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PHYLOGEOGRAPHY OF KEEN'S MOUSE (*PEROMYSCUS KEENI*) IN A NATURALLY FRAGMENTED LANDSCAPE

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Phylogenetic methods were used to analyze cytochrome-*b* sequences ($n = 257$; 560 base pairs) from *Peromyscus keeni* (Keen's mouse) collected from 23 islands of the Alexander Archipelago and 6 mainland locations in southeast Alaska and western Canada. Although *P. keeni* is ubiquitous across this region, island populations are genetically distinctive. Genetic structure of extant populations of *P. keeni* appears to have been more heavily influenced by vicariance than post-glacial colonization. Populations of *P. keeni* might have survived in coastal refugia during the last glacial maximum (15×10^3 years ago). Island area was significantly correlated with 2 of 3 genetic diversity measures whereas island isolation was not. Areas with divergent populations were discovered, but were largely inconsistent with 3 of 5 currently recognized subspecies. Cryptic variation was detected in 8 areas not previously identified by morphologic analyses.

Key words: Alexander Archipelago, cytochrome *b*, endemism, island, phylogeography

The Alexander Archipelago and mainland of southeast Alaska have a rich glacial history (Hamilton 1994). However, the extent of glaciation and the role this dynamic history played in shaping the structure of the region's extant biota is not fully understood (Conroy et al. 1999; Cook and MacDonald 2001; Fleming and Cook 2002; Heaton et al. 1996). Until recently, knowledge of the distribution of the region's endemic mammalian taxa has been sparse and was based on field collections made primarily in the late 1800s and early 1900s by the United States Bureau of Biological Survey and the Alexander Alaska Expeditions of the University of California at Berkeley Museum of Vertebrate Zoology (Hall 1981; MacDonald and Cook 1996). Over the last decade renewed collecting efforts in the region, combined with molecular studies of geographic variation, are beginning to reveal previously undetected patterns of geographic structure (e.g., Cook et al. 2001; Heaton et al. 1996; Hogan et al. 1993; Paetkau et al. 1998; Tomasik 2003).

A large number of endemic mammals have been described from the region (Cook and MacDonald 2001; Hall 1981). A solid taxonomic framework for these endemic mammals is imperative because of intense anthropogenic activities in these coastal forests, most notably alteration of mature habitat and associated road building (Durbin 1999; United States Department of Agriculture 1997). A better comprehension of island biogeography also is required for conserving this region's rich mammalian fauna.

The northwestern deer mouse is southeast Alaska's most widespread non-volant terrestrial mammal (MacDonald and Cook 1996). These mice are considered to belong to 5 subspecies of *Peromyscus keeni* (Keen's mouse—Cook and MacDonald 2001; Hogan et al. 1993); however, no taxonomic study has included a broad geographic sampling from southeast Alaska since Hogan et al. (1993) first recognized *P. keeni* as occurring near the town of Skagway and on Baranof Island.

To elucidate a general framework of geographic structure of northwestern deer mice across this complex landscape, the mitochondrial cytochrome-*b* (*Cytb*) gene was analyzed for specimens from across the islands and mainland. Mitochondrial DNA (mtDNA) has been widely used in phylogeographic studies of *Peromyscus* (Riddle et al. 2000; Sullivan et al. 2000; Tiemann-Boege et al. 2000; Zheng et al. 2003) and has been established as a reliable indicator of insular peromyscine phylogeography (Chirhart et al. 2001; Hafner et al. 2001; Hogan et al. 1993; Vucetich et al. 2001).

The objectives of this study were to 1) provide insight into whether genetic structure of extant island populations was influenced more heavily by post-glacial colonization or vicariant events, 2) test if island area or isolation influence levels of genetic diversity, 3) explore whether genetic structure is consistent with taxonomy, and 4) test whether areas of southeast Alaska might host genetically distinctive populations.

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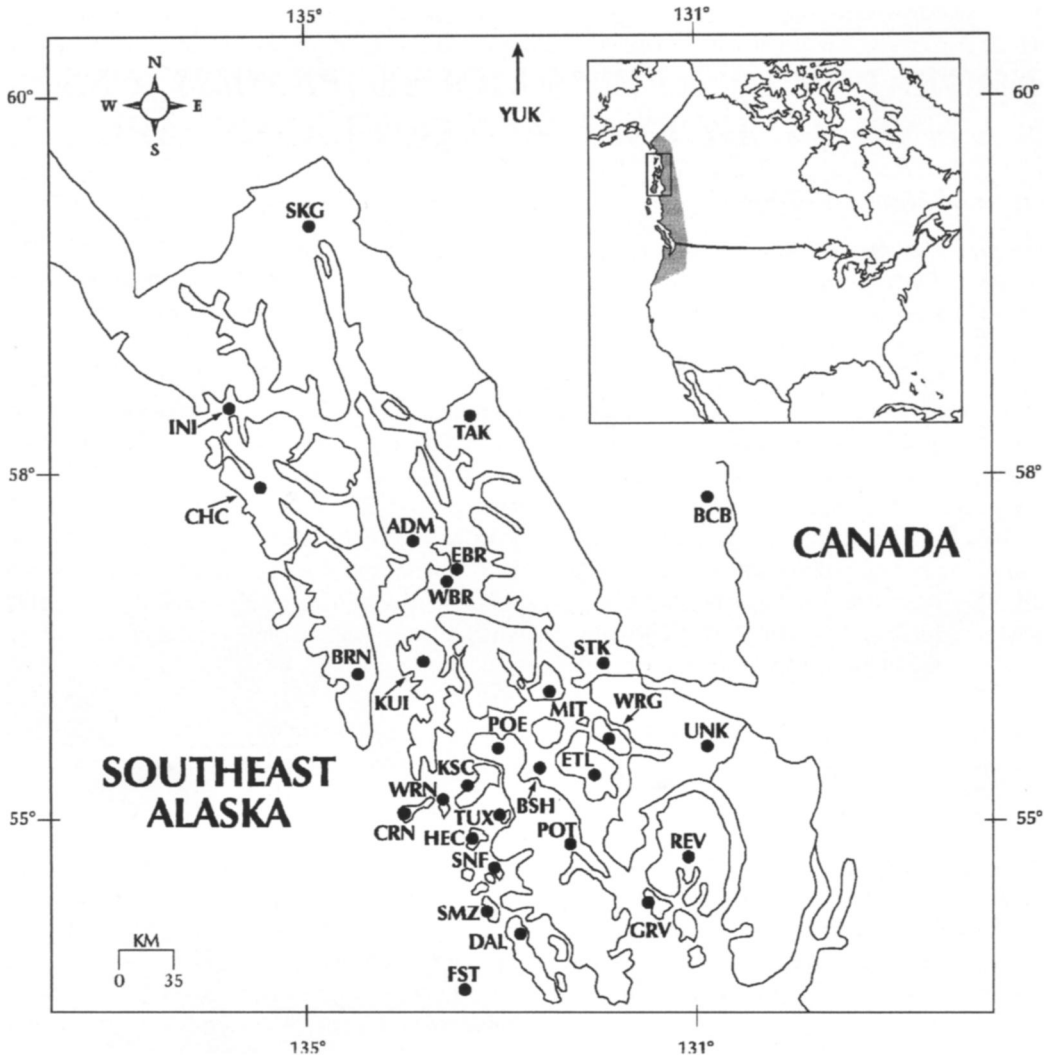

Fig. 1.—Approximate collection locations of *Peromyscus keeni* (abbreviations in Appendix I). Shaded area denotes approximate geographic distribution of *Peromyscus keeni*, as modified from Zheng et al. (2003).

Materials and Methods

Study area.—Southeast Alaska is a naturally fragmented landscape (Fig. 1) consisting of over 2,000 named islands (<1 to >5,000 km^2). The region was most recently at glacial maximum about 15×10^3 years ago (Pielou 1991). Much of the terrain is still glaciated or is characterized by heavily forested mountains that rise abruptly from a complex system of deep fiords (Gehrels and Berg 1994). This coastal biome is isolated by the Saint Elias Mountains to the north and the Coast Mountains to the east (MacDonald and Cook 1996). Southeast Alaska, when combined with coastal British Columbia, embodies the most extensive mature temperate rainforest in the world (Alaback 1991).

Sampling.—Frozen tissues were obtained through fieldwork conducted between 1990 and 1999 with voucher specimens deposited at the University of Alaska Museum. Specimens (n = 256) were obtained from 29 populations throughout southeast Alaska and western Canada (Fig. 1; Appendix I). A reference sequence from Bushy Island was obtained from GenBank (AF119261—Conroy and Cook 1999) and increased the sample size to 257.

The sampling scheme was designed to include 5 subspecies of *P. keeni* and represent 5 previously identified biogeographic subregions (MacDonald and Cook 1996). Samples were obtained (Table 1) from islands of varying size (5–5,778 km^2) and isolation from the mainland (1–33.75 km). A population was defined as a group of samples taken from the same island or mainland locality within a 5-km radius. Skagway and Yukon Territory were sampled more widely (within a 25-km radius) due to limited availability of specimens. Mainland population samples were spaced at least 100 km apart. Nine to 11 individuals were sequenced from each population, although fewer samples were available from 6 populations (Appendix I).

DNA sequences.—DNA was extracted from frozen tissue using the NaCl extraction protocol outlined in Fleming and Cook (2002), except the 2nd desalting wash was omitted. The *Cytb* gene was sequenced using the primers MVZ05 (Smith and Patton 1993) and Micro06 (Cook et al. 2003). These primers produced reliable sequences of 560 base pairs (position 10–570) for both DNA strands of all samples (GenBank accession numbers AY529204–AY529459). Sequencing followed Fleming and Cook (2002), except products were analyzed on an Applied Biosystems 377 automated sequencer. Sequences were compared to a reference sequence and aligned by eye using Sequence Navigator (Applied Biosystems, Inc., Foster, California).

Analyses.—Several measures of genetic diversity and subdivision were used in conjunction with a neighbor-joining tree and a nested

TABLE 1.—Number of haplotypes, nucleotide diversity (π), and haplotype diversity (h) of each population with $n \geq 5$ (excluding SKG and YUK). Isolation from the mainland and island area are indicated for each population. Islands are grouped as large (>1,000 km^2), medium (100–1,000 km^2), and small (<100 km^2). Arlequin 2.000 software was used to calculate *SE* for each population and SAS was used to calculate mean *SE*. Population abbreviations are defined in Appendix I.

Population	n	Area (km^2)	Isolation (km from mainland)	Number of haplotypes	π (%)	π *SE*	h	h *SE*
Mainland								
UNK	10			2	0.02	0.01	0.47	0.13
STK	10			3	0.01	0.01	0.38	0.18
TRN	10			3	0.04	0.03	0.51	0.16
BCB	10			6	0.07	0.04	0.84	0.10
Mean	10			3.50 (*SE* 0.87)	0.04	(0.01)	0.55	(0.10)
Large Islands								
POT	10	5778	6.25	2	0.01	0.01	0.36	0.16
POE	10	5778	6.25	3	0.02	0.01	0.51	0.16
CGF	9	5449	5.00	4	0.03	0.02	0.78	0.11
ADM	9	4310	5.00	2	0.02	0.02	0.56	0.09
BRN	9	4163	6.00	4	0.03	0.02	0.59	0.18
REV	9	3024	2.00	4	0.05	0.03	0.56	0.09
KUI	10	1933	3.00	3	0.01	0.01	0.6	0.13
Mean	9.43	4348	4.79	3.14 (*SE* 0.34)	0.02	(0.01)	0.57	(0.05)
Medium Islands								
ETL	10	889	2.00	1	0.00	0.00	0.00	0.00
DAL	10	658	7.25	2	0.02	0.02	0.36	0.16
WRG	10	569	1.00	3	0.01	0.01	0.64	0.10
MIT	10	547	1.00	2	0.01	0.01	0.2	0.15
KSC	10	482	7.25	2	0.01	0.01	0.36	0.16
GRV	10	233	3.00	2	0.00	0.01	0.20	0.15
HEC	10	189	8.75	4	0.01	0.01	0.64	0.15
SMZ	10	140	6.75	1	0.00	0.00	0.00	0.00
Mean	10	463	4.63	2.13 (*SE* 0.35)	0.01	(0.00)	0.30	(0.09)
Small Islands								
TUX	10	85	6.50	2	0.01	0.01	0.47	0.13
SNF	10	71	9.75	3	0.03	0.02	0.60	0.13
WRN	10	51	10.50	1	0.00	0.00	0.00	0.00
FST	10	10	33.75	2	0.01	0.01	0.56	0.08
WBR	5	5	11.00	1	0.00	0.00	0.00	0.00
Mean	9	60	14.30	1.8 (*SE* 0.37)	0.01	(0.01)	0.33	(0.13)

clade analysis. The software package Arlequin 2.000 (Schneider et al. 2000) was applied to calculate measures of genetic diversity and subdivision including analysis of molecular variance (AMOVA), nucleotide diversity (π), haplotype diversity (h), genetic divergence (F_{st}), and number of female migrants per generation (N_m). With the exception of AMOVA, populations with sample sizes <5 (Coronation, Bushy, East Brother, and Inian) were excluded from the calculations of genetic diversity and subdivision. Number of haplotypes for each population also was used as an indicator of genetic diversity.

Measures of island isolation and area were obtained from Conroy et al. (1999) or determined from United States Geological Survey 1:250,000 quadrangle maps. Island isolation is the shortest island to mainland distance, or sum of island-to-island distances, excluding the distance across islands. Island isolation was classified by order of magnitude as 1 km, 2–10 km, and >10 km from the mainland. Island size also was classified by order of magnitude as small (<100 km^2), medium (100–1,000 km^2), or large (>1,000 km^2; Table 1).

Genetic diversity measures (π, h, and number of haplotypes) were used to compare mainland and island populations by analysis of variance (ANOVA). The assumption of homogeneity of variance could not be met even after a transformation of the data. Therefore, an ANOVA using ranked values of the data was used to determine if diversity values differed among groups. These analyses were computed with SAS version 8.2 software (SAS Institute Inc. 1999). Similar results were obtained when these analyses were analyzed with and without the Skagway and Yukon Territory populations. However, these 2 populations were ultimately omitted from the ANOVA and genetic diversity calculations because samples from each were from a much larger geographic area than other populations.

The program PAUP* (Swofford 2000) was used to generate an intraspecific neighbor-joining tree (bootstrapped 1,000 iterations) under the Jukes-Cantor model of evolution (Jukes and Cantor 1969). The Jukes-Cantor model was chosen because sequence divergence was low (Nei and Kumar 2000).

The software program TCS (Clement et al. 2001) was used in the 1st step of the nested clade analysis to generate an unrooted haplotype genealogy following the algorithm of Templeton et al. (1992). Cladogram ambiguities were resolved using frequency, topological, and geographic criteria (Pfenninger and Posada 2002). Nesting design was constructed by hand (Templeton et al. 1995) and GeoDis 2.0 software (Posada et al. 2000) was used to calculate 4 distance measures (D_c, D_n, IT_c, and IT_d). Statistical significance of these measures was calculated at the 0.05 level by comparison with a null distribution derived from 10,000 random permutations of the pro-

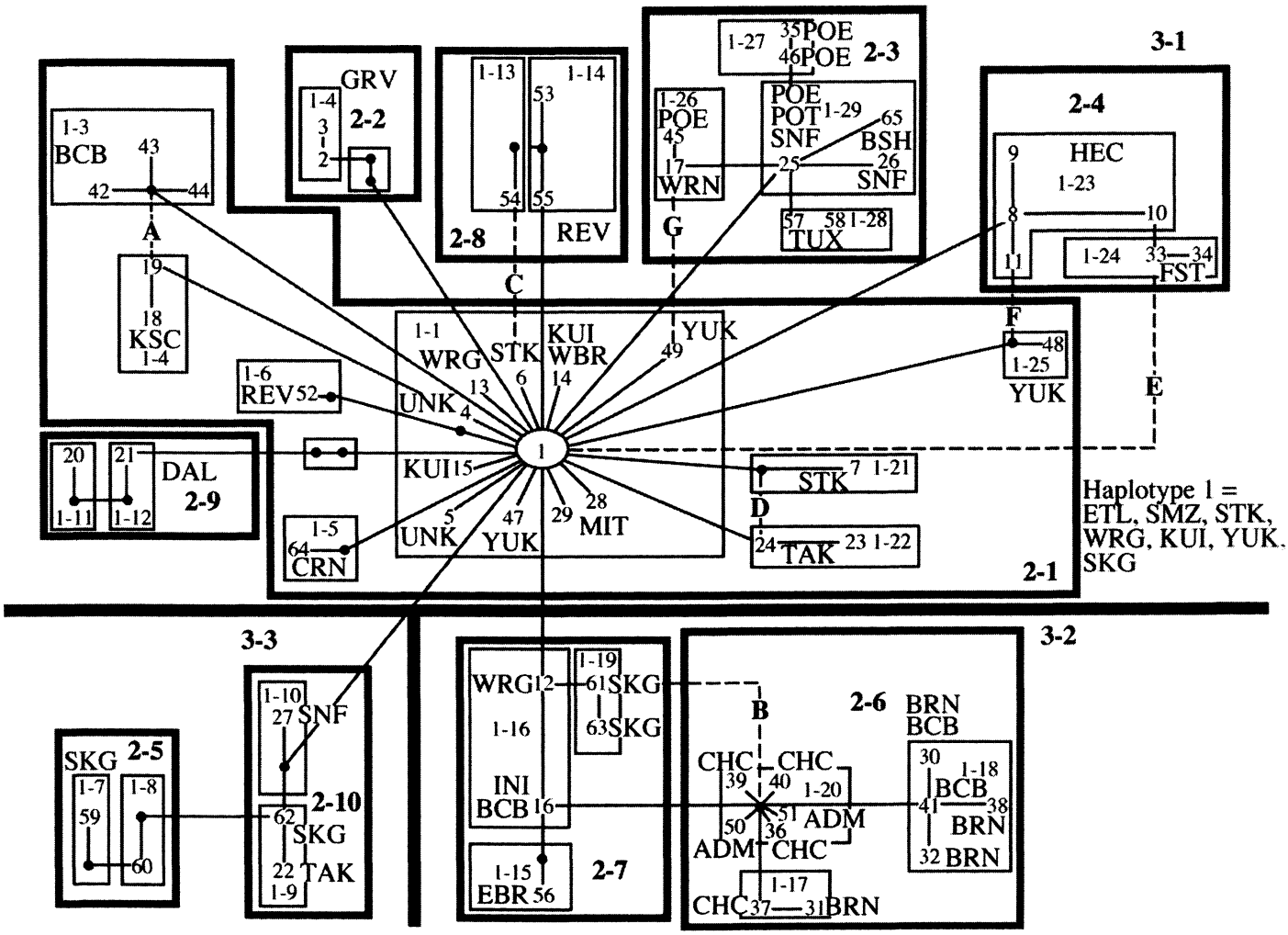

FIG. 2.—Statistical parsimony cladogram. Potential connections that were broken are indicated by letters A–G and black dots indicate missing intermediates. Clade numbers correspond to nested clades in Table 2, haplotype number is indicated at nodes, population abbreviations are in Appendix I.

cedure. Significant distance values were used in congruence with the nested clade analysis key (http://biog.byu.edu/zoology/crandal_lab/geodis.htm—updated from Templeton et al. 1995) to make inferences of population history.

RESULTS

Cytb sequences exhibited expected codon biases and base composition reported for other mammals (Irwin et al. 1991). Across these samples (n = 257), nucleotide composition was 33% adenine, 29% thymine, 24% cytosine, and 14% guanine. Nucleotide substitutions were observed at 67 (12%) of 560 sites with 12 (18%) substitutions at 1st positions, 4 (6%) at 2nd positions, and 51 (76%) at 3rd positions. There were 63 (94%) transitions and 4 (6%) transversions. Of the 11 amino acid changes, 10 were at sites previously described as highly variable in mammals (Irwin et al. 1991). The other change was fixed in the haplotypes from Forrester (haplotypes 33 and 34) and shared with 1 haplotype from Heceta (haplotype 10).

Sixty-five haplotypes were identified (Figs. 2, 3), of which 60 were unique for a particular population and 5 were shared among >1 population. Haplotype 1 occurred on Etolin (n = 10), Wrangell (n = 5), Suemez (n = 10), Kuiu (n = 6), Skagway-Haines (n = 5), Stikine River (n = 8), and Yukon Territory (n = 3). This haplotype, the most interior in the nested clade analysis (Fig. 2) and most frequent (n = 47), was assumed to be the ancestral haplotype (Fig. 2—Templeton et al. 1995). Other shared haplotypes included haplotype 14 (Kuiu [n = 3] and West Brother [n = 5]), haplotype 16 (Inian [n = 2] and British Columbia [n = 1]), haplotype 25 (San Fernando [n = 6], Prince of Wales, Thorne Bay [n = 8], and Prince of Wales, El Capitan [n = 7]), and haplotype 30 (Baranof [n = 6] and British Columbia [n = 4]). Fifty-five percent (n = 16) of all populations >1 individual (n = 29) consisted entirely of unique haplotypes, including 61% (n = 14) of islands and 33% (n = 2) of mainland populations (Figs. 2 and 3).

The AMOVA revealed approximately 72% of genetic variation was partitioned among populations and 28% was partitioned within populations. Ninety-nine percent of significant pair-wise F_{st} values were >0.25. Most significant pair-wise F_{st} values <0.25 share the ancestral haplotype 1 (Yukon

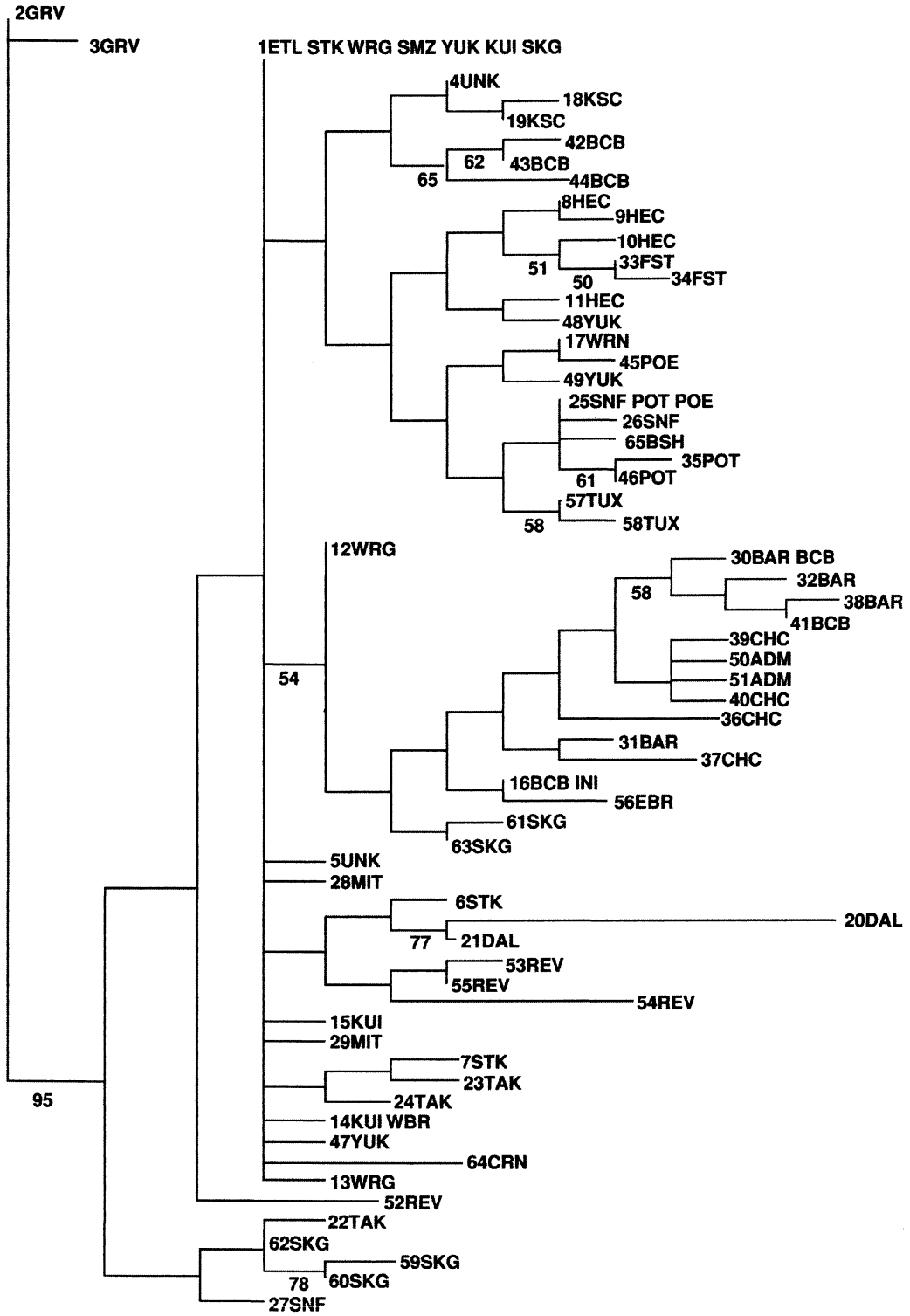

FIG. 3.—Neighbor-joining tree (stretched horizontally to make internal nodes more visible) based on Jukes-Cantor distances calculated for the 65 *Peromyscus keeni* haplotypes (labeled by haplotype and population [abbreviations in Appendix I]) examined in this study. Numbers under branches indicate bootstrap values based on 1,000 iterations.

Territory and Etolin, Yukon Territory and Wrangell, Yukon Territory and Kuiu, Yukon Territory and Suemez, Kuiu and Wrangell, Skagway-Haines and Etolin, and Skagway-Haines and Kuiu). Exceptions are Skagway-Haines and Taku River and Skagway-Haines and San Fernando. N_m values further demonstrate significant genetic subdivision of populations with 97% of all pair-wise comparisons indicating ≤ 1 female migrant per generation (Table 2).

The genetic diversity measures π (Fig. 4A; ANOVA, $F = 4.74$, $d.f. = 3, 23$, $P = 0.01$) and number of haplotypes (Fig.

TABLE 2.—Permutational chi-square statistics and probabilities for the nesting clades of *Peromyscus keeni* as shown in Fig. 3. $P < 0.05$ indicate significant geographic structure.

Nested clades	Chi-square	*P*	Chain of inference	Inference
1–1	272.16	0.00	1-2-3-4-NO	Restricted gene flow with isolation by distance
1–9	8.00	0.13	1-NO	Null hypothesis not rejected
1–16	7.00	0.03	1-2-11-17-4-9-NO	Null hypothesis not rejected
1–17	3.00	0.33	1-NO	Null hypothesis not rejected
1–18		0.81	1-NO	Null hypothesis not rejected
1–20	16.00	0.00	1-2-11-17-NO	Null hypothesis not rejected
1–26	12.00	0.02	1-2-3-5-15-NO	Past fragmentation
1–27	3.00	0.33	1-NO	Null hypothesis not rejected
1–29	25.35	0.02	1-2-11-17-NO	Inconclusive
2–1	445.73	0.00	1-2-3-4-NO	Restricted gene flow with isolation by distance
2–3	89.88	0.00	1-2-3-5-15-NO	Past fragmentation
2–4	20.00	0.00	1-2-3-5-15-NO	Past fragmentation
2–6	32.63	0.00	1-2-3-5-15-NO	Past fragmentation
2–7	22.00	0.00	1-2-11-17-NO	Inconclusive
2–10	10.00	0.01	1-2-11-17-4-9-NO	Past fragmentation
3–1	943.03	0.00	1-2-3-5-15-NO	Past fragmentation
3–2	37.96	0.00	1-NO	Null hypothesis not rejected
3–3	9.55	0.01	1-NO	Null hypothesis not rejected
Total cladogram	336.63	0.00	1-2-3-5-6-13-YES	Long distance colonization

4C; ANOVA, $F = 3.10$, $d.f. = 3, 23$, $P = 0.05$) increased from small island to mainland populations; however, h (Fig 4B; ANOVA, $F = 1.60$, $d.f. = 3, 23$, $P = 0.22$) did not. The most distinctive differences in genetic diversity are between small and medium, and large islands and the mainland (Fig. 4A, C). Although similar trends were found using isolation, a significant difference was not detected among the 4 groups for π (Fig. 4D; ANOVA, $F = 2.52$, $d.f. = 3, 23$, $P = 0.09$), h (Fig. 4E; ANOVA, $F = 0.75$, $d.f. = 3, 23$, $P = 0.54$), or number of haplotypes (Fig. 4F; ANOVA, $F = 2.07$, $d.f. = 3, 23$, $P = 0.14$).

To define nesting for the statistical parsimony network, ambiguities in the cladogram were resolved by breaking the loops marked by letters A–G (Fig. 2). Two criteria (frequency and geographical) were used to resolve loop G and all 3 criteria were used to resolve loops A, C, D, and F. More than 1 criterion could not be met to resolve loops B and E, so the neighbor-joining tree (Fig. 3) and N_m values were considered.

The statistical parsimony network revealed haplotypes separated by up to 11 mutational steps (Fig. 2). The null hypothesis of no geographical association of haplotypes was rejected for 10 geographically significant ($P < 0.05$) nested clades (Fig. 2 and Table 2). The chain of inference indicated past fragmentation (3–1, 2–10, 2–6, 2–4, 2–3, and 1–26), restricted gene flow with isolation by distance (2–1 and 1–1), and long distance colonization (total cladogram) events. An inconclusive outcome was inferred for clade 1–29.

Seven (29%) island populations (Dall, Kosciusko, Gravina, Heceta, Coronation, Tuxekan, Forrester) and no mainland populations formed monophyletic clades in the nested clade analysis. Except Heceta, all of these island populations clustered in the neighbor-joining tree (Fig. 3). Higher bootstrap values supported Dall, Gravina, and Tuxekan. Haplotypes from 2 groups of islands (Admiralty, Baranof, and Chichagof; Prince of Wales and nearby islands [Prince of Wales Island Complex]) tended to form largely single clusters.

DISCUSSION

Colonization or vicariance?—Southeast Alaska experienced many cycles of glaciation coupled with oceanic transgressions, isostatic rebound, and ecosystem change (Ager 1983; Barrie and Conway 1999; Mann and Hamilton 1995; Mobley 1988). This region of North America's north Pacific coast was thought to have been overridden with ice during the last maximum of the Cordilleran Ice Sheet (Klein 1965), 15,000 years ago (Pielou 1991). Klein (1965) presumed that extant flora and fauna became established after post-glacial retreat, but did not preclude the possibility of small mammal populations surviving in glacial refugia. More recently, historic ice-free regions have been documented in areas of southeast Alaska including present-day Chichagof, northern Kuiu, central Prince of Wales, and among islands found off the west coast of Prince of Wales. (Hamilton 1994). Conroy et al. (1999) suggested that colonization ability of particular taxa is responsible for the nested structure of southeast Alaska's mammals, but also did not rule out the possibility of some mammals persisting in regional refugia. Subsequent molecular studies have identified a divergent genetic lineage in ermine (*Mustela erminea*) from the Prince of Wales Island Complex that is consistent with the existence of a coastal glacial refugium (e.g., Cook and MacDonald 2001; Fleming and Cook 2002).

Zheng et al. (2003) sampled *P. keeni* ($n = 50$) and *P. maniculatus* ($n = 78$) from 43 localities along the north Pacific coast. They suggested a refugium for *P. keeni* occurring on Vancouver Island and southern continental British Columbia. However, their sampling (10 individuals representing 2 localities) north of Vancouver Island (their only insular

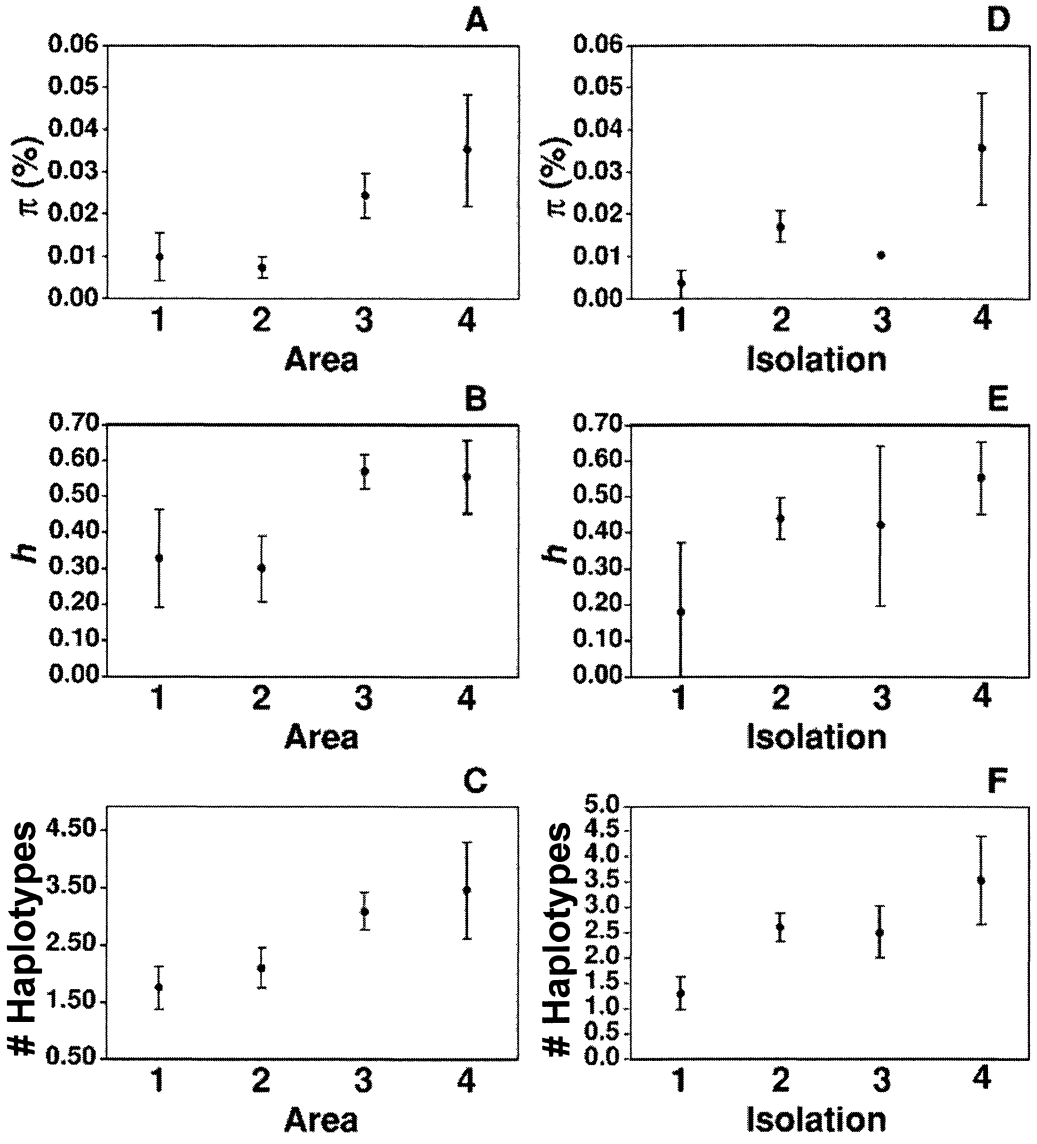

FIG. 4.—Mean and standard error of nucleotide diversity (π), haplotype diversity (h), and number of haplotypes of *P. keeni* populations. In A–C, populations are grouped by size of the landmass they were collected from: small islands (<100 km^2), medium islands (100–1,000 km^2), large islands (>1,000 km^2), and mainland. In D–F, populations are grouped by degree of isolation from the mainland at >10 km, 2–10 km, 1 km, and 0 km (i.e., on mainland).

population) was limited and does not preclude the possibility of a more northern refugium, perhaps in southeast Alaska.

Five factors indicate that vicariance has been influential in shaping current population structure of *P. keeni*: the fossil record indicates an extended history of the species in the region; predominance of past fragmentation events indicated in the nested clade analysis; low π and high h values for mainland populations; limited over-water dispersal capabilities; and the ubiquitous distribution of *P. keeni* in southeast Alaska. Whether *P. keeni* occupied southeast Alaska throughout the last glacial maximum or colonized the region early in the Holocene from a separate refugium, this phylogeographic perspective suggests that these mice might have been widespread along the coast before rising seawater created the Alexander Archipelago.

Fossils from On Your Knees Cave on Prince of Wales suggest a long history of *P. keeni* in the region. These fossils have not been radiocarbon dated; however, they are present throughout sediment that predates the Holocene (T. Heaton, pers. comm.).

In addition to the predominance of past fragmentation indicated by the nested clade analysis, clades 2–1 and 1–1 were likely affected by past fragmentation events. An inference of restricted gene flow with isolation by distance is expected with confounding historical events such as past fragmentation and range expansion (Neigel and Avise 1993). Clades 2–1 and 1–1 contain individuals from both island and mainland populations. As sea levels fluctuated, island populations of these mice might have been fragmented whereas mainland populations expanded inland with retreating ice sheets. Demographic expansion on the mainland is consistent with Zheng

et al. (2003). Using coalescent methods, they suggested these mice experienced demographic expansion about 12×10^4 years ago; however, Zheng et al. (2003) acknowledged they could not differentiate between expansion during last glacial retreat (12×10^3 years ago) and earlier expansions.

Inland expansion of mainland populations also is supported by the diversity measures h and π (Table 1). The low mean π (0.04) and a high mean h (0.55) of mainland populations are indicative of rapid population growth from an ancestral population (Grant and Bowen 1998). The variety of possible interpretations of low π ($\bar{X} = 0.01$) and low h ($\bar{X} = 0.31$) for island populations might be the result of confounding geologic and ecologic events that ultimately resulted in a fragmented landscape (Avise 2000; Grant and Bowen 1998).

Chirhart et al. (2001) suggested, based on ND3 and ND4 mtDNA sequences, that *P. keeni* might be able to disperse long distances between oceanic islands (46 km) using intermittent islands as stepping stones. However, mtDNA and behavioral studies of other peromyscine species indicate poor ability to disperse long distances over water (Loxterman et al. 1998; Sheppe 1965; but see Vucetich et al. 2001). Further, low N_m and high F_{st} values suggest limited over-water dispersal of *P. keeni* in the Alexander Archipelago.

Two of several possible scenarios that could have led to the ubiquitous presence of *P. keeni* in the Alexander Archipelago are post-glacial over-water dispersal from the mainland to many of the Alexander Archipelago's 2,000 islands or vicariance of populations that had spread to the region prior to the fragmentation of the archipelago by rising sea levels.

Given the ubiquitous presence of *P. keeni* in southeast Alaska, these data are more consistent with populations of *P. keeni* existing in refugia somewhere along the north Pacific coast and inhabiting southeast Alaska before it was fragmented by rising seawater.

Genetic diversity, area, and isolation.—A reasonable expectation is that, across islands of varying size, area should be positively related to genetic diversity (MacArthur and Wilson 1967). With regard to colonization, however, we suspect that greater isolation (and hence less gene flow) should result in less genetic variation. For *P. keeni* the degree of island isolation was not related to genetic diversity, a finding consistent with the vicariance model (Whittaker 1998). Thus, the dichotomy between the significance of area and isolation as variables affecting genetic diversity is consistent with a primary role for vicariance in populations of *P. keeni* in the Alexander Archipelago.

Phylogeography and taxonomy.—Taxonomy of mammals, particularly *Peromyscus*, along the north Pacific coast has a long and rather complicated history (Hall 1981; Hogan et al. 1993). Hall (1981) recognized 2 species in southeast Alaska, *P. maniculatus* and *P. sitkensis* (Sitka mouse). The widespread sampling herein builds on earlier studies (Hogan et al. 1993; Zheng et al. 2003) which indicate *P. keeni* (not *P. maniculatus* or *P. sitkensis*) is found throughout southeast Alaska. Further, the presence of *P. keeni* in the British Columbia population indicates the species ranges further inland than previously believed (Hall 1981).

Generally, 5 subspecies of *P. keeni* are currently recognized in southeast Alaska (Cook and MacDonald 2001; Hall 1981). Of these, *Cytb* data are consistent with a distinctive population on Forrester (*P. k. oceanicus*) and a distinctive group of populations (Admiralty, Baranof, and Chichagof) that partially correspond with the range of *P. k. sitkensis*. The molecular data are inconsistent with the described range of *P. k. hylaeus* (northern mainland and Admiralty south to Prince of Wales), *P. k. algidus* (Skagway-Haines north to Yukon Territory), and *P. k. macrorhinus* (mainland restricted). Cryptic variation, not previously identified by morphological comparisons, also was uncovered.

The disjunct range of *P. k. sitkensis* includes Baranof, Duke (south of Gravina), Chichagof, Coronation, and Warren Islands (Cook and MacDonald 2001; Hall 1981; Hogan et al. 1993). The nested clade analysis (clade 2–6) and neighbor-joining tree (Figs. 2 and 3) suggest that *P. keeni* from Admiralty, Baranof, and Chichagof are closely related. However, *P. keeni* from Warren and Coronation form independent monophyletic clades in the nested clade analysis and these populations are not consistent with the described range of *P. k. sitkensis*. Further analyses are needed to address the relationship of British Columbia populations and *P. k. sitkensis* (Figs. 2, 3).

Sampling from the described range of *P. k. algidus* (MacDonald and Cook 1996; Hall 1981; Hogan et al. 1993) did not produce a monophyletic clade or fixed mutations that would corroborate the findings of Hogan et al. (1993) that identified mice from the Skagway area as *P. k. algidus*. The Skagway-Haines population was the most genetically widespread population in the cladogram with up to 11 mutational steps separating individuals. Previous studies of mtDNA variation that explored relationships between peromyscine subspecies frequently found reciprocal monophyly characterizes these forms (Chirhart et al. 2001; Pergams et al. 2000; Sullivan et al. 1997). If these criteria are applied to *P. keeni* in southeast Alaska, then island populations from Coronation, Dall, Kosciusko, Gravina, Tuxekan, Bushy, and Heceta might be taxonomically distinct. The Prince of Wales Island Complex that clustered together in the cladograms also could warrant further investigation as a distinct taxon (Figs. 2 and 3). It is clear the existing taxonomic framework for mammals of this complex region should be used with caution.

Endemism and geology.—Centers of endemism along the north Pacific coast suggest common geologic events shaped population structure of various terrestrial mammal species including *P. keeni*. The contiguous presence of the ancestral haplotype from the mainland Stikine River site to the middle islands of the Alexander Archipelago could indicate that Wrangell, Etolin, Mitkof, and Kuiu were part of an historic peninsula that was fragmented later than the rest of the archipelago. This ancestral haplotype was not detected on Mitkof; however, both Mitkof haplotypes were only 1 mutational step from the ancestral haplotype. If *P. keeni* did persist in a southeast Alaska refugium, the presence of the ancestral haplotype in an area hypothesized to be ice free during last glacial maximum (Kuiu—Hamilton 1994) could be interpreted as evidence of the potential refugium's location.

In contrast to the widespread ancestral haplotype, haplotypes from Gravina are divergent (Figs. 2 and 3). Gravina also harbors a morphologically unique population of *Taricha granulosus* (rough-skinned newt—Myers 1942; Petranka 1998).

The northern island haplotypes largely form a single cluster suggesting that Admiralty, Baranof, Chichagof, and surrounding smaller islands were linked after they were separated from remaining landmasses. Swarth (1911, 1936) defined Admiralty, Baranof, and Chichagof islands as a single biogeographic subregion. Based on unique combinations of taxa and endemics, MacDonald and Cook (1996) suggested that Baranof and Chichagof should comprise a subregion separate from Admiralty. However, the mitochondrial perspective for *P. keeni* is more consistent with Swarth's groupings (1911, 1936). Populations on islands of the Prince of Wales Island Complex cluster together (Figs. 2 and 3), a pattern repeated in other southeast Alaska mammals (Conroy and Cook 2000; Demboski et al. 1998; Fleming and Cook 2002).

Six of the seven 1-step monophyletic clades (Coronation, Dall, Forrester, Kosciusko, Tuxekan, and Heceta) in the statistical parsimony cladogram (Fig. 2) are outer island populations. The high level of endemism displayed in the outer islands west of Prince of Wales might be indicative of earlier isolation and is consistent with the geologic record, which suggests the outer Alexander Archipelago was deglaciated prior to most of the inner archipelago (Mann 1986).

Conclusions.—Most mammals are thought to have arrived in southeast Alaska after the last glacial maximum as post-glacial colonizers (e.g., Conroy et al. 1999; Klein 1965), but *P. keeni* might be an exception to this hypothesis. More extensive sampling throughout the range of this species is needed to rigorously assess this proposition. Limited over-water dispersal ability, in combination with vicariant geologic events early in the Holocene, appears to have shaped many genetically distinctive populations of *P. keeni* in the Alexander Archipelago. Island area was significantly correlated with 2 (π and number of haplotypes) of 3 genetic diversity measures, whereas island isolation was not significantly correlated with genetic diversity. *Cytb* data were inconsistent with 3 of 5 currently recognized subspecies. Cryptic variation in 8 other areas was identified, 6 of which were isolated islands west of Prince of Wales. This phylogeographic perspective provides another piece in the puzzle of how the dynamic glacial history of the north pacific coast shaped terrestrial mammal populations.

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APPENDIX I

Specimens examined.—The 257 specimens examined are listed below by collection locality followed by the abbreviation used in Figs. 1, 2, 3, and Table 1, sample size, and a reference number for samples which are archived in the Alaska Frozen Tissue Collection (AF).

Admiralty Island (ADM).—n = 9, AF19754–19758, AF19760–19763.

Baranof Island (BRN).—n = 9, AF7615, AF7617–7622, AF7624–7625.

British Columbia, Canada (BCB).—n = 10, AF12738–12747.

Bushy Island (BSH).—n = 1, AF17750.

Chichagof Island (CHC).—n = 9, AF6877–6879, AF6881–6882, AF6890, AF6893, AF6899, AF6901.

Coronation Island (CRN).—n = 4, AF17160, AF17164–17165, AF15200.

Dall Island (DAL).—n = 10, AF34202–34203, AF34205, AF34309, AF34367–34372.

East Brother Island.—n = 2, AF12606, AF12608.

Etolin Island (ETL).—n = 10, AF15785–15787, AF15792, AF15805, AF15811–15815.

Forrester Island (FST).—n = 10, AF16756–16765.

Gravina Island (GRV).—n = 10, AF26160–26162, AF26168–26171, AF26173, AF26175–26176.

Heceta Island (HEC).—n = 10, AF4794–4795, AF4797–4803, AF4809.

Inian Island (INI).—n = 2, AF7952, AF8709.

Kosciusko Island (KSC).—n = 10, AF26410–26415, AF26417–26420.

Kuiu Island (KUI).—n = 10, AF7019–7022, AF7024–7028, AF7038.

Mitkof Island (MIT).—n = 10, AF2420–2424, AF2468–2472.

Prince of Wales Island, El Capitan (POE).—n = 10, AF35782–35791.

Prince of Wales Island, Thorne Bay (POT).—n = 10, AF30562–30563, AF35522–35529.

Revillagigedo Island (REV).—n = 9, AF34541–34549.

San Fernando Island (SNF).—n = 10, AF16600—03, AF16770–16772, AF16774–16776.

Skagway-Haines (SKG).—n = 11, AF4593–4594, AF8758, AF8767, AF12505–12506, AF12534, AF12570, AF22088, AF39601–39602.

Stikine River (STK).—n = 10, AF2624–2628, AF2630–2634.

Suemez Island (SMZ).—n = 10, AF16866–16872, AF17904–17906.

Taku River (TAK).—n = 10, AF10099–10108.

Tuxekan Island (TUX).—n = 10, AF10343, AF12464–12465, AF12468, AF12488–12489, AF12491, AF17882, AF17878, AF17961.

Unuk River (UNK).—n = 10, AF4409–4418.

Warren Island (WRN).—n = 10, AF10322, AF10326–10334.

West Brother Island (WBR).—n = 5, AF12609–12613.

Wrangell Island (WRG).—n = 10, AF25644–25649, AF25651–25654.

Yukon Territory, Canada (YUK).—n = 6, AF29706–29707, AF29750, AF29752, AF29789, AF29790.