

EVOLUTIONARY HISTORY OF THE ARCTIC GROUND SQUIRREL (*SPERMOPHILUS PARRYII*) IN NEARCTIC BERINGIA

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Pleistocene glaciations had significant effects on the distribution and evolution of arctic species. We focus on these effects in Nearctic Beringia, a high-latitude ice-free refugium in northwest Canada and Alaska, by examining variation in mitochondrial cytochrome *b* (*Cytb*) sequences to elucidate phylogeographic relationships and identify times of evolutionary divergence in arctic ground squirrels (*Spermophilus parryii*). This arctic-adapted species provides an excellent model to examine the biogeographic history of the Nearctic due to its extensive subspecific variation and long evolutionary history in the region. Four geographically distinct clades are identified within this species and provide a framework for exploring patterns of biotic diversification and evolution within the region. Phylogeographic analysis and divergence estimates are consistent with a glacial vicariance hypothesis. Estimates of genetic and population divergence suggest that differentiation within Nearctic *S. parryii* occurred as early as the Kansan glaciation. Timing of these divergence events clusters around the onset of the Kansan, Illinoian, and Wisconsin glaciations, supporting glacial vicariance, and suggests that *S. parryii* survived multiple glacial periods in Nearctic Beringia. Across the Arctic, Beringia has been identified as an important regional refugium for a number of species. Within Nearctic Beringia, genetic differentiation across populations of arctic ground squirrels further reflects the effect of glacial patterns on a finer scale. The arctic ground squirrel has had a long evolutionary history in the Nearctic, with strong phylogeographic structure and stable clades persisting through multiple glacial cycles.

Key words: arctic ground squirrel, Beringia, cytochrome *b*, phylogeography, *Spermophilus parryii*

Pleistocene glaciations had a major effect on the biota of northern latitudes (Hewitt 1996, 2001). Species distributions were modified repeatedly by the advance and retreat of glacial ice, which isolated some species in refugia and potentially led to differentiation. Beringia was a large glacial refugium, or series of refugia, throughout the glacial advances of the Pleistocene (Hoffmann 1981, 1986; Hopkins 1967, 1982; Hultén 1937; Macpherson 1965; Pielou 1991). Beringia consisted of portions of Palearctic eastern Siberia, Nearctic Alaska, and western Yukon Territories in Canada. The region has played a central role in understanding the effects of glaciation on Holarctic species. During glacial periods, water was sequestered in glaciers, causing sea levels to drop, exposing the Bering Land Bridge, and providing a corridor for the migration of species between Asia and North America (Pielou 1991). Research on a variety

of taxa has demonstrated that Beringia was key to structuring extant diversity in the Arctic (Hoffmann 1981, 1986; Hopkins 1967; Hultén 1937; Macpherson 1965; Pielou 1991).

Nearctic Beringia has been considered a source for post-glacial recolonization of northern North America following the recession of the Laurentide and Cordilleran glaciers, which extended over much of the higher latitudes of the continent (Pielou 1991). Recent phylogeographic and population genetic studies of plants (Abbott et al. 2000; Tremblay and Schoen 1999), fish (Bernatchez and Wilson 1998), birds (Holder et al. 1999), and mammals (Brunhoff et al. 2003; Demboski and Cook 2003; Fedorov et al. 2003; Fedorov and Stenseth 2002) have demonstrated that species persisted through 1 or more glacial maxima in Nearctic Beringia refugia. For several species, the region was an important source for postglacial colonization of recently deglaciated regions.

The arctic ground squirrel (*Spermophilus parryii*) also is thought to be closely tied to the glacial changes of Beringia. The evolutionary history of this species has been examined through morphology (Hall 1981), parasitology (Holland 1963; Rausch 1994), karyology (Nadler 1966), and enzymology (Nadler

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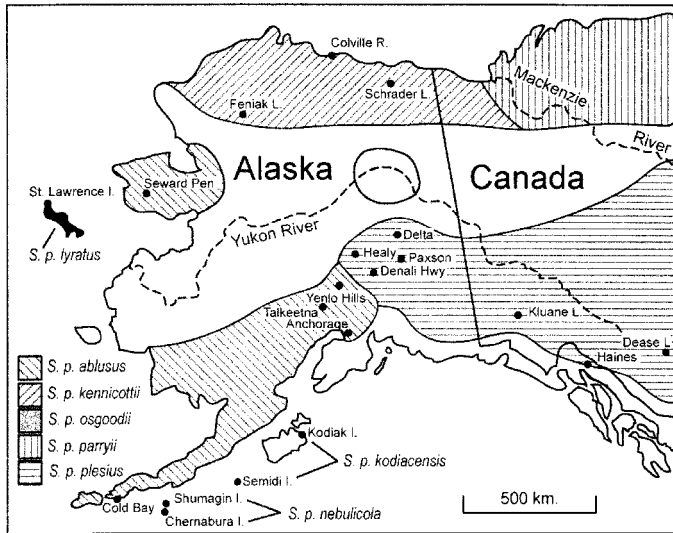


FIG. 1.—Subspecies ranges of *Spermophilus parryii* and localities sampled in this study.

1968; Nadler and Hoffmann 1977; Nadler and Hughes 1966; Nadler and Youngman 1969, 1970; Nadler et al. 1973, 1974). On the basis of morphology and the fossil record, *S. parryii* likely diverged in Beringia during the late Pleistocene, from either *Spermophilus columbianus* (Holland 1963; Howell 1938; Macpherson 1965; Rand 1954; Vorontsov and Lyapunova 1986) or *Spermophilus undulatus* (Gromov et al. 1965; Nadler and Hoffmann 1977; Robinson and Hoffmann 1975). More recent fossil evidence questions the late Pleistocene origin of *S. parryii* and suggests that the species could have occurred in Beringia earlier than previously thought (Guthrie 1985; Jopling et al. 1981). For Nearctic Beringia, all studies agree that a major evolutionary split exists between populations of *S. parryii*, found north of the Brooks Range in Alaska and in arctic Canada, and populations found further south in interior Alaska and subarctic Canada. Nadler and Hoffmann (1977) reviewed these studies and concluded that *S. parryii* arose in Asia from *S. undulatus* and crossed the Bering Land Bridge to North America during the Illinoian or Wisconsin glaciations; in North America, *S. parryii* then diverged into arctic and subarctic groups, and with the melting of the glaciers at the end of the Pleistocene, the arctic and subarctic groups dispersed to their present distributions and further diverged from each other.

The close relationship between arctic ground squirrels and the glacial history of Beringia, along with the ecology of this species, provides an excellent model for research on the genetic effects of glaciation. Typically found in both arctic and alpine tundra, the species is distributed throughout eastern Siberia, Alaska, and northern Canada to Hudson Bay (Wilson and Ruff 1999). Habitat changes of the Pleistocene should have substantially affected their evolution. In Nearctic Beringia, Pleistocene glaciations could have contributed to the extensive morphological variation that has been documented, with Hall (1981) recognizing 8 subspecies (*S. p. ablusus*, *kennicottii*, *kodiakensis*, *lyratus*, *nebulicola*, *osgoodii*, *parryii*, and *plesius*; Fig. 1). Populations are distributed patchily because the species

requires well-drained soils to create burrows for predator avoidance and hibernation (Carl 1971; Mayer 1953). This heterogeneous distribution leads to high natal philopatry and low levels of gene flow (Byrom and Krebs 1999), which could enhance diversification (Baker and Marler 1980; Chesser 1991).

Variation in the mitochondrial cytochrome-*b* gene (*Cytb*) of the arctic ground squirrel in Nearctic Beringia is examined to explore the effects of this dynamic geologic history on this species. High levels of genetic diversity within these Nearctic squirrels would support the evolutionary hypotheses of Nadler and Hoffmann (1977) that Nearctic Beringia was an important region for the persistence and diversification of the species. Lack of diversity would suggest that the species is a recent colonizer of the region and would not support the nominal sub-specific diversity of Hall (1981) or the extensive evolutionary history proposed by Nadler and Hoffmann (1977). Genetic and population divergence times are estimated from the phylogenetic results by moment-based and coalescent methods to better understand the timing and causes of divergence. Divergence dates in the late Pleistocene would support previous research that suggests *S. parryii* arose during this period (Nadler and Hoffmann 1977; Repenning et al. 1964). Earlier divergence dates would support fossil evidence that *S. parryii* occurred in Nearctic Beringia prior to the Late Pleistocene (Guthrie 1985; Jopling et al. 1981). Finally, the evolutionary history of the arctic ground squirrel is placed within the context of research on other high-latitude species as we attempt to understand the role of Nearctic Beringia in biotic diversification.

MATERIALS AND METHODS

Sampling.—Tissues of 48 arctic ground squirrels from 21 localities (Appendix I) throughout Alaska and northwest Canada encompass a majority of the species range in North America and represent all described North American subspecies except *S. p. parryii* and *S. p. osgoodii* (Fig. 1). Samples were collected through field expeditions and are archived at the University of Alaska Museum, Fairbanks, Alaska.

Molecular methods.—The complete *Cytb* gene (1,140 base pairs) was sequenced (GenBank accession numbers AY427977–AY428024) by the methods of Fleming and Cook (2002). DNA was extracted from tissue by digestion for approximately 4 h at 65°C in 550 μ l of buffer composed of 50 mM Tris-HCl (pH 8.0), 50 mM EDTA (pH 8.0), 1% sodium dodecyl sulfate, 100 mM β -mercaptoethanol, and 10 mg/ml Proteinase K. Precipitation of proteins from DNA was performed with sodium chloride, and DNA was precipitated with ethanol.

The entire *Cytb* gene was amplified by polymerase chain reaction (PCR) in 2 fragments with the use of primer pairs MVZ 05 (Smith and Patton 1993) and SPERMO 06 (5'-TAT GGG TGA AAG GGR AYT TTA TCT GA-3'), and MVZ 14 (Smith and Patton 1993) and SPERMO 07 (5'-TTC GCA TTC CAC TTT ATT CCT CCA TTT-3'). Double-stranded amplifications were performed in 50- μ l total reaction volumes with final concentrations of 1 μ M primer, dNTPs at 19 μ M, 10 \times PCR buffer, and MgCl₂ at 1.5 mM. Each PCR was performed with 35 cycles, alternating denaturation at 94°C for 10 s, annealing at 45°C for 15 s, and extension at 72°C for 45 s, with a cloned version of DNA polymerase from the bacterium *Thermus aquaticus* (Ampli-taq, Applied Biosystems, Foster City, California). All PCR experiments included a negative control. Aliquots (5 μ l) of PCR products and negative controls were visualized in 1.5% agarose minigels stained with ethidium bromide.

Remaining PCR product was precipitated with polyethylene glycol, recovered by vacuum centrifugation, and resuspended in 10 mM Tris-HCl (pH 8.0). The purified PCR products were then used as a template in dideoxy terminal-cycle sequencing reactions with a Perkin Elmer kit (FST-RR, 402119, Wellesley, Massachusetts) on a Perkin Elmer GeneAmp PCR (systems 2400 and 9700). The products were then run on 4% polyacrylamide gels on an automated sequencer (373-A, Applied Biosystems). Both heavy and light strands were sequenced for all samples. Partial *Cytb* sequences were examined, and the complete gene was assembled on the basis of areas of overlap and aligned by eye with the Sequence Navigator program (Version 1.01, Applied Biosystems).

Data analysis.—The MODELTEST program (Posada and Crandall 1998) was used to determine the simplest substitution model that was not significantly different from more complex models for these data. That model was then used to generate phylogenetic relationships. Relationships among the *Cytb* sequences were visualized with a maximum likelihood tree constructed with PAUP* (Version 4.0b8—Swofford 2001). The tree was rooted with *Spermophilus beecheyi* sequences from GenBank (AF157919 and AF157918). *S. beecheyi* was included to use the estimated divergence date of 5.16×10^6 years ago between *S. beecheyi* and *S. parryii* (Smith and Coss 1984). This divergence estimate, based on geologic and fossil evidence, is the best available date for calibrating a molecular clock for the arctic ground squirrel. Support for relationships was estimated with 1,000 bootstrap replicates.

Estimates of divergence times rely on assumptions of neutrality and constant population size, which were tested with Fu's F_s -test of neutrality (Fu 1997). Fu's F_s values for all clades were calculated for 1,000 replicates with ARLEQUIN (Schneider et al. 2000). A test of rate heterogeneity also was conducted to determine whether the gene is evolving in a clocklike manner. A likelihood ratio test with 24 degrees of freedom ($26 \text{ haplotypes} - 2$) was performed to compare maximum likelihood values for trees with and without a molecular clock constraint (Huelsenbeck and Rannala 1997).

Divergence times between and within major clades of *S. parryii* were calculated on the basis of an estimated rate of mutation (μ). Mutation rate (μ) under the molecular clock is equal to the number of nucleotide substitutions along 1 lineage divided by the time since divergence (Harris and Hey 1999). Mutation rate was calculated for *S. parryii* *Cytb* on the basis of the average pairwise divergence, or the average number of differences between *S. beecheyi* and *S. parryii* (Nei and Li 1979). Average pairwise divergence is the average number of substitutions between 2 groups of sequences and is related to μ by the equation average pairwise divergence = $2\mu t$, where t is the divergence time (Arbogast et al. 2002). Average pairwise divergence between *S. beecheyi* and *S. parryii* was calculated with ARLEQUIN (Hartl and Clark 1997). To calculate μ , this estimate of divergence was divided by 2 to estimate the amount of divergence for 1 branch and then divided by the date of divergence (5.16×10^6 years ago) between *S. beecheyi* and *S. parryii* (Smith and Coss 1984). The combination of phylogenetic data and μ makes it possible to estimate times of divergence of genealogical lineages with both moment-based estimates of divergence (Arbogast et al. 2002) and the coalescent model of Kingman (1982).

The moment-based estimates of divergence times are calculated by ARLEQUIN from average pairwise divergence between and within major clades. Within each clade, average pairwise divergence was estimated by separating the clade into 2 parts on the basis of geography. The average pairwise divergence estimates were divided by 2μ to estimate times of genetic divergence (Arbogast et al. 2002). Because genetic divergence predates population divergence, net di-

vergence (Nei and Li 1979), which corrects for within-clade variation, was calculated to estimate the time of population divergence between major clades. Net divergence is equal to between-clade average pairwise divergence minus within-clade average pairwise divergence. Net divergence was divided by 2μ to estimate times of population divergence.

Genetic and population divergence also were estimated by the coalescent approach of Kingman (1982). The coalescent model is stochastic. It estimates genealogy and population parameters from a sample of genetic sequences (Arbogast et al. 2002; Kingman 1982). Estimates of maximum likelihood value for θ (a measure of 2 times the net effective population size [N_e] multiplied by μ ; θ_{ml}), time to most recent common ancestor, and net migration were accomplished with the MDIV software program (Nielsen and Wakeley 2001), which uses a maximum likelihood Markov chain Monte Carlo approach based on the standard coalescent model to estimate the population genetic parameters. Estimates were made for each clade and larger groups by combining clades. N_e was estimated by the equation $\theta_{ml}/2\mu$ (Hartl and Clark 1997). Genetic divergence was estimated by multiplying time to most recent common ancestor by N_e (Harris and Hey 1999). Estimates of population divergence were made by multiplying scaled time of divergence by N_e (Edwards and Beerli 2000).

RESULTS

Phylogeography.—With the use of MODELTEST, the Hasegawa-Kishino-Yano substitution model incorporating rate heterogeneity (HKY+G—Hasegawa et al. 1985) was identified as the simplest model that best fit the data. Starting parameters (base frequency A, 0.29; C, 0.26; G, 0.12; T, 0.33; substitution model Ti/Tv = 10.22, Gamma = 0.3791) are typical for mammalian *Cytb* (Irwin et al. 1991). The HKY+G model parameters determined by MODELTEST were then used to search for the maximum likelihood tree (Fig. 2). Four relatively well-supported (bootstrap support > 88) clades (north, southwest, west, and central) are identified within *S. parryii* and correspond well to geographic regions. The southwest clade shows extensive structure, consists primarily of populations of the Alaska Peninsula and associated islands, and includes individuals of *S. p. kodiacensis* and *S. p. nebulicola* and a distinct clade of *S. p. ablusus* populations found just south of the Alaska Range. The west clade consists of individuals from the Seward Peninsula (*S. p. ablusus*) and St. Lawrence Island (*S. p. lyratus*). The central clade contains a polytomy of *S. p. plesius* sequences from throughout central Alaska and northwestern Canada. The north clade consists of a polytomy of *S. p. kennicottii* from arctic Alaska.

Divergence times.—Fu's F_s -tests (Table 1) show no significant deviation from neutrality ($P > 0.05$). The χ^2 likelihood ratio test did not show a significant difference ($P > 0.24$) between maximum likelihood values for trees with and without a molecular clock. *S. parryii* and *S. beecheyi* differ by an average of 178.69 substitutions (Table 2). On the basis of the fossil record and geologic evidence, Smith and Coss (1984) estimated a date of divergence between *S. beecheyi* and *S. parryii* at 5.16×10^6 years ago. On the basis of average pairwise divergence and time of divergence, an estimated mutation rate μ is equal to $178.69/(2 \times 5.16 \times 10^6) = 1.73 \times 10^{-5}$ per year for the entire *Cytb* gene and $1.73 \times 10^{-5}/1,140 =$

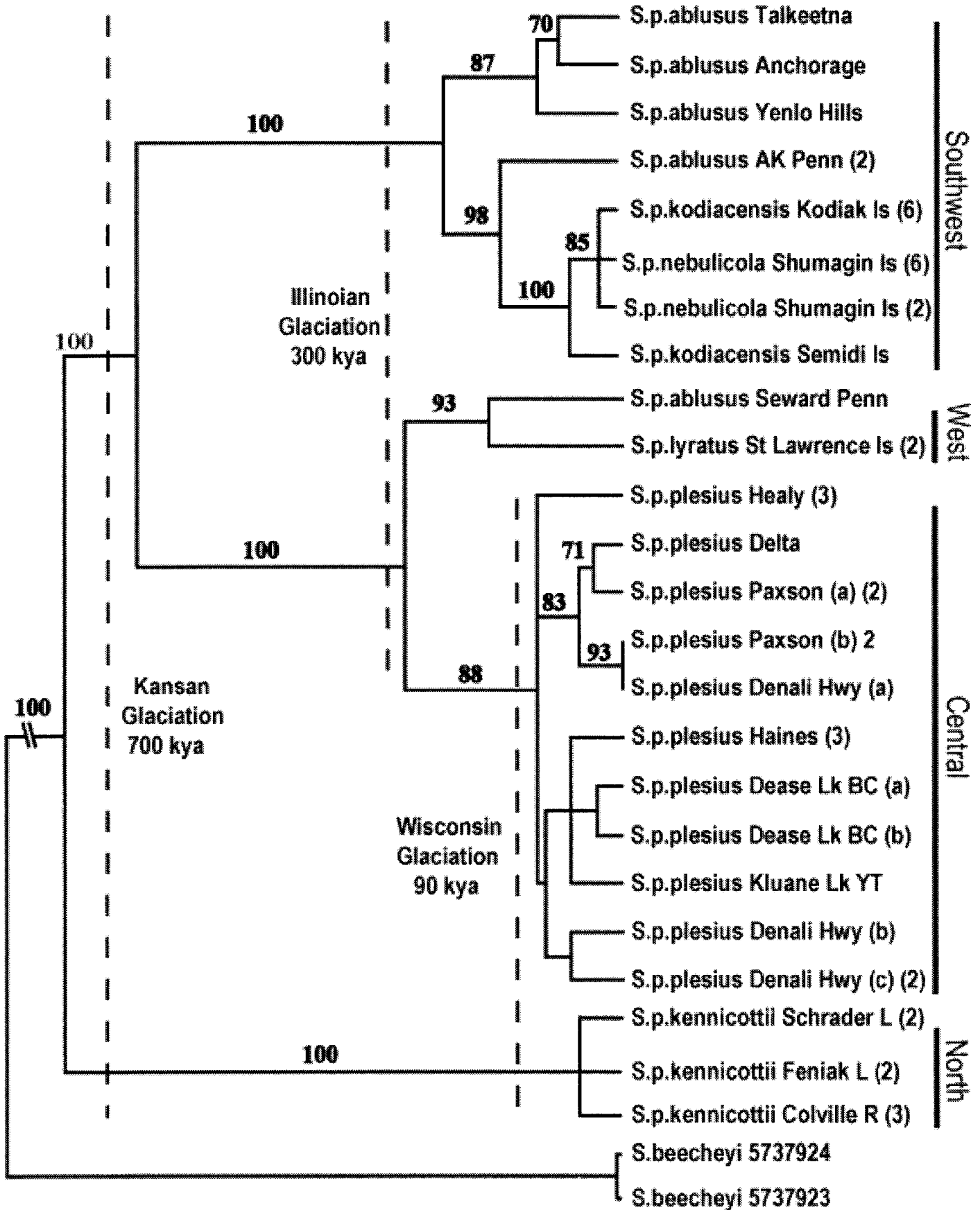


FIG. 2.—Maximum likelihood tree of relationships of populations of *Spermophilus parryii*, including *Spermophilus beecheyi* as the outgroup. Numbers above the branches represent bootstrap values >70 generated from 1,000 bootstrap replicates. Letters at the end of sample locations indicate distinctive haplotypes, and the number identifies the quantity of identical haplotypes. (Abbreviation: kya = thousands of years ago.)

TABLE 1.—Sample size (n), Fu's F_s values, and P values for Fu's F_s test of neutrality for major clades of *Spermophilus parryii*.

Group	n	F_s	P value
<i>S. parryii</i> + <i>S. beecheyi</i>	50	5.00	0.94
North + south	48	3.20	0.86
Southwest + west/central	41	2.68	0.82
West + central	21	-0.89	0.35
West	3	3.92	0.93
Southwest	20	1.29	0.71
Central	18	-1.68	0.21
North	7	1.85	0.85

1.52×10^{-8} per base pair per year. This corresponds to a mutation rate of 1.52%/million years, an estimate then used to calculate times of genetic and population divergence between and within major clades by moment-based methods (Table 2). Estimates of genetic and population divergence from the moment-based analysis range from 1×10^6 to 1×10^5 and 8×10^5 to 7×10^4 years ago, respectively. Population divergence times, important in identifying times of vicariant divergence, cluster into 3 groups around 8×10^5 , 3×10^5 , and 6×10^4 years ago. Population genetic parameters (θ_{ml} , time to most recent common ancestor, scaled time of divergence; Table 3) were estimated by the MDIV program with the use of a coalescent approach. Estimates of effective population size (N_e) based on θ_{ml} and μ suggest that populations of *S. parryii* were relatively large throughout the Pleistocene. Parameters estimated by MDIV also were used to estimate times of genetic divergence and population divergence (Table 3). Population and genetic divergence times are similar to those of the moment-based analysis, and population divergence estimates again cluster into 3 groups.

DISCUSSION

Nearctic Beringia apparently was important for species persistence in the Arctic during repeated glacial advances of the Pleistocene. Few studies to date have identified substantial geographic structure within organisms inhabiting Nearctic Beringia; however, the high levels of variation within arctic ground squirrel populations provide an opportunity to examine the effects of vicariant events on species within this region.

TABLE 2.—Average pairwise divergence (apd) and net divergence (nd = apd between groups A and B - ((apd within group A + apd within group B)/2) values and associated estimated genetic divergence (apd/(1.73147×10^{-5})) and population divergence (nd/(1.73147×10^{-5})) times in years for the major clades of *Spermophilus parryii*.

Group	Sample size	Average pairwise divergence	Genetic divergence (years)	Net divergence	Population divergence (years)
<i>S. parryii</i> + <i>S. beecheyi</i>	50	178.7	5.16×10^6	164.1	4.74×10^6
North + south	48	40.3	1.16×10^6	27.3	7.90×10^5
Southwest + west/central	41	39.1	1.13×10^6	32.5	9.38×10^5
West + central	21	15.9	4.60×10^5	9.4	2.71×10^5
West	3	13.0	3.75×10^5	13.0	3.75×10^5
Southwest	20	11.9	3.43×10^5	7.0	2.03×10^5
Central	18	5.2	1.51×10^5	1.7	4.81×10^4
North	7	3.5	1.01×10^5	2.5	7.22×10^4

Phylogeography.—Significant phylogeographic structuring of Nearctic arctic ground squirrels appears tied to the glacial history of the region and might be enhanced by the suspected low vagility of the species. The patchy distribution of this semifossorial rodent likely results in low levels of gene flow (Chesser 1991), an effect magnified for mitochondrial DNA because female *S. parryii* are highly philopatric (Byrom and Krebs 1999). Together, the patchy distribution and strong philopatry increase the chances for diversification. The 4 major clades identified are geographically distinct and largely match subspecific designations. Comparison between the geographic distribution of the 4 clades and the geologic history of Nearctic Beringia is consistent with the hypothesis that glacial vicariance lead to divergence.

The distinctive north clade indicates an early split into arctic and subarctic groups, a pattern previously identified by Nadler and Hoffmann (1977). These tundra populations are found north of the Brooks Range and are separated from subarctic clades by boreal forest. Glaciation and the invasion of boreal forests following glacial retreat might have formed a barrier between the arctic and subarctic groups leading to isolation and diversification. Alternatively, populations of the north clade might have persisted in a refugium in arctic Canada and then expanded westward into northern Alaska following glacial retreat, as has been hypothesized for other taxa (Ehrlich et al. 2000; Fedorov and Stenseth 2002; Holder et al. 1999; Tremblay and Schoen 1999). *S. parryii* does not now occur in the areas of arctic Canada that are considered to be a possible refugium on the basis of glacial patterns (Dyke et al 2002; Pielou 1991), and no fossils exist for this region, possibly corroborating Macpherson's (1965) idea that there is an ecological limit to their northward expansion. The polytomy formed by the populations in the north clade could reflect a recent population expansion, possibly due to an increase in suitable habitat. Values of Fu's F_s test contradict recent expansion, but the small sample size for this clade might compromise this test.

Along with the major dichotomy between arctic and subarctic groups, a substantial split exists between populations in southwestern Alaska and those found in interior Alaska (west, central). Hall (1981) depicted a zone of contact between *S. p. ablusus* and *S. p. plesius* in central Alaska; however, contact between the 2 groups has not been verified. Populations from

TABLE 3.—Coalescent method estimates of population genetic parameters, including sample size, maximum likelihood value for θ (θ_{ml}), effective population size ($N_e = \theta_{ml}/2\mu$), time to most recent common ancestor (TMRCAs), genetic divergence in years (TMRCAs $\cdot N_e$), scaled time of divergence, and population divergence in years (time of divergence $\times N_e$), for major clades of *Spermophilus parryii*.

Group	Sample size	θ_{ml}	Effective population size (N_e)	TMRCAs	Genetic divergence (years)	Time of divergence	Population divergence (years)
<i>S. parryii</i> + <i>S. beecheyi</i>	50	27.41	791,427	7.86	6.22×10^6	7.32	5.79×10^6
North + south	48	18.38	530,686	2.49	1.32×10^6	1.36	7.22×10^5
Southwest + west/central	41	11.37	328,381	3.82	1.25×10^6	2.60	8.54×10^5
West + central	21	8.61	248,596	2.02	5.03×10^5	1.50	3.73×10^5
West	3	3.74	108,116	3.62	3.92×10^5	3.26	3.52×10^5
Southwest	20	3.70	106,710	3.25	3.47×10^5	2.42	2.58×10^5
Central	18	4.69	135,304	1.64	2.22×10^5	0.68	9.20×10^4
North	7	0.88	25,459	3.10	7.90×10^4	2.94	7.48×10^4

Talkeetna and Anchorage are in the southwest clade, whereas populations north of the Alaska Range (Healy, Paxson, Denali Highway) are in the central clade. Glaciation of the Alaska Range during the Pleistocene could have acted as an isolating barrier that promoted divergence. Current distribution of members of these clades might reflect range expansion following glacial retreat. A negative value of Fu's F_s test for the central clade might be consistent with the idea of recent population expansion in the region, but the test is not significant at the 0.05 level.

The southwest clade shows substantial structuring (Fig. 2), with a strong dichotomy existing between the populations south of the Alaska Range (Talkeetna, Anchorage, Yenlo Hills) and those of the Alaska Peninsula and associated islands. Island populations (Chernabura, Kodiak, Semidi, Shumagin) also are strongly differentiated from those on the Alaska Peninsula. This identifiable geographic structure could be due to limited sampling along the Alaska Peninsula or might suggest that these island populations have been isolated longer than other Nearctic populations. Extensive structure suggests in situ diversification of *S. parryii* in the region, a pattern not seen previously in other studies of *S. parryii* (Nadler and Hoffmann 1977) or other species of the region (e.g., Holder et al 1999). Alternatively, the extensive structure could be due to selective sweeps within each population or higher diversification in these populations. Fu's F_s tests are not statistically significant and suggest that these alternative explanations are unlikely.

Insular populations (Kodiak, Semidi, Shumagin) of the southwest clade are of particular interest. Small refugia in the region have been proposed, possibly on Kodiak Island or on the continental shelf exposed by lowered sea level during glacial advances (Karlstrom 1964). Endemic subspecies (*kodiakensis*, *nebulicola*) named on the basis of morphologic differences are consistent with the existence of possible refugia and suggest long-term in situ diversification. Bailey (1993), on the basis of historic records related to fox farming, instead suggests that insular populations of arctic ground squirrels are due to human introductions to more than 22 islands in the region (Bailey 1993; Hall 1981; Murie 1959). Although insular populations are distinct from the rest of the southwest clade, discriminating between these 2 hypotheses was difficult due to our limited sampling of islands in the region.

Populations located between the Brooks and Alaska mountain ranges are placed in the west and central clades. The west

clade comprises populations of the Seward Peninsula and St. Lawrence Island, and members of this clade are separated from the central clade by boreal forest and from the southwest clade by the Yukon River Delta. Nadler and Hoffmann (1977) identified the Yukon River Delta as a major geographic break in the distribution of the arctic ground squirrel, and not surprisingly, the west clade is more closely related to the central clade than the southwest clade. Similarly, Nadler and Hoffmann (1977) suggested that populations north of the Yukon River on the Seward Peninsula described as *S. p. ablusus* were more closely related to the subspecies *S. p. plesius* of central Alaska than to *S. p. ablusus* of southwest Alaska. The close relationship of Seward Peninsula populations and St. Lawrence Island populations also was suggested by Nadler and Hoffmann (1977) and agrees with the geologic history of the Bering Land Bridge. Strong bootstrap support for the St. Lawrence Island population is consistent with the subspecific designation of *S. p. lyratus*. Members of the central clade are found in alpine tundra north of the Alaska Range in central and eastern Alaska and in western Canada. Central Alaska was a likely refugium for members of the west and central clades because it was ice free during the Pleistocene and vegetated primarily by tundra or steppe plant communities (Guthrie 2001; Hoffmann 1981; Hopkins 1967, 1982), and fossil evidence from the region indicates that squirrels were present and widespread in central Alaska throughout the late Pleistocene (Guthrie 1985; Jopling 1981; Repenning et al. 1964).

Glacial patterns within the localized region of Nearctic Beringia had a significant effect on arctic ground squirrel diversification, a finding that largely has not been noted in the few studies of other species within Nearctic Beringia. Studies of *Rangifer tarandus* (Flagstad and Roed 2003) and *Ursus arctos* (Leonard et al. 2000) suggest that minimal phylogeographic structure is perhaps due to high levels of gene flow. In contrast, studies on *Microtus oeconomus* (Brunhoff et al. 2003; Galbreath and Cook 2003), moose (Hundertmark et al. 2003), and lemmings (*Dicrostonyx groenlandicus*—Ehrich et al. 2000; *Lemmus*—Fedorov et al. 2003) concluded that recent colonization or expansion in this region limits phylogeographic structure.

Divergence times.—Most estimated times of genetic differentiation are deeper than the late Pleistocene, suggesting differentiation of these populations likely persisted over several glacial cycles. Nadler and Hoffmann (1977) hypothesized that

the arctic ground squirrel migrated into North America during either the Wisconsin or Illinoian glaciation (8×10^4 and 3×10^5 years ago, respectively—Bowen et al. 1986). The estimated time of divergence for the north, southwest and, west-central clades are near 1×10^6 years ago, suggesting a migration into North America with subsequent diversification in the early to mid-Pleistocene. Separation of members of the west and central clades, as well as diversification within the west and southwest clades, all date near the beginning of the Illinoian glaciation. Only divergence within the central and north clades is estimated to have been during the late Pleistocene (1.32×10^5 to 1×10^4 years ago—Bowen et al. 1986).

Genetic divergence within a population is initiated prior to population divergence (Arbogast et al. 2002; Edwards and Beerli 2000). Population divergence patterns in *S. parryii* largely coincide with the onset of glacial periods. Divergence of ancestors of the north clade from those of southern clades and separation of the southwest clade from those of central Alaska (west, central) occurred between 9.4 and 7.9×10^5 years ago near the onset of the Kansan glaciation (9×10^5 years ago—Bowen et al. 1986). Population divergence times for the west and central clades, as well as divergence within the southwest and west clades, occur between 2 and 3.8×10^5 years ago, the beginning of the Illinoian glaciation (3×10^5 years ago—Bowen et al. 1986). The divergence of populations within the north and central clade occurs between 4 and 7×10^4 years ago near the beginning of the Wisconsin glaciation (8×10^4 years ago—Bowen et al. 1986). Hence, major dichotomies in the tree (Fig. 2) correspond to the onset of glacial periods.

Conclusions based on a molecular clock are necessarily suspect (Ayala 1999) because confidence limits around estimates of divergence are extremely large due to the Poisson mutation model of the molecular clock and potential inaccuracies in the mutation rate, phylogenetic tree, and calibration dates (Hillis et al. 1996). A species-level clock based on well-supported phylogenies can increase the accuracy of the clock (Marshall 1990). Divergence dates are stronger when based on accurately dated fossil records, documented vicariant events, and multiple calibration points (Marshall 1990). Smith and Coss (1984) use all of these approaches to estimate the divergence of *S. parryii* and *S. beecheyi* at 5.16×10^6 years ago to provide the best date of divergence for *S. parryii* available. Smith and Coss (1984) use fossil data from the split of the 2 species as well as fossil data of the divergence of the subspecies *S. b. beecheyi* and *S. b. douglasii* and the rise of the Sacramento and San Joaquin River drainages that form an absolute barrier between the 2 subspecies to estimate divergence.

Additionally, 2 tests support the clocklike evolution of *Cytb* in *S. parryii* (Wayne and Simonsen 1998). A likelihood ratio test failed to reject a molecular clock. Fu's F_s tests detected no significant departures from neutrality, indicating that no population expansion or selective sweeps have occurred. Alternatively, lack of significance could be attributed to low sample size, especially for the north and west clades.

Comparisons to other studies also suggest that these estimates are reasonable. The estimated μ of 1.52% per million

years is higher than the universal rate for *Cytb* sequence divergence μ of 1% per million years based on the divergence of primates (Brown et al. 1979). A higher rate for rodents has been documented (Martin and Palumbi 1993). Spradling et al. (2001) attributed differences to small effective population size and population subdivision that increased rates of evolution through a higher rate of fixation due to genetic drift. Small local populations and extensive population subdivision are characteristics of *S. parryii*. However, the estimated rate of mutation for *S. parryii* is on the low end for this group (3.75–6% per million years—Arbogast et al. 2002; 1.9–5.7% per million years—Martin and Palumbi 1993). Martin and Palumbi (1993) related differences in divergence rates to generation time and metabolic rate. Generation time for *S. parryii* is relatively long for a rodent, and the species lowers its metabolic rate while hibernating (Buck and Barnes 1999), which can last for up to 7 months a year (Carl 1971; Mayer 1953) and can lead to a lower mutation rate. Spradling et al. (2001) also found mutation rates in *Spermophilus* to be low relative to other rodents but found no evidence that this pattern was due to generation time or metabolic rate.

Additional support for clocklike evolution in *Cytb* is provided by both moment-based and coalescent methods. Moment-based estimates are based on a neutral mutation rate (μ), constant through time, and average pairwise divergence. The coalescent method estimates are based on a maximum likelihood Markov chain Monte Carlo approach to estimate θ_{ml} , a measure of μ , and N_e . Despite these distinctive approaches, both methods provide similar estimates of divergence times.

Additional fossil evidence suggests that the Late Pleistocene migration hypothesis of Nadler and Hoffmann (1977) might be incorrect. Their hypothesis is based on a report by Repenning et al. (1964) that fossil evidence of *S. parryii* was found first in deposits from the Riss (Illinoian) glacial period in Siberia and then later in the Wisconsin period in Alaska. However, subsequent fossil discoveries indicate widespread populations in the Nearctic throughout the Illinoian glaciation (Guthrie 1985; Jopling et al. 1981).

Nearctic Beringia appears to have been central to the diversification of arctic ground squirrels, with their evolutionary history intricately linked to the glacial cycles of the Pleistocene. Nearctic populations are aligned into 4 geographically distinct clades. This structure suggests that, in addition to previous studies that demonstrated that Beringia was an important refugium for diversification of high-latitude species (e.g., Abbott et al. 2000; Brunhoff et al. 2003; Holder et al. 1999; Tremblay and Schoen 1999), glacial events on a finer scale within Nearctic Beringia were important in shaping diversity. Divergence of major clades began as early as the Kansan glaciation, with major divergences initiated at the onset of the Kansan, Illinoian, and Wisconsin glaciations, respectively, and suggesting persistence in multiple refugia throughout the Pleistocene within Nearctic Beringia. Similar spatial and temporal patterns can be expected for other species in the region (Riddle 1996).

Analyses with independent markers and more intensive sampling are necessary to test these patterns of differentiation

in arctic ground squirrels. The examination of additional described Nearctic and Palearctic subspecies of *S. parryii*, including the final 2 North American subspecies (*osgoodii* and *parryii*), would provide a more comprehensive view of variation. Examination of populations from eastern Siberia might clarify the role of the Bering Land Bridge on diversification between and within the 2 regions. An enlarged sampling scheme also would allow for finer scale perspectives on diversification of the arctic ground squirrel.

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APPENDIX I

Specimens examined are listed by haplotype name, location by latitude and longitude, and specimen number of Alaska Frozen Tissue Collection. All specimens are deposited in the University of Alaska Museum, Fairbanks, Alaska.

Spermophilus parryii ablusus.—Alaska Peninsula, 54.98°N 162.46°W, 27001. Alaska Peninsula, 54.98°N 162.46°W, 27002. Anchorage, 61.19°N 149.63°W, 8823. Seward Peninsula, 65.09°N 164.91°W, 7444. Talkeetna, 62.75°N 150.10°W, 1351. Yenlo Hills, 62.08°N 151.27°W, 30778.

Spermophilus parryii kennicottii.—Colville River, 70.43°N 150.40°W, 24001. Colville River, 70.43°N 150.40°W, 24002. Colville River, 70.43°N 150.40°W, 24003. Feniak Lake, 68.26°N 158.50°W, 13024. Feniak Lake, 68.26°N 158.50°W, 13025. Schrader Lake, 69.36°N 145.00°W, 13019. Schrader Lake, 69.36°N 145.00°W, 13048.

Spermophilus parryii kodiacensis.—Kodiak Island, 57.73°N 152.50°W, 1246. Kodiak Island, 57.73°N 152.50°W, 1368. Kodiak Island, 57.73°N 152.50°W, 1369. Kodiak Island, 57.73°N 152.50°W, 1370. Kodiak Island, 57.73°N 152.50°W, 5757. Kodiak Island, 57.73°N 152.50°W, 5758. Semidi Island, 56.07°N 156.07°W, 17759. Semidi Island, 56.07°N 156.07°W, 17760.

Spermophilus parryii lyratus.—St. Lawrence Island, 63.78°N 171.74°W, 5740. St. Lawrence Island, 63.78°N 171.74°W, 5741.

Spermophilus parryii nebulicola.—Shumagin Island, 55.00°N 159.76°W, 16103. Shumagin Island, 55.00°N 159.76°W, 17856. Shumagin Island, 55.00°N 159.76°W, 17861. Shumagin Island, 55.00°N 159.76°W, 17874. Shumagin Island, 55.00°N 159.76°W, 17935. Shumagin Island, 55.00°N 159.76°W, 17965. Chernabura Island, 54.82°N 159.76°W, 18029. Chernabura Island, 54.82°N 159.76°W, 18633.

Spermophilus parryii plesius.—Delta, 64.28°N 145.75°W, 1338. Dease Lake BC(a), 58.18°N 129.88°W, 12792. Dease Lake BC(b), 58.18°N 129.88°W, 12804. Denali Highway (a), 63.03°N 147.49°W, 24781. Denali Highway (b), 63.03°N 147.49°W, 22856. Denali Highway (c), 63.03°N 147.49°W, 22858. Denali Highway (c), 63.03°N 147.49°W, 22859. Haines, 59.62°N 135.16°W, 8755. Haines, 59.62°N 135.16°W, 8769. Haines, 59.62°N 135.16°W, 12523. Healy, 63.75°N 149.00°W, 1848. Healy, 63.75°N 149.00°W, 3295. Healy, 63.75°N 149.00°W, 18012. Kluane Lake YT, 60.99°N 138.15°W, 8773. Paxson(a), 63.50°N 145.50°W, No voucher specimen. Paxson(a), 63.50°N 145.50°W, No voucher specimen. Paxson(b), 63.50°N 145.50°W, No voucher specimen. Paxson(b), 63.50°N 145.50°W, No voucher specimen.