

Insular arctic ground squirrels (*Spermophilus parryii*) of the North Pacific: indigenous or exotic?

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We lack critical information for oceanic archipelagos worldwide related to the origin and status of insular faunas. In southwestern Alaska, in particular, a need exists to tease apart whether specific insular populations are naturally occurring or are the result of exotic introductions by humans. We analyzed variation in mitochondrial sequences of the cytochrome-*b* gene and 8 nuclear microsatellite loci across 215 individuals representing 17 populations (12 insular) to refine our understanding of the history of the previously identified Southwest clade of arctic ground squirrels (*Spermophilus parryii*). We found significant geographic structure that suggests long-term isolation and diversification (Ushugat Island and Cold Bay), but we also documented closely related populations that are likely the result of human-mediated introductions. The latter instances (Kavalga and Unalaska islands) corroborate reports from early Alaska explorers. DOI: 10.1644/09-MAMM-A-386.1.

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Introductions of exotic mammals have been a primary driver of extinctions worldwide, with an impact that is especially acute when exotics enter island ecosystems and encounter insular endemics (Quammen 1996; Whittaker and Fernández-Palacios 2007). Predation by exotics, for example, has been advanced as the key factor in depletion or extinction of insular, tropical seabirds of the Pacific (Steadman 2006). Heavy declines of nesting seabirds on several islands of southwestern Alaska (Aleutian Chain and islands south of the Alaska Peninsula) provided 1 of the earliest published examples of environmental impacts on insular systems due to introduction of exotic mammals (Dall 1870; Murie 1959; Turner 1886), but relatively few assessments of the impact of exotic species on high-latitude archipelagos have been completed (Anderson et al. 2006; Silva and Saavedra 2008).

Prior to the exploratory voyage of Vitus Bering in 1741, the central and western Aleutians largely were devoid of terrestrial mammals (Hopkins 1967; Murie 1959), but islands in the eastern Aleutians and south of the Alaska Peninsula supported a limited mammalian fauna (MacDonald and Cook 2009). These eastern islands are nearer mainland source populations and could have been colonized naturally by species dispersing from the central Beringian refugium (Waltari et al. 2007) across land and ice bridges at the close

of the Last Glacial Maximum (LGM). Smaller ice-age refugia in this coastal region also have been proposed, possibly on Kodiak Island or on the outer continental shelf that was exposed by dramatically lowered sea levels (>100 m) during glacial advances (Karlstrom 1964).

After the arrival of Europeans to the region in the 18th century, mammals such as Sitka black-tailed deer (*Odocoileus hemionus sitkensis*), wapiti (*Cervus canadensis*), red fox (*Vulpes vulpes*), brown rat (*Rattus norvegicus*), and others were introduced by humans to islands or nearby mainland sites (Bailey 1993; MacDonald and Cook 2009). The location and chronology of many island introductions, however, remains obscure. Arctic foxes (*Vulpes lagopus*) and red foxes were moved to more than 450 islands in Alaska by fur ranchers (Bailey 1993). In addition to these furbearers, fox ranchers “filled barrels” with arctic ground squirrels (*Spermophilus parryii*) and other small rodents (e.g., root voles [*Microtus oeconomus*]) and released them on numerous islands (Peterson 1967:123) to supplement existing prey for these fox ranches, especially as insular bird populations declined (Bailey 1993;



Black 1984). In the case of ground squirrels, introductions might have been initiated by the indigenous Alutiiq and Aleut people of the region, who highly valued them for garment making (Black 2001; Clark 2005).

Murie (1959) visited a number of islands in southwestern Alaska in 1936 and 1937 and recognized the negative impact of alien predators on insular bird populations. His subsequent reports to the United States Fish and Wildlife Service instigated efforts to eradicate exotics and establish programs aimed at conserving the native insular faunas (Ebbert and Byrd 2002). Many of the islands initially studied by Murie later became incorporated into the Alaska Maritime National Wildlife Refuge (Ebbert and Byrd 2002). Due to declining populations of a number of native species, maintaining or restoring insular faunas is becoming a major conservation issue in Alaska's archipelagos (Cook et al. 2006). Similar to other archipelagos worldwide (Whittaker and Fernández-Palacios 2007), critical information on the origin and status of these northern insular faunas is lacking (MacDonald and Cook 2007). In southwestern Alaska, in particular, a need exists to tease apart whether specific insular populations are naturally occurring or the result of introductions by humans.

A preliminary investigation of mitochondrial sequence variation (Eddingsaas et al. 2004) provided a broad view of phylogeographic structure in ground squirrels throughout Alaska and the adjoining Yukon Territory. That effort suggested that southwestern Alaska populations were strongly differentiated from all other ground squirrels. Within southwestern Alaska, populations on some islands are highly distinctive, suggesting that they may be insular paleoendemics (i.e., older relictual lineages that persisted in situ since before the LGM). Work by Eddingsaas et al. (2004) was based on relatively few specimens ($n = 20$) representing southwestern populations of ground squirrels and a single mitochondrial marker. Those limitations, combined with questions surrounding possible human introduction of ground squirrels onto islands throughout the region, necessitate a finer-scale examination of insular populations based on multiple, independent markers.

We tested whether the preliminary mitochondrial patterns uncovered by Eddingsaas et al. (2004) reflected the evolutionary history of these populations by investigating variation in a suite of nuclear loci. In addition to our primary goal of exploring the influence of LGM refugia on genetic relationships among ground squirrel populations across southwestern Alaska, we used biogeographic analyses to identify distinctive clusters of island populations. We then looked at individual islands to begin the process of discriminating introduced arctic ground squirrels from distinctive (indigenous) populations. Mapping the human imprint of species introductions has direct implications for the management of these insular mammals. Arctic ground squirrels prey on the eggs and chicks of waterfowl and seabirds to the extent that storm petrels and other burrow nesters are absent on islands with this rodent but present on nearby islands without them (Ebbert and Byrd 2002). Indigenous populations could be candidates for

conservation efforts, but recently introduced populations might be considered harmful exotics that should be removed to protect nesting bird populations and other aspects of North Pacific insular ecosystems.

MATERIALS AND METHODS

Sampling.—Island populations included in this analysis extend from the Kodiak Archipelago westward along the southern edge of the Alaska Peninsula and into the Aleutian Archipelago to Kavalga Island (Fig. 1). Through fieldwork beginning in 1991 and continuing through 2003, we collected tissue samples from ground squirrels representing 12 (32%) of the 37 southwestern islands believed to harbor extant populations (Table 1). We followed guidelines approved by the American Society of Mammalogists (Gannon et al. 2007). Specimens are archived in the University of Alaska Museum of the North in Fairbanks or Museum of Southwestern Biology in Albuquerque, New Mexico. We selected 5 individuals from each population for mitochondrial DNA (mtDNA) sequence analyses (total $n = 75$; Appendix I) and 2–41 individuals (total $n = 215$) for nuclear (microsatellite) analyses (Appendix I). To test whether island populations were closely related to each other, to particular mainland populations (indicating a possible history of translocation), or were more distantly related (indicating possible refugial isolation), we used analyses of geographic variation in mitochondrial sequences and 8 nuclear microsatellite loci. In general, microsatellite loci are expected to be more variable than the mitochondrial cytochrome-*b* (*Cytb*) gene in mammals and should provide finer resolution of patterns of within- and among-population variation (Hillis et al. 1996).

Molecular methods.—We used a salt extraction method (Miller et al. 1988) modified by Fleming and Cook (2002) to extract DNA from tissue (heart, liver, spleen, or muscle). The entire *Cytb* gene of the mitochondria was amplified in 2 sections by polymerase chain reaction using primer pairs MVZ 05 (Smith and Patton 1993)–SPERMO 06 (Eddingsaas et al. 2004) or MVZ 14 (Smith and Patton 1993)–SPERMO 07 (Eddingsaas et al. 2004). Amplification protocols are detailed in Eddingsaas et al. (2004).

Eight nuclear microsatellite loci (GS3, GS12, GS14, GS17, GS20, GS22, GS25, and GS26) were assayed using primers developed for the Columbian ground squirrel (*Spermophilus columbianus*—Stevens et al. 1997). Polymerase chain reaction amplification was carried out in 20- μ l volumes containing 50 mg of genomic DNA, 0.3 mM of deoxynucleoside triphosphates, 0.16 μ M concentrations of each primer, 1X polymerase chain reaction buffer, 0.04 U/ μ l of *taq*, and either 1.5 mM (GS14, GS17, GS22, GS25, and GS26) or 2.0 mM (GS3, GS12, and GS20) of MgCl₂. Amplification conditions were 94°C for 1 min, 33 cycles of 94°C for 15 s, 54°C for 20 s, 72°C for 5 s, and a final extension at 72°C for 30 s. Amplified products were electrophoresed on 5% polyacrylamide gels (BMA, Rockland, Maine) using an Applied Biosystems Inc. (Foster City, California) 377 automated sequencer. Microsat-

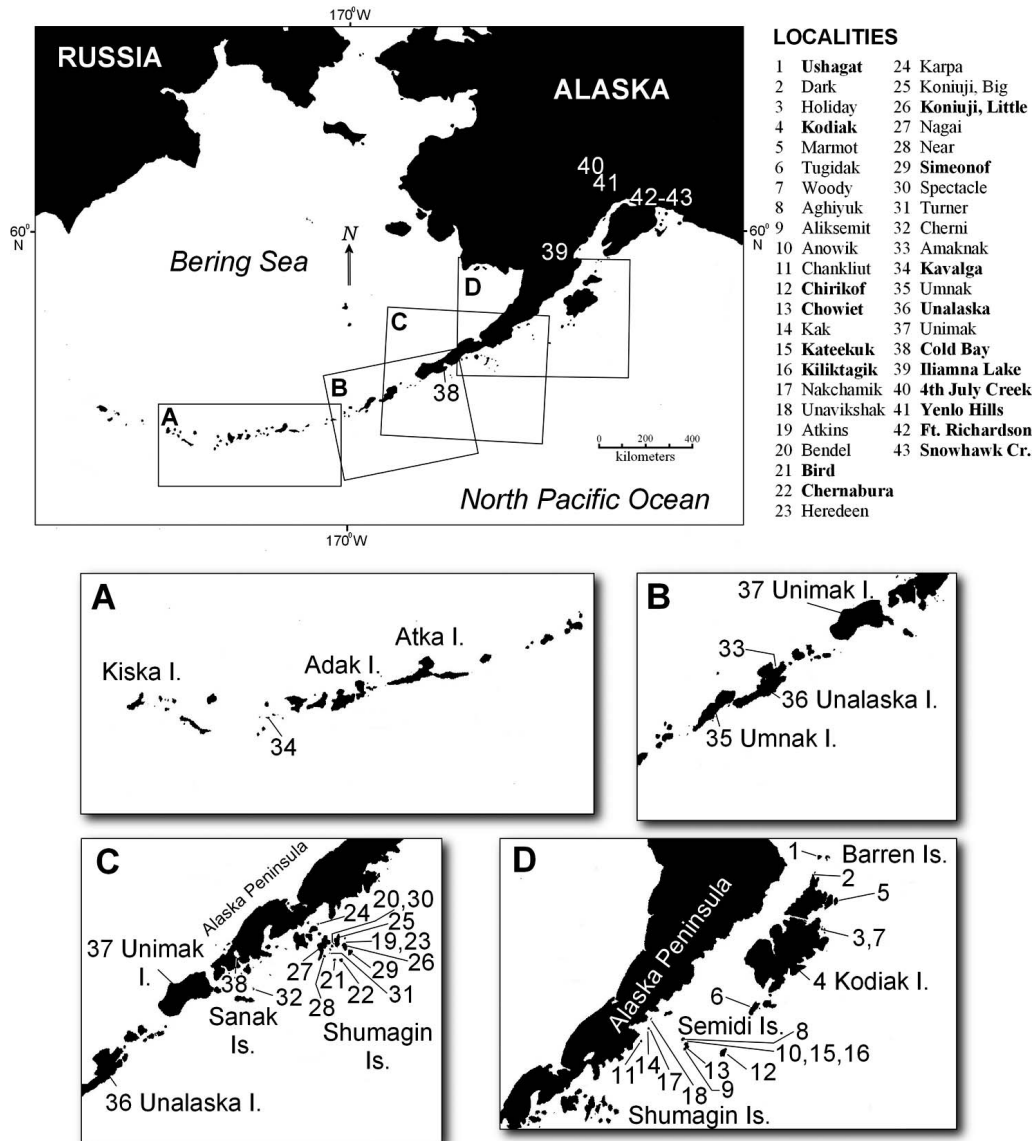


FIG. 1.—Maps of southwestern Alaska showing the location of insular and mainland populations of *Spermophilus parryii* sampled (in boldface type) in this study.

ellite alleles were sized using an internal GS 350 TAMRA (Applied Biosystem Inc.) standard. In addition to the internal size standard, a control individual was amplified and electrophoresed on each gel to ensure consistent allele sizing. We used GENESCAN 3.1 (Applied Biosystem Inc.) and GENOTYPER 2.1 (Applied Biosystem Inc.) software to collect the microsatellite data.

In addition to exploring interpopulation relationships in southwestern Alaska, *Cytb* sequences allowed us to place these populations within the larger context of phylogeographic variation in North American arctic ground squirrels (Eddingsaas et al. 2004). We used PAUP* (version 4.0b8—Swofford 2001) to build a maximum-likelihood phylogeny for unique *Cytb* haplotypes based on the HKY model of nucleotide substitution selected (via standard Akaike information criterion) using MODELTEST version 3.7 (Posada and Crandall 1998). The transition:transversion ratio was determined by

MODELTEST. The phylogeny was rooted with representatives of the other major clades of *S. parryii* (Eddingsaas et al. 2004), and nodal support was assessed via 1,000 bootstrap replicates.

Microsatellite analyses.—We calculated microsatellite allele frequencies for each locus in each population and in total using the computer program MSA 3.12 (Dieringer and Schlotterer 2003). Descriptive statistics (average number of alleles per locus, allelic richness, observed heterozygosity [H_o], and F_{IS}) were obtained using MSA and FSTAT version 2.9.3.2 (Goudet 2001). Tests for Hardy–Weinberg equilibrium and linkage disequilibrium were performed on each locus for each population using GENEPOP version 3.3 (Raymond and Rousset 1995). Tests of Hardy–Weinberg equilibrium for loci with 4 or fewer alleles were performed using the exact method of Louis and Dempster (1987), and loci with 5 or more alleles were tested using a Markov chain method (10,000 demorizations, 100 batches, and 5,000 iterations—Guo and Thomson

TABLE 1.—Locality and status of southwestern Alaska arctic ground squirrels (modified from MacDonald and Cook 2009). USNM = United States National Museum; MSB = Museum of Southwestern Biology; PSM = Slater Museum of Natural History; UAM = University of Alaska Museum of the North; MVZ = Museum of Vertebrate Zoology.

Map locality	Island group	Island ^a	Status
1	Barren Islands	Ushagat	Indigenous (Hall 1981); probably indigenous (this study). Only Barren Island with ground squirrels, where they are abundant (Bailey 1978). Archived specimens at USNM, MSB.
2	Kodiak Archipelago	Dark	Present (Alaska Department of Natural Resources 2008). No archived specimens.
3		Holiday	Probably introduced from Kodiak stock. Archived specimens at MSB, PSM.
4		Kodiak	Probably introduced (Howell 1938; Rausch 1953; this study) from Shumagin Islands (this study). Lectotype (USNM 9242/38543) for <i>kodiacensis</i> (Allen). Extant populations reported from Kodiak Airport, Buskin Lake, northeast of Ugak Bay, and possibly near Karluk Lake (MacDonald and Cook 2009).
5	Semidi Islands and vicinity	Marmot	Probably introduced; possibly indigenous. Reported present as early as 1792 (D. W. Clark, pers. comm.) and abundant on island (Chumbley et al. 1997). No archived specimens.
6		Tugidak	Present (Alaska Department of Fish and Game 1995; Bailey 1993). No archived specimens.
7		Woody	Probably introduced from Kodiak stock. No archived specimens.
8		Aghiyuk	Present (Hatch and Hatch 1983). No archived specimens.
9		Aliksemit	Present (Hatch and Hatch 1983). No archived specimens.
10		Anowik	Present (Hatch and Hatch 1983). No archived specimens.
11		Chankliut	Introduced 1918 (Bailey and Faust 1981). No archived specimens.
12		Chirikof	Possibly indigenous; probably introduced (this study). Excavated skeletal remains radiocarbon dated at 450 ± 50 years old (P. Saltonstall, pers. comm.), suggesting possible pre-Russian era transport by Alutiiq people. Archived specimens at MSB.
13		Chowiet	Possibly introduced (this study). Archived specimens at MSB.
14		Kak	Present (Bailey and Faust 1981). No archived specimens.
15		Kateekuk	Probably introduced (this study). Archived specimens at UAM, MSB.
16		Kiliktagik	Probably introduced (this study). Archived specimens at UAM, MSB.
17	Nakchamik	Present (Bailey and Faust 1981). No archived specimens.	
18	Unavikshak	Present (Bailey and Faust 1981). No archived specimens.	
19	Shumagin Islands	Atkins	Present (Byrd et al. 1997).
20		Bendel	Present (Bailey 1993; A. Eddingsaas, pers. obs.). No archived specimens.
21		Bird	Status undetermined (this study). Archived specimens at UAM, MSB.
22		Chernabura	Status undetermined (this study). Archived specimens at UAM, MSB.
23		Heredeen	Present (Byrd et al. 1997).
24		Karpa	Present (A. Eddingsaas, pers. obs.). No archived specimens.
25		Koniuji, Big	Probably introduced 1916 (Bailey 1993). Specimens archived at MSB.
26		Koniuji, Little	Probably introduced; possibly indigenous (this study). Archived specimens at UAM, MSB.
27		Nagai	Observed on the island by Georg Steller in 1741 (Ebbert and Byrd 2002), which suggested to Bailey (1978) indigenous status on some of the larger Shumagins. Type locality of <i>nebulicola</i> Osgood, 1903 (USNM 59145).
28	Near		Present (Bailey 1978, 1993). No archived specimens.
29		Simeonof	Probably indigenous (Ebbert and Byrd 2002; this study). Archived specimens at USNM, UAM, MSB.
30	Sandman Reefs	Spectacle	Present (Bailey 1993) before 1960 (D.W. Clark, pers. comm.). No archived specimens.
31		Turner	Present (Bailey 1993). No archived specimens.
32		Cherni	Present (A. Eddingsaas, pers. obs.). No archived specimens.
33		Aleutian Islands	Amaknak
34		Kavalga	Introduced (Murie 1959; Burris and McKnight 1973; this study) about 1920 from Unalaska (Murie 1959). Archived specimens at USNM, UAM, MSB.
35		Umnak	Probably introduced (Eyerdam 1936; Howell 1938), source unknown. No archived specimens.
36		Unalaska	Introduced (Burris and McKnight 1973; this study) ca 1896 or earlier (MacDonald and Cook 2009) from Nushagak (Osgood 1904). Additional specimens at MSB, MVZ.
37	Mainland	Unimak	Indigenous (Hall 1981; Murie 1959; others). Archived specimens at USNM, MVZ.
38		Cold Bay	Indigenous.
39		Iliamna Lake	Indigenous.
40		4th of July Creek	Indigenous.
41		Yenlo Hills	Indigenous.
42		Fort Richardson	Indigenous.
43		Snowhawk Creek	Indigenous.

^a Island name in boldface type indicates DNA sequenced for this study.

1992). Pairwise tests of linkage disequilibrium between loci also were performed using a Markov chain method (10,000 demorizations, 100 batches, and 5,000 iterations). We report Bonferroni corrected P -values for all tests.

Estimates of genetic differentiation between all population pairs (F_{ST}) were calculated using FSTAT (Goudet 2001) and tested for statistical significance using 10,000 permutations. The level of significance was held at $P < 0.05$ using the Bonferroni correction.

To determine the number and identity of genotypically distinct populations we used the program STRUCTURE 2.2.3 (Pritchard et al. 2000), which implements a Bayesian clustering algorithm based on a Markov chain Monte Carlo approach to assign individuals to a given number (K) of population clusters. Ten runs were performed at each K (1–25) with no admixture, without prior information of individual origin, and burn-ins of 10,000 and 50,000 replications. To determine the number of clusters we compared the log likelihood of each K as outlined in Pritchard et al. (2000) and calculated ΔK (Evanno et al. 2005). Assignment tests were conducted based on the optimal number of clusters detected in our STRUCTURE analysis.

We assessed patterns of recent migration in the program BayesAss+ (Wilson and Rannala 2003) using 3,000,000 iterations, a burn-in of 1,000,000, and a sampling frequency of 2,000. This analysis does not assume Hardy–Weinberg equilibrium within populations and can detect patterns of asymmetrical migration between populations. BayesAss+ estimates the rate of gene flow (m) and associated confidence interval using a Bayesian Markov chain Monte Carlo sampling method to determine if m is significantly different than 0.

Relationships among populations based on microsatellite data were investigated further using PHYLIP version 3.6 (Felsenstein 1993). Pairwise chord distances (Cavalli-Sforza and Edwards 1967) among populations were calculated in the GENDIST subroutine using the allele frequency matrix created in MSA 3.12 (Dieringer and Schlotterer 2003). Chord distances were used to construct a neighbor-joining tree in the NEIGHBOR subroutine. Bootstrap replicates (1,000) were generated in MSA 3.12 and analyzed in PHYLIP version 3.6 (Felsenstein 1993) using the GENDIST subroutine. A bootstrap consensus tree was created using the CONSENSE subroutine. Tree files were viewed using TREEVIEW version 2.0 (Page 1996).

RESULTS

Cytochrome b.—Base frequencies (A: 0.28, C: 0.27, G: 0.13, and T: 0.32) were typical of mammalian *Cytb* (Irwin et al. 1991). The transition : transversion ratio was 32.04, and this ratio was used in the substitution model. Phylogeographic analysis of the *Cytb* sequences expanded on the preliminary patterns of differentiation reported by Eddingsaas et al. (2004). The maximum-likelihood *Cytb* tree (Fig. 2) identified 3 major groups: a polytomy of mainland populations found south of the Alaska Range and Iliamna Lake at the base of the

Alaska Peninsula (Southwest Alaska Mainland), a Peninsula/Island group that includes all islands south of the Alaska Peninsula and a mainland population near the tip of the Alaska Peninsula (Cold Bay), and an Aleutian Islands group represented by Unalaska and Kavalga islands of the Aleutian Chain. Only the Peninsula/Island group shows well-supported substructure, with the deepest split between the mainland (Cold Bay) and island populations. Within these islands Ushagat is the most distinctive, with populations on the Semidi Islands and Chirikof Island forming a clade separate from a polytomy that includes populations of the Shumagin Islands and Kodiak Island.

Microsatellites.—All 8 microsatellite loci were highly polymorphic, with variation ranging from 8 (GS12) to 19 (GS25) alleles. For populations with >10 individuals, estimates of genetic diversity (number of alleles per locus, allelic richness, and heterozygosity) were generally higher in mainland populations than island populations (Table 2). Tests for deviations from Hardy–Weinberg equilibrium indicated that 6 populations exhibited heterozygote deficiencies (Table 2) at 5 loci (GS3, GS12, GS14, GS20, and GS26). No significant linkage disequilibrium was detected between loci within populations, thus we retained all loci for subsequent analyses.

Tests for genetic differentiation between populations (F_{ST}) indicated extensive structuring among southwestern Alaska populations of *S. parryii* (Table 3). When populations with small sample sizes (<10 individuals) were removed from consideration, all but 2 populations (Kavalga and Unalaska) were significantly distinct.

STRUCTURE analyses determined that assignment of individuals to 21 discrete population clusters had the highest log likelihood ($-3,511.50$). However, using the method of Evanno et al. (2005), the highest number of clusters was $K = 19$. These results contrast with the 17 localities originally sampled; however, 2 of these clusters represent a mix of the introduced populations of Kavalga and Unalaska, and 2 are a mix of Unalaska/4th of July/Iliamna Lake. Where there is overlap of populations in clusters, shared individuals generally are related by geographic proximity (Table 4). Populations that shared assignments to particular clusters (at >0.05) include Unalaska/Chowiet/Kateekuk; Chirikof/Kiliktagik; Chirikof/Iliamna Lake/Chowiet/Kateekuk; 4th of July Creek/Yenlo Hills/Iliamna Lake/Kateekuk; Kavalga/Unalaska; Unalaska/4th of July Creek/Iliamna Lake; Chernaburna/Little Koniuzzi/Simeonof; Bird/Chernaburna; Chowiet/Kateekuk; 4th of July Creek/Fort Richardson/Simeonof; and Unalaska/Iliamna Lake/Kateekuk/Simeonof.

Estimates of migration from BayesAss+ indicated that most rates of gene flow between populations were not detectable; that is, they were not distinguishable from rates generated when the data lack sufficient variation to detect dispersal events with high confidence (Wilson and Rannala 2003). The few measurable rates of recent migration were low and indicated directional patterns ranging from $m = 0.085$ to 0.169 (Table 5).

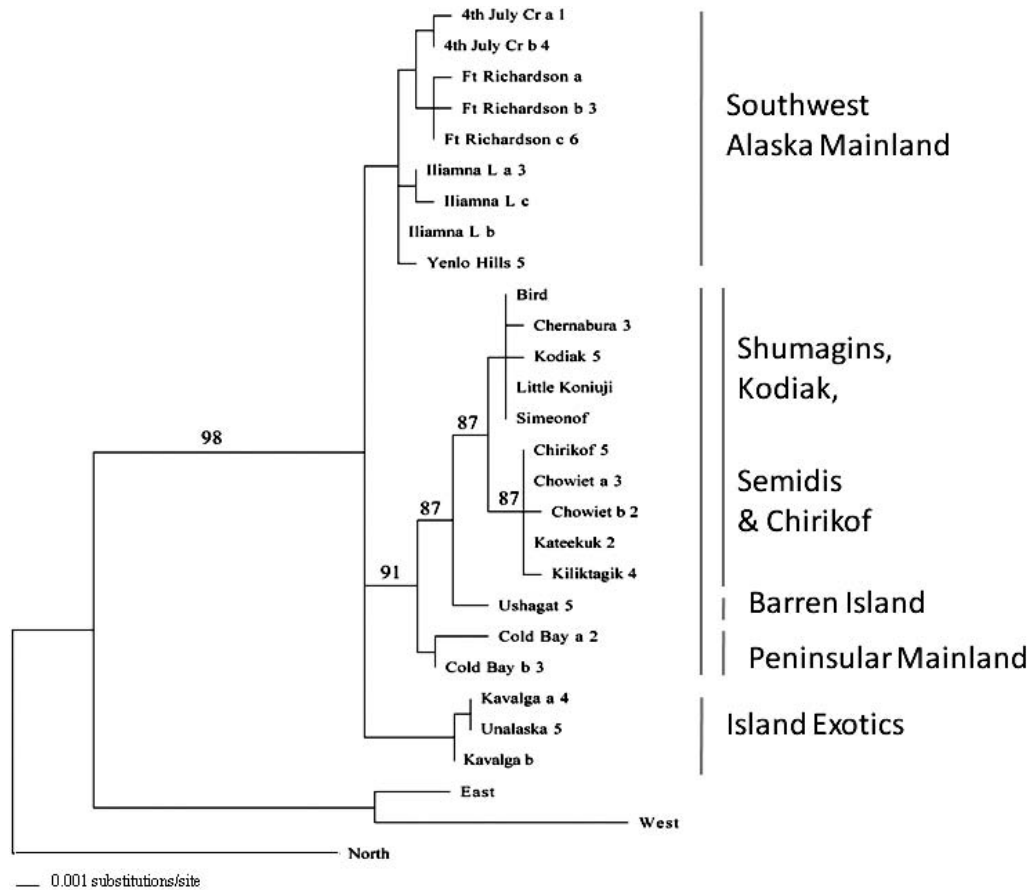


FIG. 2.—Maximum-likelihood tree of cytochrome-*b* haplotypes indicating the relationships of southwestern Alaska populations of *Spermophilus parryii*. Tree is rooted with haplotypes from the other major clades (East, West, and North) of *S. parryii* identified by Eddingsaas et al. (2004). Numbers on branches represent bootstrap values > 80% based on 1,000 bootstrap replicates. Letters at the end of population name represent distinct haplotypes, and numbers indicate number of individuals with that haplotype.

Neighbor-joining assessment of microsatellite variation revealed relationships similar to those found with mtDNA analyses, although few are supported strongly by bootstrap resampling. Geographic clusters (Fig. 3) include mainland populations south of the Alaska Range, a group of populations on the Alaska Peninsula and Aleutian Islands of Unalaska and Kavalga, and the 4 Shumagin island populations. Relationships of individuals from islands of the Kodiak region (Kodiak, Ushagat, and Chirikof) and the Semidi Islands are less well defined.

DISCUSSION

Eddingsaas et al. (2004) analyzed mitochondrial sequence variation in Nearctic populations of arctic ground squirrels and identified 4 geographically distinct clades. Of these, the Southwest clade is composed of populations found south and west of the Alaska Range. Our expanded exploration of variation in both mitochondrial sequences and nuclear microsatellite loci serves to refine our understanding of the history of ground squirrels in southwestern Alaska during the late Quaternary, including populations that inhabit several North Pacific island complexes.

Regionwide variation.—Terrestrial species in southwestern Alaska might have persisted during the repeated glacial advances of the late Pleistocene in a series of smaller ice-free refugia that were isolated along the southern edge of the Alaska Peninsula (Cook and MacDonald 2001; Holder et al. 2000; Hopkins 1967; Rausch 1969). Evidence for hypothesized LGM refugia along the North Pacific Coast is controversial (Demboski et al. 1999). For ground squirrels, identification of 3 distinct groups in the southwestern region suggests the possibility of 3 glacial refugia where arctic ground squirrels could have persisted during the LGM, if not earlier. Refugial populations of *S. parryii* in southwestern Alaska based on molecular data were 1st postulated in Eddingsaas et al. (2004) and supported by estimates of deep divergence dates (300,000 years ago at the onset of the Illinoian glaciations). Populations of arctic ground squirrels on mainland Alaska might have persisted during the LGM in the ice-free regions of Beringia, most likely in the Yukon River delta area (Hamilton 1986; Hopkins 1967). Potential coastal refugia for these insular southwestern populations are unclear because relatively little is known about glacial patterns along the Alaska Peninsula during the late Pleistocene (Mann and Hamilton 1995; Mann and Peteet 1994; Stilwell and Kaufman

TABLE 2.—Population information and general diversity estimates for 8 microsatellite loci from 17 populations of *Spermophilus parryii* in southwestern Alaska. Population information, including region, group, and sample size (*n*), is indicated. Estimates of the average number of alleles per locus (alleles), mean allelic richness (richness), *F_{IS}* (the inbreeding coefficient within populations; boldface type if out of Hardy–Weinberg equilibrium, * if statistically significant at *P* < 0.0005), and observed heterozygosity (*H_O*) are given.

Population	Region	Group	<i>n</i>	Alleles	Rich- ness	<i>F_{IS}</i>	<i>H_O</i>
Kavalga Island	Aleutians	Aleutians	41	4.25	1.51	0.214*	0.402
Unalaska Island	Aleutians	Aleutians	16	4.75	1.64	0.364*	0.410
Ushagat Island	Barrens	Islands	11	2.50	1.30	0.160	0.250
Chirikof Island	Kodiak	Islands	22	4.63	1.54	0.363*	0.350
Kodiak Island	Kodiak	Islands	14	3.38	1.47	0.127	0.410
Chowiet Island	Semidis	Islands	22	4.25	1.40	0.251*	0.299
Kateekuk Island	Semidis	Islands	2	1.75	1.33	−0.200	0.375
Kilikitagik Island	Semidis	Islands	4	1.63	1.21	−0.050	0.219
Bird Island	Shumagins	Islands	8	2.63	1.43	0.320	0.297
Chernabura Island	Shumagins	Islands	2	2.38	1.63	0.391	0.438
Little Koniuji Island	Shumagins	Islands	5	2.63	1.38	0.890	0.350
Simeonof Island	Shumagins	Islands	2	1.50	1.27	−0.250	0.313
4th of July Creek	Central	Mainland	5	3.63	1.70	−0.193	0.800
Fort Richardson	Central	Mainland	18	5.25	1.73	0.132	0.639
Yenlo Hills	Central	Mainland	14	4.00	1.56	0.270*	0.412
Cold Bay	Peninsula	Mainland	23	5.88	1.65	0.215*	0.513
Iliamna Lake	Peninsula	Mainland	6	3.75	1.73	0.039	0.706

1996). Small refugia have been proposed throughout southwestern Alaska, possibly on Kodiak Island (Karlstrom and Ball 1969), the inner Shumagin Islands, or on the continental shelf, or a combination of these, exposed by sea levels that could have been lowered >100 m during glacial maxima (Harlin-Cognato et al. 2006; Hopkins 1967; Karlstrom 1964). Holder et al. (2000) found significant phylogeographic structure across populations of rock ptarmigan (*Lagopus muta*) in southwestern Alaska and concluded that patterns of

morphologic and genetic variation were consistent with differentiation in independent glacial refugia that was later reinforced by the insular nature of these populations.

Within the Southwest clade the geographic variation (Fig. 2) we uncovered is not surprising given life-history characteristics of *S. parryii* (e.g., high natal philopatry) that lead to a patchy distribution and, in turn, relatively low dispersal rates (Byrom and Krebs 1999). Arctic ground squirrels create burrows for predator avoidance and hibernation. Burrows require well-drained soils that are discontinuously distributed in Alaska (Carl 1971; Mayer 1953) and hence promote local diversification (Baker and Marler 1980; Chesser 1991). Among the oceanic islands of the southwestern region gene flow is limited further because arctic ground squirrels apparently do not colonize naturally across the oceanic barrier, as demonstrated by their spotty occurrence on islands throughout the region (Bailey 1993; MacDonald and Cook 2009) and elsewhere (Vorontsov et al. 1984). Hence, we would suspect that individual island populations should be genetically distinguishable if they were established prior to the LGM and isolated from other populations following sea-level rise after the LGM. In contrast, populations that were introduced by humans should show minimal differentiation.

Relatively high levels of genetic differentiation among some populations within the Southwest clade suggest that arctic ground squirrels potentially persisted in southwestern Alaska in multiple coastal refugia and that subsequent isolation on islands in the Holocene has reinforced divergence, as proposed for rock ptarmigan of the region (Holder et al. 1999, 2000). The locations of potential coastal refugia, where members of the divergent Southwest Clade persisted during the LGM, are not clear and will require paleoreconstruction of glacial advances. New perspectives also are being developed based on excavation of paleomiddens on islands in the region that should provide further insight into the presence and persistence of mammals (Maschner et al. 2008).

TABLE 3.—Matrix of pairwise estimates of genetic differentiation (*F_{ST}*) calculated for 8 microsatellite loci between populations of *Spermophilus parryii* from southwestern Alaska. Significant (*P* < 0.05, Bonferroni corrected) estimates are noted in boldface type.

	KAV	UNA	USH	CHI	KOD	CHO	KAT	KIL	BIR	CHE	LIT	SIM	FOU	FOR	YEN	COL	ILI
Kavalga Island (KAV)	0.000																
Unalaska Island (UNA)	0.035	0.000															
Ushagat Island (USH)	0.432	0.365	0.000														
Chirikof Island (CHI)	0.347	0.307	0.330	0.000													
Kodiak Island (KOD)	0.445	0.434	0.591	0.429	0.000												
Chowiet Island (CHO)	0.440	0.425	0.496	0.180	0.511	0.000											
Kateekuk Island (KAT)	0.439	0.383	0.457	0.092	0.523	0.016	0.000										
Kilikitagik Island (KIL)	0.477	0.474	0.566	0.275	0.578	0.517	0.594	0.000									
Bird Island (BIR)	0.360	0.319	0.518	0.449	0.473	0.557	0.546	0.593	0.000								
Chernabura Island (CHE)	0.305	0.214	0.461	0.327	0.417	0.473	0.386	0.525	0.102	0.000							
Little Koniuji Island (LIT)	0.468	0.408	0.433	0.372	0.561	0.464	0.476	0.598	0.371	0.267	0.000						
Simeonof Island (SIM)	0.425	0.345	0.582	0.452	0.529	0.551	0.677	0.726	0.231	0.145	0.431	0.000					
4th of July Creek (FOU)	0.233	0.173	0.370	0.224	0.391	0.335	0.279	0.456	0.306	0.160	0.288	0.332	0.000				
Fort Richardson (FOR)	0.304	0.211	0.339	0.306	0.418	0.384	0.324	0.405	0.385	0.221	0.339	0.296	0.107	0.000			
Yenlo Hills (YEN)	0.310	0.232	0.301	0.252	0.408	0.325	0.296	0.479	0.254	0.221	0.165	0.307	0.142	0.250	0.000		
Cold Bay (COL)	0.240	0.187	0.295	0.179	0.300	0.238	0.203	0.386	0.216	0.126	0.258	0.246	0.088	0.258	0.125	0.000	
Iliamna Lake (ILI)	0.146	0.102	0.377	0.213	0.320	0.344	0.306	0.456	0.241	0.125	0.326	0.292	0.067	0.183	0.104	0.058	0.000

TABLE 4.—Bayesian assignment test results for 17 populations of *Spermophilus parryii* from southwestern Alaska. Values indicate the proportion of membership (>0.01) of each predefined population in each of the 19 clusters, without using any prior population of origin.

Population	Cluster																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Kavalga	—	0.02	—	—	—	—	—	0.01	—	—	0.45	0.45	—	—	0.03	—	—	—	0.03
Unalaska	—	0.24	—	—	—	—	—	—	—	—	0.31	0.27	—	—	0.11	—	—	—	0.06
4th of July	—	—	—	—	0.03	—	0.25	—	—	—	—	—	—	—	0.25	—	0.43	—	0.03
Fort Richardson	—	—	—	—	—	—	0.02	—	—	—	—	—	—	0.81	—	—	0.16	—	—
Yenlo Hills	0.03	—	—	—	—	—	0.22	0.72	—	—	—	—	—	—	—	—	0.01	—	0.01
Ushagat	—	—	—	1.00	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Chirikof	0.04	0.01	0.19	—	0.54	—	—	—	0.02	—	—	—	—	—	—	—	—	0.01	—
Kodiak	—	—	—	—	—	—	—	—	—	—	1.00	—	—	—	—	—	—	—	—
Cold Bay	0.33	0.01	—	—	0.01	0.60	0.02	—	—	—	—	—	—	—	—	—	—	—	0.02
Iliamna Lake	0.02	0.02	—	—	0.08	0.01	0.12	0.03	0.01	—	0.03	0.03	—	—	0.27	—	0.04	0.03	0.33
Chowiet	—	0.06	0.03	—	0.08	0.01	0.02	—	—	—	—	—	0.03	—	0.02	—	0.01	0.71	0.03
Kateekuk	—	0.17	0.05	—	0.27	—	0.20	0.01	0.01	—	—	—	0.03	—	—	—	—	0.12	0.13
Kiliktagik	—	—	1.00	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Bird	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.00	—	—	—
Chernabura	—	—	—	—	—	—	—	—	—	—	—	—	0.50	—	—	0.50	—	—	—
Little Koniuji	—	—	—	—	—	—	0.20	—	—	—	—	—	—	0.95	—	—	0.01	—	0.02
Simeonof	—	—	—	—	—	—	—	—	—	—	—	—	—	0.65	—	0.05	0.21	—	0.09

Geographic structure can be identified as 1st, populations from Kavalaga and Unalaska that are the result of documented human-caused introductions originating from a mainland population near the base of the Alaska Peninsula (Nushagak), and 2nd, populations on the mainland that are north of the Alaska Peninsula (mainland north) that appear to be basal to, 3rd, a well-supported clade of populations that are found on the far western Alaska Peninsula and islands south of the Peninsula (Fig. 2). Within this latter peninsula-island group populations from Cold Bay (mainland Alaska Peninsula) and Ushagat (Barren Islands) are distinctive, as are a group that includes the Semidi islands (Chowiet, Kateekuk, and Kiliktagik) and Chirikof Island. A 4th, less well-defined group, consists of the Shumagin Islands plus Kodiak Island. As expected, microsatellite variation provides a finer view of differentiation in these populations, but major clades still are generally supported. Shared population assignment of individuals (Table 4) is generally restricted to populations that are closely related based on mitochondrial sequence analysis. Populations that are assigned exclusively to a single cluster

(Table 4) include the island populations of Ushagat, Kodiak, Bird, and Kiliktagik. In general, mainland populations, known human-mediated introductions (Kavalga and Unalaska), or island populations within the same island group (Shumagins or Semidis) shared cluster membership.

TABLE 5.—Estimates (BAYESASS+—Wilson and Rannala 2003) of the 6 significant rates of migration (*m*), and associated confidence intervals (*CI*s), from one population into another population from a total of 17 populations of *Spermophilus parryii* examined from southwestern Alaska.

Population		<i>m</i>	<i>CI</i>
From	Into		
Kavalga Island	Chirikof Island	0.130	(0.050, 0.220)
	Kodiak Island	0.113	(0.028, 0.220)
Little Koniuji Island	Ushagat Island	0.099	(0.025, 0.217)
Unalaska Island	Chowiet Island	0.085	(0.026, 0.161)
Yenlo Hills	Chowiet Island	0.169	(0.087, 0.257)
	Bird Island	0.124	(0.029, 0.259)

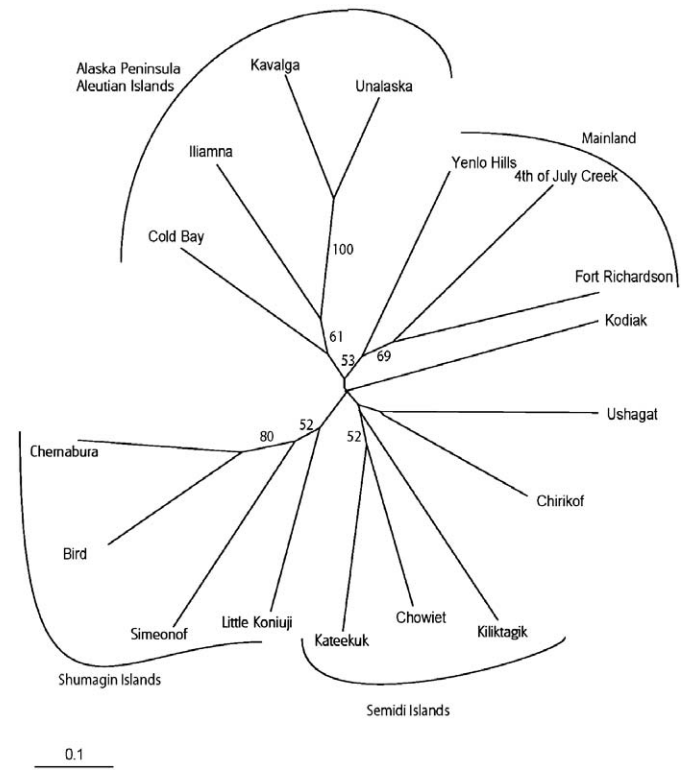


FIG. 3.—Consensus tree from 1,000 unrooted neighbor-joining trees based on Cavalli-Sforza and Edwards chord distance generated for 8 microsatellite loci for 17 populations of *Spermophilus parryii*. Numbers on branches represent bootstrap values >50%, and the scale bar refers to the chord distance.

Island endemism.—Questions related to whether particular insular populations in Alaska are indigenous or exotic (i.e., the result of human introduction) remain unclear in most cases; however, relationships among particular islands and island groups are beginning to emerge (Cook et al. 2001). Molecular signatures indicate that *S. parryii* persisted in the region for a long time with distinctive populations in Cold Bay, Ushagat Island, and the Semidi/Shumagin island groups. Low levels of differentiation within the Semidi/Shumagin island groups, including the close genetic relationship and pattern of gene flow between Chirikof and Kodiak Island populations, suggest possible human-mediated introductions across these islands. Identifying the source and recipient of these transplants is confounded by a lack of sampling; however, there may be some indication that the original mainland source population for Kavalga Island was similar to that for other introduced island populations, such as Chirikof and Kodiak islands (Table 5). When phylogenetic results are viewed in light of the glacial history of the region, it is possible to refine hypotheses that distinguish indigenous and introduced populations.

The distinctiveness of squirrels from the Shumagin Islands group (*S. p. nebulicola*) was recognized previously (Howell 1938; Osgood 1904), based on morphological examination of 15 specimens from 3 outer (southeast of West Nagai Strait) islands, namely Nagai, Simeonof, and 1 of the 2 Koniuji islands (Howell 1938). Hopkins (1967) suggested that the outer Shumagins might have been ice free during the LGM, raising the possibility that this nominal subspecies represents populations that persisted through the LGM (i.e., paleoendemics). The mtDNA sequences are inconsistent with the idea that these island populations have such a deep, independent history from other populations such as Kodiak (Fig. 2). Because no evidence indicates that the very outermost Shumagins (Bird, Chernaburna, and Simeonof) were ever connected to the Alaska Peninsula (Hopkins 1967), Ebbert and Byrd (2002) postulated that these populations could have been introduced by humans, a hypothesis that gains support from the low levels of migration (Table 5) into Bird Island from the mainland (Yenlo Hills). Ground squirrels on Big Koniuji Island are reported to be the result of an introduction in 1916 (Bailey 1993). If that is the case, these outermost island populations might have originated from either island stock like that on Little Koniuji or Nagai islands or, less likely, from distant Kodiak Island. Squirrels were observed on Nagai Island by Georg Steller in 1741, thus indicating their presence on this island by approximately 1733, but we were unable to include representatives of that population in this analysis. Lack of material also remains a problem for Atkins, Bendel, Herdeen, Karpa, Big Koniuji, Near, Spectacle, and Turner islands (Table 1). Ground squirrels apparently are absent from all of the Shumagins closer to the mainland (i.e., those northwest of West Nagai Strait and including the 3 major islands of Unga, Popof, and Korovin) with 1 exception, the small island of Karpa, located 8 km northeast of Korovin (A. A. Eddingsaas, pers. obs.).

Finally, the Kodiak Island population is monomorphic for a unique mitochondrial haplotype that is closely related to

haplotypes of the Shumagin Islands group, but Bayesian assignment tests based on nuclear microsatellite variation (Table 4) place Kodiak in a cluster separate from all other populations. The large geographic distance between these islands (>350 km) and the extremely limited range of arctic ground squirrels on Kodiak Island (restricted to the extreme northeastern corner of the island in the vicinity of the town of Kodiak and on nearby Woody and Holiday islands in Chiniak Bay) allow the possibility of introduction of *S. parryii* to Kodiak Island from the Shumagin Island group, with subsequent genetic divergence of this introduced population. If Kodiak Island arctic ground squirrels are introduced, the validity of *S. p. kodiacensis* J. A. Allen, 1874, is questionable.

The history of either persistence or human introductions of *S. parryii* on the Semidi Islands group is also unclear. This island group has a poorly known glacial history, and some likelihood exists that it contains a mix of indigenous and introduced populations. Bailey and Faust (1981) reported that ground squirrels were introduced on Chankliut Island (also in the Semidis) in 1918. We examined Chowiet, Kateekuk, and Kilihtagik islands in the Semidis, and, with the exception of Kateekuk, all show relatively high among-island microsatellite differentiation (Table 3). Kateekuk, although represented by a sample of only 2, is very closely aligned with Chowiet and to a lesser extent Chirikof at levels that might reflect human introduction. The mtDNA analyses also indicate that the Chirikof Island population is closely related to the Semidi Islands group (Fig. 2). Chirikof Island, at the southern end of the Kodiak Archipelago, is >70 km distant from the Semidis. We hypothesize that Chirikof Island supports an introduced population—a view also held by Dall (1870)—and the likely source based on our sampling data is Kateekuk Island. However, radiocarbon dates (450 years ago \pm 50 SE) for arctic ground squirrel bones from Chirikof Island indicate that squirrels occurred on Chirikof before the Russians arrived in North America in 1733. Ground squirrels long have been highly valued by Alutiiq and Aleut people, raising the possibility of prehistoric introduction of squirrels to islands by humans. Aleksandr Baranov, governor of Russian America from 1799 to 1818, established a special outpost on Chirikof (then known as Ukamak) for ground squirrel procurement.

Other cases of human introduction of arctic ground squirrels are better documented. In the Aleutians, arctic ground squirrels occur naturally only as far west as Unimak Island (MacDonald and Cook 2009). They were introduced on Unalaska Island in 1896 or earlier from Nushagak stocks (Osgood 1904) and on Kavalga Island from Unalaska stocks around 1920 (Murie 1959; Swanson 1982). Kavalga and Unalaska are very similar genetically (Fig. 2; Table 3). Squirrels were reported to be introduced on Amaknak Island about 1895 from an unknown source (Peterson 1967), and also onto Umnak Island (Dufresne 1946; Eyerdam 1936; Howell 1938). No specimens from these islands are available for study. Thus, all Aleutian squirrel populations west of Unimak Island (i.e., Unalaska, Amaknak, Umnak, and Kalvaga) are likely exotics, but several populations remain to be examined in a molecular framework.

In summary, we expand on the conclusion of Eddingsaas et al. (2004) that populations of ground squirrels on the Alaska Peninsula and associated islands form the distinctive Southwest clade and are divergent from all other arctic ground squirrel populations. Within the Southwest clade, a deep split occurs between populations south of the Alaska Peninsula (primarily island populations) and mainland populations north of the Alaska Peninsula extending to the southern flanks of the Alaska Range. A significant caveat to this conclusion is the lack of representative specimens from a 700-km expanse along the Alaska Peninsula (between Cold Bay and Iliamna Lake). Specimens also are unavailable for 25 of 37 islands of probable occurrence in this region. Although we have demonstrated introductions in a few cases and identified clusters of islands that are distinct from other populations, central questions remain as to how many of these populations were established and when they began to differentiate.

Mitochondrial and microsatellite (Table 4) structure identified in these rodents could be related to higher diversification rates purported to be typical of insular populations, combined with the low vagility of ground squirrels. With the possible exception of Ushagat, none of the island populations is divergent at the deep level that would suggest persistence as paleoendemics. Nonetheless, because of the difficulty of applying appropriate evolutionary models to single mitochondrial loci or microsatellite loci (Brito and Edwards 2009), molecular dating of the divergence of these populations should await analyses based on a coalescent assessment of sequence variation across multiple independent loci (Hare 2001; Rosenberg and Nordborg 2002).

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- Alaska Peninsula, Cold Bay: UAM50603 (AY427977), UAM50604 (AY427978), MSB196853, MSB196854, MSB196857, MSB196890, MSB196893, MSB196895, MSB196902, MSB196903 (GU220840), MSB196914, MSB196915, MSB196916, MSB196933, MSB196923, MSB196925, MSB196926, MSB196929, MSB196931, MSB196934 (GU220839), MSB196937, MSB196939, MSB196942 (GU220838). Iliamna Lake: MSB151124, MSB151428 (GU220868), MSB151429 (GU220871), MSB151432 (GU220869), MSB151434 (GU220870), MSB151437 (GU220872).
- Aleutians, Kavalga Island: UAM60416 (GU220874), UAM60417 (GU220875), UAM60418, UAM60419, UAM60420, UAM60421 (GU220877), UAM60422 (GU220876), UAM60423 (GU220873), UAM60424, UAM60425, MSB151410, MSB151411, MSB151412, MSB151413, MSB151711, MSB196858, MSB196859, MSB196865, MSB196866, MSB196869, MSB196874, MSB196875, MSB196876, MSB196878, MSB196883, MSB196884, MSB196896, MSB196897, MSB196898, MSB196899, MSB196900, MSB196901. MSB196905, MSB196924, MSB196927, MSB196928, MSB196944, MSB196945, MSB196946, MSB196947, MSB196948. Unalaska Island: MSB151370, MSB151371, MSB151409, MSB151414 (HM204709), MSB151415 (HM204710), MSB151438 (HM204711), MSB196871, MSB196872 (HM204712), MSB196873, MSB196880, MSB196881, MSB196882 (HM204713), MSB196917, MSB196918, MSB196919, MSB196920.
- Barren Islands, Ushagat: MSB196867 (GU220850), MSB196870, MSB196868, MSB196877 (GU220851), MSB196879 (GU220853), MSB196906 (GU220849), MSB196907, MSB196908 (GU220852), MSB196910, MSB196909, MSB196921.
- Semidi Islands, Chowiet Island: MSB151341, MSB151342 (GU220833), MSB151347, MSB151348, MSB151349, MSB151350, MSB151351, MSB151352, MSB151353, MSB151361, MSB151362, MSB151363, MSB151364 (GU220836), MSB151365, MSB151366 (GU220834), MSB151367, MSB151369 (GU220837), MSB151372, MSB151373 (GU220835), MSB151374, MSB196911, MSB196913. Kateekuk Island: UAM73565 (GU220841), UAM73569 (GU220842). Kiliktagik Island: UAM41852 (GU220844), UAM43112 (AY427996), UAM47267 (GU220843), UAM54611 (AY427997).

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APPENDIX I

Subspecies, location, and University of Alaska Museum of the North (UAM) or Museum of Southwestern Biology (MSB) catalog number of specimens. The GenBank accession number (GU, HM, or AY) is included for those individuals that were sequenced. All specimens were included in microsatellite analyses with the exception of UAM31890 and UAM31892.

Spermophilus parryii ablusus.—Anchorage, 4th of July Creek: UAM64828 (GU220855), UAM65791 (GU220857), UAM65792 (GU220858), UAM65808 (GU220856), UAM65820 (GU220854). Fort Richardson: UAM31883 (GU220867), UAM31884 (GU220860), UAM31885, UAM31886 (GU220861), UAM31887 (AY427979), UAM31888, UAM31889 (GU220859), UAM31890 (GU220863), UAM31891, UAM31892 (GU220864), UAM31893, UAM31894, UAM31909, UAM32934, UAM61960, UAM61961,

Spermophilus parryii nebulicola.—Shumagin Islands, Bird Island: UAM43096 (AY420005), UAM50454, UAM50761 (GU220824), UAM50840 (GU220826), UAM50842 (GU220825), UAM50845 (AY428005), UAM50601, UAM50602. Chernaburna Island: UAM43098 (AY428006), UAM43099 (AY428007). Little Koniuji Island: UAM43095 (AY428001), UAM50843 (GU220845), UAM50844 (GU220846), UAM50846 (GU220848), UAM51646 (GU220847). Simeonof Island: UAM43097 (AY428004), UAM50841 (AY428003).

Spermophilus parryii kodiacensis.—Chirikof Island: MSB196848, MSB196849, MSB196850, MSB196852, MSB196855, MSB196885, MSB196886, MSB196887, MSB196888, MSB196889, MSB196891, MSB196892, MSB196904 (GU220830), MSB196912, MSB196930 (GU220831), MSB196932, MSB196935 (GU220832), MSB196936, MSB196938, MSB196940, MSB196941, MSB196943. Kodiak Island: UAM24121 (AY427993), UAM24123 (AY427994), UAM24125, UAM24126 (AY427992), UAM24127 (AY427991), UAM24347 (AY427990), MSB196856, MSB196860, MSB196861, MSB196862, MSB196863, MSB196864, MSB196894, MSB196926.