PHYLOGENETIC DIVERSIFICATION WITHIN THE
SOREX CINEREUS GROUP (SORICIDAE)

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Phylogenetic relationships among 8 members of the Sorex cinereus group (S. camtschatica, S. cinereus, S. haydeni, S. jacksoni, S. portenkoi, S. preblei, S. pribilofensis, and S. ugyunak) and S. longirostris were estimated using DNA sequence data from 2 mitochondrial genes, cytochrome b (1,140 base pairs) and nicotinamide adenine dinucleotide dehydrogenase 4 (582 base pairs). S. hoyi, S. monticolus, S. palustris, S. tenellus, S. trowbridgii, and S. vagrans also were included in our analyses. Phylogenetic analyses of the combined data recovered 2 major clades within the species group: a northern clade that includes the Beringian species (S. camtschatica, S. jacksoni, S. portenkoi, S. pribilofensis, and S. ugyunak), S. haydeni, and S. preblei and a southern clade that includes S. cinereus and S. longirostris. Mitochondrial DNA clades generally corresponded to previously identified morphological groups with 2 exceptions: inclusion of S. longirostris with S. cinereus in the southern clade and inclusion of S. preblei within the northern clade. With the exception of the 5 Beringian species, taxa were readily differentiated with strong bootstrap support in our topologies. We also noted phylogenetic concordance with the general ecological affiliations of each species; the northern clade generally includes xeric-affiliated species, whereas the southern clade includes species with mesic habitat affinities.

Key words: Beringia, biogeography, mitochondrial DNA, molecular systematics, shrew

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The Sorex cinereus group comprises 11 species of long-tailed shrews with members distributed across North America and into northeastern Asia (Hutterer 1993; Fig. 1). This group includes the only Palearctic representatives of the subgenus Otisorex (S. camtschatica, S. leucogaster, and S. portenkoi), the most widespread species of Sorex in North America (S. cinereus), and several oceanic (e.g., S. jacksoni) or continental (e.g., S. lyelli) island forms (Hall 1981). These species occur in a wide variety of habitats including deciduous, boreal and temperate rain forests, prairie, shrub-steppe, and tundra (French 1980; Tomasi and Hoffmann 1984; Youngman 1975).

Jackson (1928) originally proposed the S. cinereus group to include S. cinereus, S. fontinalis, S. lyelli, and S. preblei. Later studies added S. milleri (Findley 1955), the Beringian species (S. jacksoni, S. pribilofensis, and Russian forms—Hoffmann and Peterson 1967; Stroganov 1956), and S. haydeni (van Zyll de Jong 1980) to the group. Additional studies have presented different hypotheses concerning the taxonomy, biogeography, and evolutionary relationships of the group (summarized in Table 1). However, most studies were restricted in taxonomic and geographic scope, and a
comprehensive evolutionary synthesis of the *S. cinereus* group was not available until the expansive morphological treatment of the group by van Zyll de Jong (1991). His cladistic examination of quantitative (13 cranial measurements) and qualitative (e.g., dental pigmentation and pelage coloration) characters revealed 2 major clades within the *S. cinereus* group: “Beringian” (*S. camtschatica*, *S. jacksoni*, *S. haydeni*, *S. leucogaster*, *S. portenki*, and *S. pribilensis*) and “southern” (*S. lyelli*, *S. milleri*, *S. preblei*, and subspecies of *S. cinereus*). Taxa within the Beringian clade are generally characterized by shorter rostrums and tails and tricolored pelage (in some cases). Expanding on previous hypotheses that stressed the importance of Pleistocene glaciations (Hoffmann and Peterson 1967), van Zyll de Jong (1982, 1991) speculated that major diversification within the group coincided with the last major glacial expansion (Wisconsinan) and continued into the Holocene. van Zyll de Jong (1991) hypothesized that the Beringian clade diversified in Beringia and suggested that *S. haydeni* represented a southward migrant from this refugium to the northern Great Plains. Biochemical and molecular evidences have provided additional support for the close relationship of *S. cinereus*, *S. fontinalis*, *S. haydeni*, and *S. preblei* (including *S. longirostris*) within the group (Fumagalli et al. 1999; George 1988; Stewart and Baker 1994a, 1997; Stewart et al. 1993). However, these studies did not include the Beringian species, thus limiting insight into broader evolutionary and biogeographic questions for western North America and Asia. Mitochondrial DNA (mtDNA) introgression between *S. cinereus* and *S. haydeni* also has been reported that further complicates assessment of phylogenetic relationships within the group (Brunet et al. 2002; Stewart and Baker 1997).

Our goal was to provide an expanded molecular reexamination of the morphological phylogeny presented by van Zyll de Jong (1991). We estimated phylogenetic relationships on the basis of sequence variation from 2 mitochondrial genes, cytochrome *b* and nicotinamide adenine dinucleotide dehydrogenase 4, for 8 of 11 species within the *S. cinereus* group. We tested whether our mitochondrial DNA phylogeny is consistent with the existing morphological framework and examined biogeography of the *S. cinereus* group within the context of general ecological affiliations.

**Materials and Methods**

We examined 46 individuals (Appendix I) representing 8 species of the *S. cinereus* group recognized by van Zyll de Jong (1991). Two specimens tentatively identified as *S. cinereus* from New Mexico (J. Findley, pers. comm.) were also included. In addition, *S. longirostris* was included in our analyses based on its close relationship with *S. cinereus* (George 1988). Given a lack of unambiguous sister taxa for the *S. cinereus* group (Fumagalli et al. 1999), we included 5 additional species from within the subgenus Otisorex: *S. hoyi*, *S. monticolus*, *S. palustris*, *S. tenellus*, and *S. vagrans*. *S. trowbridgii* has been shown to be a basal species within *Otisorex* (Fumagalli et al. 1999) and was the designated out-group for all phylogenetic analyses.

Genomic DNA was extracted from heart, kid-
Table 1.—Summary of some major studies that have examined members of the Sorex cinereus group. Data include studies of allozymes (A), DNA sequences (D), ecology and distribution (ED), karyotype (K), cranial morphology (MC), external morphology (ME), and penile morphology (MP).

<table>
<thead>
<tr>
<th>Type of data</th>
<th>Study</th>
<th>Taxonomic conclusion</th>
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<tbody>
<tr>
<td>MC, ME</td>
<td>Jackson (1928)</td>
<td>Sorex cinereus group proposed</td>
</tr>
<tr>
<td>MC, ME, ED</td>
<td>Hoffmann and Peterson (1967)</td>
<td>Beringian species allied with S. cinereus</td>
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<tr>
<td>K</td>
<td>Meylan (1967)</td>
<td>Specific distinction of S. cinereus</td>
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<td>ME, MP</td>
<td>Yudin (1969)</td>
<td>Specific distinction of S. cinereus, S. beringianus (=S. leucogaster), and S. hydrodromus (=S. pribilofensis)</td>
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<tr>
<td>ME, MC</td>
<td>van Zyll de Jong (1980)</td>
<td>Specific distinction of S. haydeni</td>
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<tr>
<td>A</td>
<td>Stewart et al. (1993)</td>
<td>Paraphyly (S. cinereus and S. haydeni)</td>
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<tr>
<td>D</td>
<td>Stewart and Baker (1994b, 1997), Brunet et al. (2002)</td>
<td>Paraphyly or mtDNA introgression (or both) of S. haydeni and S. cinereus</td>
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<tr>
<td>K, MP</td>
<td>Rausch and Rausch (1995)</td>
<td>Sorex jacksoni subsumed to S. c. jacksoni</td>
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<tr>
<td>ED</td>
<td>Dokuchaev (1997)</td>
<td>Multiple colonization of Asia by members of the S. cinereus group</td>
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<tr>
<td>D</td>
<td>Fumagalli et al. (1999)</td>
<td>Sister relationship of S. cinereus and S. haydeni</td>
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Previous analyses of both the cytochrome-\(b\) and nicotinamide adenine dinucleotide dehydrogenase 4 sequences by Demboski (1999) recovered the same major clades separately and in combination; therefore, all results presented in this study are based on the combined data (1,722 base pairs). Analyses were conducted using parsimony and maximum-likelihood (ML) methods. Initially, PAUP* 4.0b2-8a (Swofford 2001) was used to conduct an equal-weight parsimony search (heuristic, stepwise addition, 10 random addition replicates, and tree-bisection–reconnection branch swapping) using all sequences (\(n = 46\)). Nodal support was estimated using bootstrap analysis with 500 replicates (Felsenstein 1985). Unequal base composition of sequences was tested using the chi-square test implemented in PAUP*. 
Due to the computational constraints of ML, the data set was pruned to 32 unique haplotypes. A neighbor-joining topology based on p-distances served as the starting point for evaluating different models of sequence substitution. These included the Jukes–Cantor (Jukes and Cantor 1969), Kimura 2-parameter (Kimura 1980), Hasegawa–Kishino–Yano (Hasegawa et al. 1985), Tamura–Nei (Tamura and Nei 1993), and general time-reversible (Lanavé et al. 1984) models. Additional parameters estimated with each model included the proportion of invariable sites (Hasegawa et al. 1985), discrete approximation to the Γ-distribution (Yang 1994), and a mixed distribution model (Gu et al. 1995). Because all 20 models evaluated were hierarchically nested, likelihood-ratio tests were used to determine the most appropriate model from among those evaluated (Yang et al. 1995). A fully defined ML search (heuristic, stepwise addition, and tree-bi-section–reconnection branch swapping) was then conducted using the chosen model (general time-reversible + I + Γ). Model parameters were reestimated from the 1st tree recovered and then used in a subsequent ML search (e.g., Sullivan et al. 1997). This strategy continued until the likelihood score and parameter estimates stabilized. Nodal support for the ML tree was assessed using both ML (general time-reversible + I + Γ; 100 replicates) and parsimony (500 replicates) bootstrap analyses (Felsenstein 1985). We also tested the molecular clock hypothesis for our data using a likelihood-ratio test (Felsenstein 1988).

We used the Shimodaira–Hasegawa test (Shimodaira and Hasegawa 1999) as implemented in PAUP* (Swofford 2001) to compare van Zyll de Jong’s (1991) Wagner parsimony topology (Fig. 1) with the best ML tree recovered in our analysis. The Shimodaira–Hasegawa test allowed us to compare both an a priori–selected and a posteriori–selected relationships; the appropriate use of this test is discussed in Goldman et al. (2000). We were specifically interested in the placement of S. preblei within the southern clade, a finding reported by van Zyll de Jong (1991). We estimated the optimal ML tree constrained to fit relationships revealed by van Zyll de Jong’s (1991) midpoint-rooted topology [[preblei, cinereus, longirostris], remaining taxa]. The likelihood score of the constrained tree (\(L_{VZDJ}\)) was then compared with the score of the best ML tree (\(L_{ML}\)) using the Shimodaira–Hasegawa test. To reduce computational time associated with full likelihood optimizations for each of the 10,000 replicates, bootstrap data sets, we used resampling-estimated log-likelihood optimization. This approach bypasses reestimation of model parameters for each bootstrap replicate (Kishino et al. 1990).

RESULTS

The complete cytochrome-\(b\) gene had 1,140 base pairs. Partial nicotinamide adenine dinucleotide dehydrogenase 4 sequences (582 base pairs) corresponded to positions 10914–11495 in Mus (Bibb et al. 1981). GenBank accession numbers for these sequences are listed in Appendix I. Levels of variation between cytochrome \(b\) and nicotinamide adenine dinucleotide dehydrogenase 4 were similar. Within the complete data set (\(n = 46\)), 482 (28%) sites were observed to vary. Of these, 333 (19%) were parsimony informative. Variation was distributed among codon positions such that 1st- (18%) and 3rd-position (81%) sites accounted for 99% of the total variation. The null hypothesis of base stationarity across sequences, as determined in PAUP*, was not rejected (\(\chi^2 = 12.60, \text{ d.f.} = 135, P = 1.0\); ignoring correlation due to phylogenetic structure). Nuclear copies of mtDNA genes (pseudogenes) have been documented in some species of mammals (Smith et al. 1992); however, characteristics of pseudogenes such as a relaxation of compositional biases or sequences truncated with stop codons were not observed in our data.

The parsimony search conducted with all sequences (\(n = 46\)) recovered 165 equally parsimonious trees of 965 steps (consistency index [CI] = 0.595, retention index [RI] = 0.835, rescaled CI = 0.497). A strict consensus of these trees was similar to the 1st of the trees (Fig. 2) except for resolution of tip relationships within the S. cinereus group. Twenty models of sequence substitution were evaluated with the reduced data set (\(n = 32\)). The likelihood score obtained under the general time-reversible + I + Γ model was the best overall (\(-\ln L = \))
Fig. 2.—Equal-weight parsimony tree (heuristic search) recovered using the complete data set ($n = 46$). This is 1 of 165 equally parsimonious trees of 965 steps (consistency index [CI] = 0.595, retention index = 0.835, rescaled CI = 0.497). Tree was rooted with *Sorex trowbridgii*. Bootstrap values of $\geq 50\%$ (500 replicates) are shown below branches. The *S. cinereus* group, northern–southern dichotomy, and Beringian clades are depicted. Two tentatively identified specimens of *S. cinereus* from New Mexico are designated with an asterisk. Specimen numbers are given after specific names (Appendix I).
indicated that we must reject the assumption that molecular clock tests with both ML topologies was with the next simplest model, general time-reversible+$\Gamma$, was significantly better ($-\ln L = 6,448.77; \chi^2 = 14.80, d.f. = 1, P < 0.0001$). The remaining models evaluated also were significantly worse. A subsequent search under the optimized general time-reversible+$1+\Gamma$ model ($r_{AC} = 2.77, r_{AG} = 20.88, r_{AT} = 2.99, r_{CG} = 0.24, r_{CT} = 45.28, \alpha = 2.42, p_{inv} = 0.64$) recovered 2 equally likely trees ($-\ln L = 6,428.82$). The trees differed only with regard to placement of the branch uniting the 3 S. haydeni sequences (AFTC23162, AFTC23163, and NK7942).

Congruence between parsimony and ML topologies included the recovery of a strongly supported clade ($\geq 92\%$ bootstrap support) corresponding to the S. cinereus group with the inclusion of S. longirostris (Figs. 2 and 3). The major bifurcation corresponds to southern (S. cinereus–S. longirostris) and northern (S. preblei–S. haydeni–Beringian species) clades. Within the northern clade, 3 additional subclades were recovered. One of these consisted of the closely related Beringian species, S. camtschatica, S. jacksoni, S. portenkoi, S. pribilofensis, and S. ugyunak (Figs. 2 and 3). Sister to the Beringian species were S. haydeni and 2 samples tentatively identified as S. cinereus from northern New Mexico (J. Findley, pers. comm.). The basal subclade within the northern group consisted of the 2 samples of S. preblei from Oregon. Within the southern clade, subclades composed of S. cinereus and S. longirostris were apparent.

Members of the ‘‘S. monticolus–vagrants’’ group were monophyletic in both analyses (Figs. 1 and 2), and a weakly supported relationship between S. hoyi and this group was observed. S. tenellus was basal to all taxa excluding S. trowbridgii, the designated out-group. An unambiguous sister taxon to the S. cinereus group was not apparent in our analyses. Results of the molecular clock tests with both ML topologies indicated that we must reject the assumptions of clocklike evolution in our data ($\chi^2 = 60.88, d.f. = 30, P < 0.005$).

Results of the Shimodaira–Hasegawa test led us to reject van Zyll de Jong’s (1991) hypothesis that S. preblei is nested within the southern clade. The difference in likelihood scores between the best ML tree and an ML tree constrained to fit van Zyll de Jong’s hypothesis was $\delta = L_{ML} - L_{VZDJ} = -6,428.82 - (-6,591.52) = 162.70$. The result of the Shimodaira–Hasegawa test indicates that the inclusion of S. preblei within the mtDNA southern clade can be rejected ($P < 0.0001$).

**DISCUSSION**

**Phylogenetic and taxonomic considerations.**—Taxa within the S. cinereus group were readily differentiated in both parsimony and ML topologies with the exception of the 5 Beringian species (Figs. 2 and 3). The northern–southern mtDNA dichotomy (about 6\% uncorrected sequence divergence) that we observed within the S. cinereus group generally corresponds to the major morphological clades recognized by van Zyll de Jong (1991). One major difference is our placement of S. preblei within the mtDNA northern clade, which contrasts with the morphological phylogeny (van Zyll de Jong 1991). van Zyll de Jong (1991) allied S. preblei with the southern clade but noted that it was the most divergent taxon within this clade in the cladistic analysis. When we constrain S. preblei to the mtDNA southern clade, to reflect the existing morphological framework, we are able to easily reject this relationship based on the results of the Shimodaira–Hasegawa test. In addition, our results indicate that S. longirostris is the sister taxon to S. cinereus in the southern clade.

At this stage, we suggest no major taxonomic revisions for the S. cinereus group other than the inclusion of S. longirostris in this species group. In addition, we note that there is a lack of resolution among the 5 Beringian species (Figs. 2 and 3). The maximum level of uncorrected sequence diver-
Fig. 3.—One of 2 maximum-likelihood trees recovered (−ln L = 6,428.82) for the pruned data set (n = 32) using general time-reversible +I+Γ model. The 2 trees differed only with regard to placement of the node uniting *Sorex haydeni* samples. Tree was rooted with *S. trowbridgii*. Maximum-likelihood (100 replicates) and parsimony (500 replicates) bootstrap values (≥50%) are shown below the branches. The *S. cinereus* group, northern–southern dichotomy, and Beringian clades are depicted. A tentatively identified specimen of *S. cinereus* from New Mexico is designated with an asterisk. Specimen numbers are given after specific names (Appendix I).
gence within the Beringian clade (0.81%) is less than that in most interspecific comparisons previously reported in *Sorex* (Demboski and Cook 2001; Fumagalli et al. 1999) and in comparisons among different populations of *S. cinereus* examined in this study (1.34%). *S. jacksoni* and *S. pribilofensis* are characterized by slightly longer terminal branches (Fig. 2), and this is probably indicative of their isolation on St. Lawrence and St. Paul islands, respectively, with the flooding of the Bering Strait (about 10,000–16,000 years ago—Hopkins et al. 1982).

It might be tempting to suggest taxonomic changes within the Beringian clade based on our data; however, these taxa have an unsettled taxonomic history with many conflicting conclusions (Table 1). Rausch and Rausch (1995) concluded that *S. jacksoni* was a subspecies of *S. cinereus* (*S. c. jacksoni*) based on analysis of karyotypes (2n ‡ 66, FN ‡ 70) that were identical to *S. cinereus* from mainland Alaska. This designation requires recognition of polyphyletic relationships when compared with our mtDNA results, which indicate a nonsister relationship between *S. cinereus* and *S. jacksoni* combined with about 6% uncorrected sequence difference (Figs. 2 and 3). In contrast, *S. portenkoi*, which is indistinguishable from *S. jacksoni* and other Beringian species on our analysis, possesses a karyotype (2n = 60, FN = 62—Ivanitskaya and Kozlovsky 1985) that is distinct from that of both *S. jacksoni* and *S. cinereus*. Known karyotypes for members of the *S. cinereus* group, viewed within the context of the mtDNA results, suggest that the distinct karyotypes of *S. portenkoi* and *S. haydeni* (2n = 64, FN = 66) may represent derived states. The difficulty of identifying an unambiguous sister taxon to the *S. cinereus* group and the absence of karyological information for some taxa prevent characterization of an ancestral karyotype for the group. Taxonomic revisions within the Beringian clade should await information from additional molecular characters, expanded geographic sampling within taxa, and the inclusion of *S. leucogaster* (2n = 66, FN = 70—Ivanitskaya and Kozlovsky 1985).

Specific status for *S. haydeni* has been supported by previous biochemical, chromosomal, molecular, and morphological studies (Fumagalli et al. 1999; George 1988; van Zyll de Jong 1980; Volobouev and van Zyll de Jong 1994). Our mtDNA data corroborate those earlier studies and suggest the possibility of previously undetected phylogenetic and geographic diversities within this species (Figs. 2 and 3). The tentative classification of 2 New Mexico specimens as *S. cinereus* (NK42552 and NK42640—J. Findley, pers. comm.) appears incorrect based on the close sister relationship of these individuals to *S. haydeni* from the northern Great Plains. These may be the 1st records of “*S. haydeni*” from New Mexico, but additional sampling of the southern Rockies is needed to expand on these preliminary results. In Alberta and Minnesota, bidirectional, mitochondrial introgression between *S. cinereus* and *S. haydeni* has been reported (Brunet et al. 2002; Stewart and Baker 1994b, 1997). These species are not sister taxa based on our analyses, do not appear to exhibit morphological intergradation (van Zyll de Jong 1980), and possess distinct karyotypes (Volobouev and van Zyll de Jong 1994). Analysis of nuclear loci would provide information regarding levels of gene flow at these hybrid zones and an insight into species boundaries in *Sorex*.

Although morphologically similar to *S. cinereus* (Klippel and Parmalee 1982), *S. longirostris* was considered by Findley (1955) to be part of a group allied with western taxa; *S. vagrans*, *S. ornatus*, and Mexican species. On the other hand, Junge and Hoffmann (1981) opted not to include *S. longirostris* within any proposed species groups in *Sorex*. Our analyses corroborate the allozyme study of George (1988) that identified a close relationship between *S. longirostris* and the *S. cinereus* group.
However, contrary to the allozyme data, our results place *S. longirostris* sister to *S. c. fontinalis* (= *S. c. fontinalis*), *S. haydeni*, and *S. preblei*. These differences may reflect sampling biases or differential sorting of mtDNA relative to nuclear genes in rapidly evolving taxa (Avise 1994).

Previous biochemical and molecular work has been inconclusive regarding identification of a sister taxon for the *S. cinereus* group. The most expansive treatments of *Sorex* have concluded that deeper nodes within the subgenus *Otisorex* were the result of a rapid radiation that obscured these relationships (Fumagalli et al. 1999; George 1988). Stewart and Baker (1994a) suggested that *S. fumeus*, which was not sampled for this study, was sister to *S. cinereus* and *S. haydeni*; however, their sampling was limited to 7 species of *Sorex*. Our limited survey of other species of *Sorex* was also inconclusive. Inclusion of additional North American species of *Sorex* may shed light on this question; however, the polytomy that characterizes early diversification within *Otisorex* may still obscure resolution at internal nodes (Fumagalli et al. 1999).

Implications for historical biogeography.—Phylogenetic diversity within *Sorex* is thought to be a result of repeated climatic and geological changes that led to large-scale diversification across the Northern Hemisphere (Findley 1955; George 1988; Reumer 1989). Late Pleistocene climatic fluctuations have been postulated as having an important role in this diversification (Findley 1955; George 1988; Reumer 1989; van Zyll de Jong 1991). Indeed, it is tempting to suggest that climatic oscillations during the late Pleistocene shaped many of the phylogenetic relationships within the *S. cinereus* group as has been previously postulated (George 1988; van Zyll de Jong 1991). Fossil estimates for the 1st appearance of members of the *S. cinereus* group are also within a Pleistocene time frame (Kurtén and Anderson 1980). Unfortunately, violation of the molecular clock assumption for our data prohibits estimation of divergence dates within the *S. cinereus* group. However, with this caveat in mind, we speculate that initial divergence leading to the northern–southern dichotomy most likely predates the late Pleistocene. The beginning of extreme climatic oscillations, after a long period of relative stasis during the Pliocene, may have been the catalyst for phylogenetic diversification within the *S. cinereus* group.

Our results and those of other molecular studies suggest that multiple southern refugia were available during glacial advances in the Pleistocene (Conroy and Cook 2000a; Cook et al. 2001; Demboski and Cook 2001; Good and Sullivan 2001; Hayes and Harrison 1992). Within the northern clade, divergence between *S. preblei* and *S. haydeni*–Beringian clades could be the result of a general east–west segregation of ancestral populations. During a later climatic shift, ancestral populations leading to *S. haydeni* and members of the Beringian clade may have become isolated north and south of continental ice sheets. Given the moderate level of divergence (about 2%) between the Beringian clade and *S. haydeni*, the hypothesis that *S. haydeni* represents a late Pleistocene off-shoot of the Beringian clade that has recently colonized southward into the Great Plains through a Wisconsinan ice-free corridor is not supported (van Zyll de Jong 1991). The observation that samples of the tentatively identified “*S. cinereus*” from New Mexico (NK42552 and NK42640) are geographically separate, yet minimally differentiated (0.87%), from *S. haydeni* of the northern Great Plains suggests a recent (possibly late Pleistocene–Holocene timeframe) subdivision of a previously widespread ancestral population that was partially displaced to the southwestern United States. This level of divergence is similar to levels observed within the Beringian species. A similar biogeographic scenario leading to relictual isolates of the northern jumping mouse, *Zapus hudsonius*, in the southwestern United
States was previously observed (Hafner et al. 1981) and provides an insight into the possible assembly of continental biotas in this region (Riddle 1998).

Within the southern clade, divergence of *S. cinereus* and *S. longirostris* (about 2.6%) may represent a mid-Pleistocene north–south geographic segregation in eastern North America. During the late Pleistocene–early Holocene, western forms of *S. cinereus* may have tracked the movement of boreal forest northward into central Alaska and Canada after recession of continental ice sheets. The east–west dichotomy observed within *S. cinereus* (Fig. 2) appears to be relatively recent based on levels of sequence divergence (1.34%) but will require expanded sampling throughout the range of *S. cinereus* to be fully characterized.

**Amphiberian diversification.**—A lack of divergence among populations on opposite sides of the Bering Strait reflects the recent separation of Beringian forms by the late Pleistocene–early Holocene marine transgression of the Bering Land Bridge. As continental ice sheets contracted, *S. ugyunak* probably colonized eastward out of Beringia to occupy its current range across northern Canada and Alaska. Biogeographic hypotheses invoking multiple invasions of Asia have been posited based on ecological and morphological data (Dokuchaev 1997; Král and Ivanitskaya 1973; Okhotina 1977; Yudin 1973). These studies have suggested that *S. camtschatica*, *S. leucogaster*, and *S. portenkoi* are differentiated as a result of 2 or 3 unidirectional invasions of Asia. However, genetic divergence is minimal among taxa in the Beringian clade (Figs. 2 and 3), indicating that geographic, morphological, and ecological differentiation among these taxa is recent. Biogeographic scenarios that involve multiple amphiberingian invasions are not supported by our data. Biochemical and molecular studies of other mammals (e.g., *Lemmus*, *Lepus*, and *Microtus*) also have reported minimal amphiberingian differentiation, and these findings are consistent with the most recent flooding of the Bering Strait (Conroy and Cook 2000b; Fedorov et al. 1999; Halanych et al. 1999; Lance and Cook 1998).

The role of Beringia as a filter between the North American and Asian continents has been an important paradigm (Simpson 1940). However, the Beringian refugium also has been suggested as a major region of diversification for high-latitude organisms (Hoffmann 1981, 1984; Hopkins et al. 1982). Other genetic studies provide support for this hypothesis (Lance and Cook 1998; Steppan et al. 1999) and still others provide evidence that various refugia were available across Beringia (Abbott et al. 2000; Holder et al. 2000). The distinct Beringian clade includes members that span the 2 continents, are endemic to the Bering Sea region, and apparently originated in this high-latitude refugium.

**Ecological implications.**—Current habitat affiliations, coupled with our mtDNA phylogeny, indicate a fundamental division of the *S. cinereus* group into mesic- and xeric-affiliated clades. These broad ecological classifications generally correspond to the major mtDNA dichotomy within the *S. cinereus* group (Fig. 4). A postulated late Pliocene or early Pleistocene time frame (about $2 \times 10^6$ years ago) for initial divergence between the northern and southern clades would coincide with the general cooling trend that preceded the glacial–interglacial cycles characterizing much of the Pleistocene. During cooler glacial periods, the presence of large continental ice sheets contributed to the development of heterogeneous landscapes found in refugia. For example, xeric habitats (e.g., tundra and shrub-steppe) were found in Beringia and the southern fringe of continental ice sheets, whereas more mesic habitats (e.g., boreal and deciduous forest) were found farther south in North America (Davis 1983). Our results suggest that isolation in distinct mesic and xeric refugia may explain the primary subdivision found in this group.

Members of the Beringian clade inhabit
the xeric conditions of the “arctic desert” that have characterized Beringia during the Pleistocene and into the Holocene (Elias et al. 1996; Hopkins et al. 1982). *S. haydeni* and *S. preblei* also are found in distinctly arid habitats such as areas of the Great Plains (van Zyll de Jong 1980) and shrub-steppe regions of the western United States (Cornely et al. 1992), respectively. The observation that tentatively identified *S. cinereus* from New Mexico may actually represent a western population of the prairie-affiliated *S. haydeni* is logical. These individuals were collected in xeric Ponderosa pine stands of the Jemez Mountains (Kirkland and Findley 1996). General ecological affiliation is also a better predictor of phylogeny than is morphology with regard to *S. preblei* (Fig. 4). This species is most commonly found in arid shrub-steppe habitats in the western United States (Cornely et al. 1992). Within the mesic southern clade, *S. longirostris* is associated with marsh areas and deciduous forests (French 1980). *S. cinereus*, with the largest range of any North American shrew, is found across the continental boreal forest, with northern expansion into forest–tundra fringe and southern reaches into deciduous forest (Junge and Hoffmann 1981). There are exceptions to this general pattern of mesic and xeric diversification. For example, lack of divergence between the forest-affiliated *S. camtschatica* and tundra-affiliated shrews within the Beringian clade suggests that *S. camtschatica* has colonized boreal forest along the Sea of Okhotsk and the Kamchatka Peninsula since the late Pleistocene. MtDNA does not provide support for divergence of *S. camtschatica* in a forest refuge as suggested by Dokuchaev (1997) and van Zyll de Jong (1991). There also are other examples of mesic–xeric diversification within *Sorex* in North America. The known members of the unnamed subgenus identified by George (1988) comprise 2 xe-
monic-affiliated sister species (S. arizonae and S. merriami) and a 3rd, mesic-affiliated species, S. trowbridgii. Two closely related western United States species, S. naus and S. tenellus, also represent xeric-affiliated taxa, but the phylogenetic relationships of these 2 species with other members of Sorex remain unclear (George 1988).

Our study provides a preliminary molecular framework of phylogenetic relationships within this widespread group. Expanded sampling of the S. cinereus group should include S. leucogaster, S. lyelli, and S. milleri, in conjunction with more extensive intraspecific sampling of eastern forms of S. cinereus.

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LITERATURE CITED


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APPENDIX I

Specimens examined.—Specimens or sequences were obtained from the following institutions or individuals (with abbreviations): Alaska Frozen Tissue Collection or University of Alaska Museum (AFTC or UAM); University of Washington Burke Museum (UWBM); Museum of
Southwestern Biology (NK); Virginia Museum of Natural History (VMNH); P. Kennedy (PK); N. Dokuchaev (ND). GenBank numbers are indicated by AF and AY. Sample size in parentheses follows specific name. Localities are followed by specimen, cytochrome b GenBank, and nicotinamide adenine dinucleotide dehydrogenase 4 GenBank numbers in parentheses.

*Sorex camtschatica* (5).—RUSSIA, Magadan (AFTC6631, AY014917, AF250426), (AFTC-6632, AY014918, AF250427), (AFTC6569, AY014919, AF250425), (ND3496, AY014920, AF250428); RUSSIA, Kamchatka Peninsula (UWBM39234, AY014916, AF250424).

*Sorex cinereus* (15).—ALASKA, Alaska Peninsula (AFTC1571, AY014946, AF250454); ALASKA, Chandalar Shelf (AFTC5127, AY014942, AF250450); ALASKA, Cleveland Peninsula (AFTC4732, AY014947, AF250455); ALASKA, Fairbanks (no number, AY014944, AF250452); ALASKA, Kanuti National Wildlife Refuge (AFTC24304, AY014951, AF250460); ALASKA, Misty Fiords (AFTC4300, AY014949, AF250458); ALASKA, Haines (AFTC4587, AY014950, AF250459); ALASKA, Seward Peninsula (AFTC7380, AY014943, AF250451); (AFTC7436, AY014945, AF250453); MONTANA, Carbon County (AFTC14892, AY014948, AF250456), (AFTC14893, AF250438, AF250457); MINNESOTA, Clay County (NK7943, AY014941, AF250449); Goodhue County (AFTC25384, AY014952, AF250461); NEW MEXICO, Sandoval County, tentative identification (NK42552, AY014935, AF250442), (NK42640, AY014934, AF250443).

*Sorex haydeni* (3).—MINNESOTA, Clay County (NK7942, AY014938, AF250446); SOUTH DAKOTA, Davison County (AFTC-23162, AY014939, AF250447), (AFTC23163, AY014940, AF250448).

*Sorex hoyi* (1).—ALASKA, Hughes Quadrangle (AFTC7982, AF238040, AF250467).

*Sorex jacksoni* (5).—ALASKA, Saint Lawrence Island (UAM21518, AY014922, AF250430), (UAM21520, AY014923, AF250431), (UAM21521 AY014924, AF250432), (UAM21522, AY014925, AF250433), (UAM21526, AY014926, AF250434).

*Sorex longirostris* (2).—TENNESSEE, Perry Co. (PK4847, AY014954, AF250463); VIRGINIA, Mecklenburg County (NDM3113, AY014953, AF250462).

*Sorex monticolus* (1).—CANADA, Yukon Territory (AFTC10905, AF238014, AF250464).

*Sorex palustris* (1).—ALASKA, Petersburg Quadrangle (AFTC2806, AF238033, AF250466).

*Sorex portenki (1).—RUSSIA, Provideniya (AFTC7467, AY014921, AF250429).

*Sorex preblei* (2).—OREGON, Harney County (NK652, AY014936, AF250444), (NK656, AY014937, AF250445).

*Sorex pribilofensis* (3).—ALASKA, St. Paul Island (UAM22593, AY014931, AF250440), (UAM22594, AY014932, AF250439), (UAM22596, AY014933, AF250441).

*Sorex tenellus* (1).—CALIFORNIA, Mono County (NK5904, AY014955, AF250468).

*Sorex trowbridgii* (1).—WASHINGTON, Kittitas County (AFTC14146, AY014956, AF250469).

*Sorex ugyunak* (4).—ALASKA, Barrow (AFTC11764, AY014927, AF250435); ALASKA, Galbraith Lake (AFTC7213, AY014929, AF250437); ALASKA, Seward Peninsula (AFTC7390, AY014928, AF250436), (AFTC7431, AY014930, AF250438).

*Sorex vagrans* (1).—MONTANA, Lake County (AFTC24263, AF154551, AF250465).