other North American organisms due to postulated barriers such as the extensive glacial-melt outflow from the Laurentide ice sheet through much of the Pleistocene that became the Mississippi River and may have isolated populations longitudinally (Avice et al., 1998; Soltis et al., 2006). The Mississippi outflow fluctuated with climate cycling, especially during interglacial phases that were coincident with expansion of mesic habitats. Southern and Beringian clades (excluding *S. longirostris*) are estimated to have split during the Illinoian glacial period (~170 ka). We postulate that ancestors of the Southern clade were situated in south and central North America at this time. Few fossil shrews are available, although fossils identified as "*S. cinereus*" were found in several localities in Texas dated to the Late Pleistocene (Kurtén and Anderson, 1980) and also in Mexico (Esteve et al., 2005). Ancestors of the Beringian clade may have been isolated west of the Rocky Mountains and south of the Cordilleran ice sheet, likely in cold periglacial habitats. This is plausibly the period at which a major ecological division within the *cinereus* complex occurred. Members of the Southern clade are closely associated with coniferous forests and other mesic habitats whereas members of the Beringian clade are more closely associated with xeric and cold environments (Fig. 4).

The Sangamon interglacial (~130 ka) is coincident with division of the Beringian clade into high-latitude and mid-latitude species, with the former rapidly expanding northward following glacial retreat. High-latitude Beringian ancestors would have been periglacial and shifted north tracking tundra habitat, preceding boreal forest expansion that ultimately formed a forest barrier between high- and mid-latitude Beringian populations. This habitat barrier, much as at present, would have been continent-wide across most of Canada (Jaramillo-Correa et al., 2004; Lafontaine et al., 2010), effectively driving allopatric divergence of the non-forest species to north and south. Concurrently however, *S. cinereus* (Southern clade) likely expanded northward through this boreal forest belt.

Onset of the Wisconsinan glacial (~75 ka) would have allowed expansion of representatives of the Beringian clade across the Beringian Isthmus to occupy Siberia through this most recent glacial phase (Dokuchaev, 1997; Fig. 1). Transcontinental movement

of the *cinereus* complex westward represents the first instance of a Palearctic recolonization among shrews following initial invasion of the Nearctic during the Miocene. Mid-latitude species such as *S. preblei* and the arid-land group likely expanded to occupy western North America at lower elevations where forests were scarce. Further differentiation was potentially influenced by geographic features such as immense pluvial lakes or glaciated mountain ranges. *Sorex cinereus* receded to the south coincident with forest contraction during the glacial phase; Eastern *S. cinereus* was likely pushed south through New England and isolated in the eastern United States as postulated for other species such as *Sorex palustris* (Stewart et al., 2002). Greater divergence of *S. cinereus* (east) from other regional populations (Fig. 2) is likely due to long-term allopatry whereas other populations of *S. cinereus* may have experienced gene flow intermittently even through the Wisconsinan glacial phase. Since the LGM (~21 ka), the established ecological differences between Beringian and Southern species resulted in fragmentation of *S. sp. RM*, *S. sp. SW*, and *S. lyelli* (arid-land forms), but allowed expansion of *S. cinereus* (forest; Fig. 1). These demographic changes are reflected by mismatch distributions and expansion statistics (Supplementary Figs. 6 and 7; Table 2) where *S. cinereus* (west) and *S. cinereus* (midwest) rapidly expanded northward, but arid-land species exhibit ragged distributions indicating stable or contracting populations. A signal of expansion in the tundra-associated *S. ugyunak* may reflect population growth eastward from the Beringian refugium to occupy newly available tundra following glacial retreat.

Diversification among the *cinereus* complex is linked closely to repeated climate-influenced allopatry followed by range fluctuation (Jansson and Dynesius, 2002; Fig. 5). Importantly, the early ecological split between Beringian and Southern clades means that ongoing diversification is promoted during each warm and cold phase of climate cycles (Fig. 5). Such a dual phase of diversification may be viewed as a speciation ratchet that resulted in this rapid radiation. Fragmentation and divergence of *S. cinereus* in forest refugia during the Wisconsinan glacial period was followed by isolation and divergence of Beringian species during the current interglacial, the latter due to the sundering of Beringia, as recently as 11 ky (e.g., Fedorov et al., 1999), and fragmentation of xeric habitats

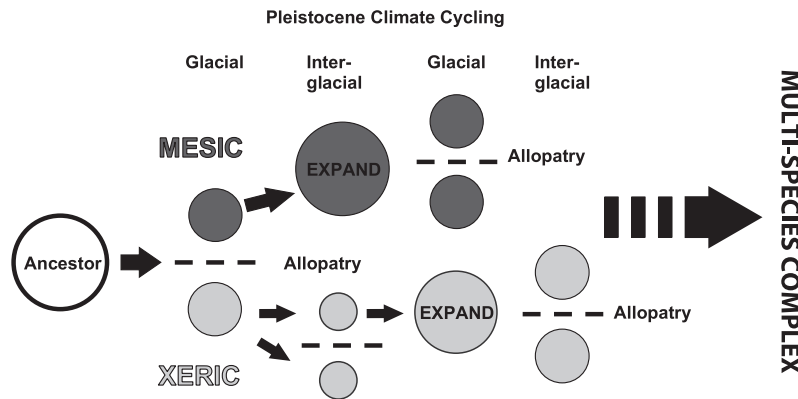


Fig. 5. A general schematic for diversification as applied to the *cinereus* complex illustrating a rapid radiation in response to Pleistocene climate change due to ecological differentiation, allopatry, and population expansion. Though not explicitly included, population contraction may also accompany allopatry.

further south in North America. A number of contact zones currently exist between species of this complex, always consisting of a Southern (i.e., *S. cinereus*, sensu stricto) and a Beringian clade species (Fig. 1). *Sorex cinereus* has been collected sympatrically with *S. haydeni* at the forest-grassland interface in Minnesota (Brunet et al., 2002), with *S. sp. RM* at the forest-sagebrush interface in Idaho, Montana, and Wyoming (Kirkland et al., 1997; pers. obs.), and with *S. ugyunak* at the forest-tundra interface within Alaska. The extent of divergence between Southern and Beringian clades may be sufficient to prevent significant hybridization. Habitats associated with these major clades appear to constitute barriers so that niche conservatism has maintained population isolation and divergence following initial episodes of allopatry (Wiens and Graham, 2005).

Hybridization within the *cinereus* complex has been recorded on at least one occasion between *S. cinereus* and *S. haydeni* (Brunet et al., 2002), although the extent of introgression appears limited to a narrow hybrid zone. Multiple instances of apparent incomplete lineage sorting among the nuclear genealogies (Supplementary Figs. S2–S5) may reflect historic hybridization or the slower sorting of nuclear alleles. With such a shallow history for the complex, repeated hybridization may have led to the underestimation of coalescence times among clades (Hewitt, 2011; Petit et al., 2003). In particular, ancient introgression in the history of the complex would have left signatures through disparate lineages that might impact phylogenies (e.g., Demboski and Cook, 2001; Good et al., 2003; Runck et al., 2009). Instances of gene flow were likely unevenly distributed, occurring particularly at the periphery of ranges for limited periods through glacial cycling. Without extensive introgression during admixture events, divergence between incipient species can increase over multiple climate cycles, resulting in rapid radiation (Hewitt, 2011).

4.2. Systematics

Demboski and Cook (2003) assessed genetic relationships among 9 of the 13 currently recognized species within the *cinereus* complex using two mtDNA loci. Subsequently Esteva et al. (2010) recognized the close alignment of *S. milleri* and *S. emarginatus* with *S. cinereus*, also using mtDNA. The present study reports the first molecular sequences for *S. leucogaster* and *S. lyelli* and includes the recently described *S. rohweri* (Nagorsen and Panter, 2009; Rausch et al., 2007). This expanded dataset also increases specimen and locality representation within species, particularly *S. cinereus* as suggested by Demboski and Cook (2003), highlights old systematic issues, and identifies potential incipient species. Demboski and Cook (2003) described two specimens from the Jemez Mountains of New Mexico that represented a disjunct and genetically distinct

population of *S. haydeni*. Multiple loci and expanded sampling (Table S1; Fig. 2) emphasize that this population (*S. sp. SW*), variably identified as *S. haydeni* (mtDNA; Demboski and Cook, 2003), *S. cinereus* (Findley et al., 1975), or *S. preblei* (Kirkland and Findley, 1996), is a member of the Beringian clade of shrews and most closely allied to *S. lyelli* (Fig. 3). *Sorex lyelli* is associated with the Beringian clade for the first time, suggesting that no member of the Southern clade occurs within the higher elevation forests of the Cascade or Sierra Nevada ranges of westernmost North America. *Sorex lyelli* likely shifted in elevation to inhabit the Sierra Nevada with aridification of the Great Basin in the Holocene, possibly coincident with the hypsithermal period (9–5 ka). A recognized phylogeographic break for other mammals north of the Cascade Range in Washington (Arbogast and Kenagy, 2001) is coincident with the western distributional limit of *S. cinereus*, although suitable forest habitat is continuous through this area and southward. *Sorex preblei* has been associated with arid sagebrush habitats from southern British Columbia (Nagorsen et al., 2001) through the Columbia Basin and Plateau of Oregon and south to northern California (Shohfi et al., 2006) and eastward to Utah, Idaho, Montana, and Wyoming (Cornely et al., 1992). Populations east of Oregon may represent an incipient species (*S. sp. RM*) that is most closely related to *S. lyelli* (Figs. 2 and 3). Systematic relationships among the high-latitude species of the Beringian clade remain unresolved, but limited divergence indicates a recent history of diversification (<50 kyr; Fig. 3) that did not extend across the multiple inundations of the Bering Strait during the Pleistocene.

Within the Southern clade, relatively high mitochondrial divergence of eastern populations of *S. cinereus* from other *S. cinereus* (Fig. 2) is consistent with George's (1988) conclusion that these are a separate species (*S. fontinalis*). However, nuclear variation indicated that *S. cinereus* (east) is well supported within *S. cinereus* (sensu stricto; Fig. 3). *Sorex milleri* was previously found to be allied with *S. cinereus* (Esteva et al., 2010) and this relationship is supported in this study, but we suggest that the validity of this species is questionable, as it is nested within *S. cinereus* (Fig. 2). Low-quality DNA from these specimens limited the number of loci recovered. Similarly the status of *S. emarginatus*, which appears to be a member of the *cinereus* complex (Esteva et al., 2010), should be further clarified. *Sorex longirostris* has been considered a member of the Southern clade based on allozymes (George, 1988) and mitochondrial DNA (Demboski and Cook, 2003; Fig. 2). However, our analyses suggest this divergence represents a deeper, basal split within the species complex (Figs. 3 and S1). We therefore recommend removal of *S. longirostris* from the Southern clade. Finally, morphological similarity of *S. rohweri* with *S. cinereus* suggests a close relationship to the *cinereus* complex (Nagorsen and Panter,

2009; Rausch et al., 2007). Although Cyt *b* genealogies have been unsuccessful in resolving the relationship of *S. rohweri* to other species (Rausch et al., 2007; current study), species tree estimation utilizing additional loci places *S. rohweri* as sister to the *cinereus* complex (Fig. 3). However, higher-level relationships among out-group taxa generally were not consistent across independent gene genealogies.

Our multi-locus assessment presents multiple avenues for future research, including further consideration of taxonomy. Although we do not formally recommend taxonomic revision, extremely low genetic diversity among all high-latitude Beringian species is less than the intra-specific differences seen among other high-latitude shrew species (e.g., Hope et al., 2010, 2011) and among other intra-specific clades identified in this study. Conversely, two distinct evolutionary units, previously considered as *S. preblei*, approach inter-specific levels of divergence that characterize other nominal shrews. Although initial morphological analyses formed the basis of species descriptions associated with this complex, our genetic assessment points toward the need for a comprehensive revision, with additional specimens from more locations for poorly represented taxa (*S. emarginatus*, *S. leucogaster*, *S. lyelli*, *S. milleri*, *S. preblei*) allowing for more detailed demographic and evolutionary analyses. Finally, novel methods for analysis of multi-locus data should provide more refined interpretations of historical demography and critical aspects of species relationships including relative lineage sorting versus gene flow, hybridization, and spatiotemporal diversification (reviewed in Hickerson et al. (2010)).

Transcontinental exchange through Beringia has been an ongoing evolutionary process across multiple taxonomic groups (Rausch, 1994; Waltari et al., 2007). There are also instances of multiple waves of colonization within a taxonomic group (e.g., Hope et al., 2011). However few instances exist of successive colonizations in both directions. An early movement of *Sorex* from the Palearctic into the Nearctic allowed for diversification within the subgenus *Otisorex* and has now culminated in a rare instance of a return to the Palearctic of high-latitude species within the *cinereus* complex.

5. Conclusions

This study characterizes the recent radiation of a diverse group of mammals consistent with phylogenetic divergence of different ecotypes during both glacial and interglacial phases of the late-Quaternary, following early ecological separation between the major clades of the complex (Fig. 5). Differentiation is stimulated by cycles of allopatry and subsequent rapid range change and expansion. The longer (100 kyr) periodicity and more extreme glacial cycles of the late-Quaternary likely helped to maintain isolation through a given cycle. A combination of abiotic (climate and geography) and biotic (habitat) factors can thus drive dramatic distributional and evolutionary change. Although the evolutionary mechanism presented here is exemplified by a complex of closely related small mammals, this mechanism need not apply only to close congeners and could be extended to include entire biotas in future comparative assessments. Quaternary climate has shaped biodiversity worldwide. It is becoming increasingly evident that such variability has promoted speciation despite relatively brief periodicity compared with the Neogene. Although formative early phylogeographic assessments have stressed contraction of species ranges into refugia and divergence during extended glacial phases (e.g., Comes and Kadereit, 1998; Hewitt, 1996; Taberlet et al., 1998), the current study provides further evidence of the importance of interglacial phases in promoting speciation among certain ecological groups (Stewart and Lister, 2001).

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ympev.2012.05.021>.

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