SYSTEMATIC AND TAXONOMIC IMPLICATIONS OF KARYOTYPIC, ELECTROPHORETIC, AND MITOCHONDRIAL-DNA VARIATION IN *PEROMYSCUS* FROM THE PACIFIC NORTHWEST

KELLY M. HOGAN, MARSHAL C. HEDIN, HUNG SUN KOH, SCOTT K. DAVIS, AND IRA F. GREENBAUM

Department of Biology, Texas A&M University, College Station, TX 77843 (KMH, MCH, IFG)
Department of Biology, Chungbuk University, Cheongju 360-763, South Korea (HSK)
Department of Animal Sciences, Texas A&M University, College Station, TX 77843 (SKD)
Present address of MCH: Department of Biology, Washington University, Box 1137, One Brokings Drive, St. Louis, MO 63130

Chromosomes, allozymes, and mitochondrial DNA (mtDNA) were analyzed to examine the taxonomic and systematic relationships of coastal *Peromyscus* from northern Washington to southern Alaska. All three datasets indicate that *Peromyscus* from this region constitute two distinct groups. One group comprises *P. oreas, P. sitkensis,* and several currently recognized subspecies of *P. maniculatus* including *P. m. algidus, P. m. hylaeus, P. m. keeni, P. m. macrorhinus,* and *P. m. prevostensis.* The second group comprises only populations of *P. m. austerus.* The former group is differentiated from the latter by number of autosomal arms, allele-frequency differences, and mtDNA haplotypes. This dichotomy in karyotype, and the level of allozymic and mtDNA divergence between the groups suggest that the groups constitute distinct species. We recommend that *P. oreas, P. sitkensis, P. maniculatus algidus, P. m. hylaeus, P. m. keeni, P. m. macrorhinus,* and *P. m. prevostensis* be recognized under the specific epithet of *Peromyscus keeni.*

Key words: *Peromyscus,* mtDNA, allozymes, chromosomes, systematics, Pacific Northwest

The taxonomy of deer mice (*Peromyscus*) inhabiting the Pacific Northwest from Washington to southern Alaska has been complicated by extensive insular endemism resulting in a plethora of recognized taxa. Hall (1981) recognized two species in this region, *P. maniculatus* and *P. sitkensis.* From the Columbia River north to southern Alaska and west of the Cascade and Coastal mountain ranges, *P. maniculatus* was considered to comprise 27 subspecies distributed over the entire mainland and on most of the associated islands (Hall, 1981). Despite considerable evidence supporting the specific status of *P. oreas* (Dice, 1949; Johnson and Ostenson, 1959; Liu, 1954; Sheppe, 1961), Hall (1981) retained the taxon as a subspecies of *P. maniculatus.*

Gunn and Greenbaum (1986) and Gunn (1988) reexamined the systematic status of *oreas* and concluded that it is distinct from sympatric populations of *P. m. austerus.* These taxa can be discretely separated based on the number of autosomal arms (fundamental number, FN) in the karyotype. Populations of *P. oreas* are characterized by a high FN (85–92), whereas *P. m. austerus* has a low FN (74–78). The absence of intermediate FNs in areas of sympatry was interpreted to indicate reproductive isolation. The specific status of *P. oreas* has been further supported by morphologic (Allard and

Greenbaum, 1988; Allard et al. 1987; Gunn and Greenbaum, 1986; Sullivan et al., 1990), and electrophoretic (Calhoun and Greenbaum, 1991) evidence.

The taxonomy and geographic bounds of *P. sitkensis* have been particularly problematic. Osgood (1909) described the species as occurring on Baranoff and Chicagof islands in the Alexander Archipelago of Alaska and on Prevost (now Kunghit) Island in the Queen Charlotte Islands of British Columbia. Subsequent investigators either have suggested broader distributions of *P. sitkensis* (Cowan, 1935; Swarth, 1911; Thomas, 1973) or questioned its specific validity (Foster, 1965; McCabe and Cowan, 1945). Although *P. sitkensis* has been suggested to extend south to include the islands in the Queen Charlotte Strait off Vancouver Island, Hall (1981) restricted the species to a disjunct subset of the outer islands in the Alexander Archipelago. Nagorsen (1990), in considering the range of *P. sitkensis* in British Columbia, apparently followed Osgood (1909) in recognizing *P. s. prevostensis* as restricted to the outer Queen Charlotte Islands.

The relationship of *P. sitkensis* to *P. maniculatus* and *P. oreas* remains incompletely resolved. From morphometric analysis of chromosomally identified specimens, Allard and Greenbaum (1988) demonstrated that populations previously thought to represent *P. sitkensis* from islands off northern Vancouver Island (Thomas, 1973) are associated with *P. oreas* from mainland Washington and Vancouver Island. Chromosomal (Gunn, 1988) and morphometric (Allard and Greenbaum, 1988) comparisons of *P. oreas* to specimens of *P. sitkensis* from Baranoff Island supported the hypothesis that these forms are conspecific, but distinct from *P. maniculatus*. Comparable data for the 13 insular and coastal mainland subspecies (currently assigned to *P. maniculatus*) that inhabit the range between Vancouver Island and southern Alaska, however, are essential to discern whether *P. sitkensis* and *P. oreas* are species or geographic variants of one species (Nagorsen, 1990).

In this paper, we present the results of karyotypic and electrophoretic analyses of deer mice from the Queen Charlotte Islands and British Columbia mainland. Additionally, for a subset of these mice and representatives of *P. oreas* and *P. m. austerus* (from Vancouver Island) and *P. sitkensis* (from Baranoff Island), we report data from an analysis of restriction-fragment-length polymorphisms (RFLPs) of mitochondrial DNA (mtDNA). These data are used to elucidate the taxonomic and systematic affinities of *Peromyscus* from Washington north to southern Alaska and west of the Cascade and Coastal mountain ranges.

Materials and Methods

For this study, 284 deer mice were live-trapped from 14 insular and mainland localities in British Columbia and southeastern Alaska. To facilitate integration of the data from this study to those of previous reports, our samples are designated as localities 31–44 (Fig. 1 and Appendix I) with localities 1–30 (Fig. 1) corresponding to the same designations in Calhoun and Greenbaum (1991). Using the geographic criteria of Hall (1981), the specimens from localities 31–44 correspond to: *P. m. algidus* (locality 44, $n = 10$), *P. m. hylaeus* (locality 43, $n = 14$), *P. m. keeni* (localities 35–39, $n = 145$), *P. m. macrorhinus* (localities 31–34, $n = 53$), *P. m. prevostensis* (locality 40, $n = 23$), and *P. sitkensis* (localities 41 and 42, $n = 39$).

Chromosomal analysis.—Somatic metaphase cells were obtained from bone marrow using the technique of Baker et al. (1982). Non-differentially stained (standard), C-banded (Sumner, 1972), and G-banded (Seabright, 1971) karyotypes were produced for each population examined. Chromosomes were identified according to the standardized karyotype for *Peromyscus* (Committee for Standardization of Chromosomes of *Peromyscus*, 1977). C-banded chromosomes were analyzed for variation in centromere position and distribution of interstitial and short-arm heterochromatin. A minimum of four metaphase karyotypes was examined to determine the diploid (2n) and fundamental number for each specimen. A chromosome was desig-

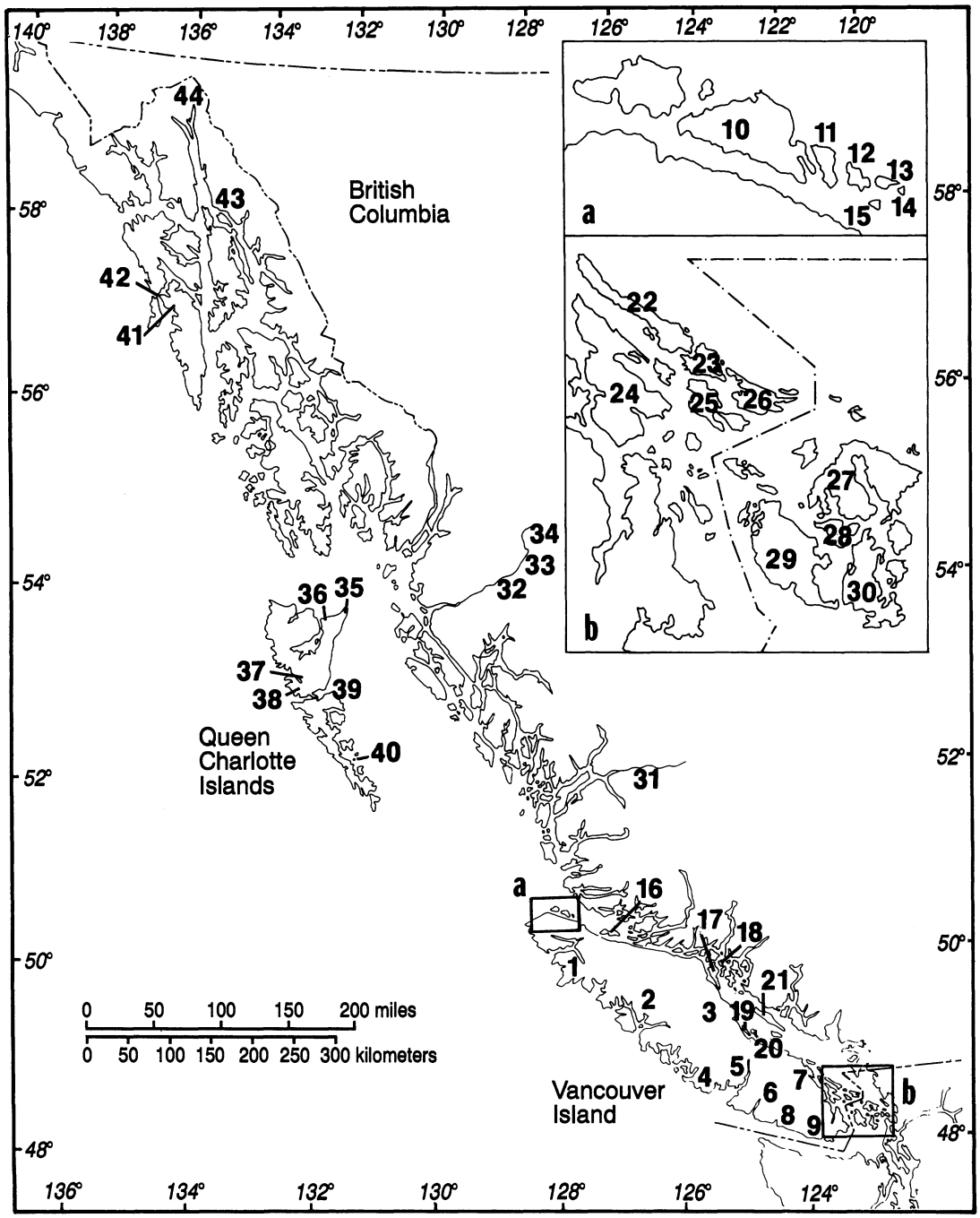

FIG. 1.—Collecting localities for *Peromyscus* used in this study.

nated biarmed if a short-arm consistently was detected using both standard and C-banded data.

Allozymic analysis.—Starch-gel electrophoresis was used to examine allozymic variation for 12 proteins encoded by 21 presumptive gene loci. Procedures for preparation of tissues (heart, liver, and kidney) and gels for electrophoretic analysis followed Calhoun et al. (1988), Richardson

et al. (1986), and Selander et al. (1971). Stains were prepared according to Selander et al. (1971) and Harris and Hopkinson (1976). Locus designations followed the standardized nomenclature for humans (McAlpine et al., 1987). To establish identity of electromorphs and provide for direct comparisons of the allelic data generated in this study to those previously reported for populations of *P. maniculatus* and *P. oreas* (localities 1–30—Calhoun and Greenbaum, 1991), reference samples of *P. m. austerus* and *P. oreas* analyzed by Calhoun and Greenbaum (1991) were used for side-by-side comparisons on gels. Alleles at each locus were designated numerically in order of mobility relative to the common anodal (100) or cathodal (−100) allele in reference samples of *P. m. austerus* (Calhoun and Greenbaum, 1991).

Buffer systems and the proteins they resolved were: poulik (electrode pH 8.2, gel pH 8.7, 200 V, 6 h)—glucose-6-phosphate dehydrogenase (Enzyme Commission no. 1.1.1.49; G6PD) and superoxide dismutase (1.15.1.1; SOD-1, -2); tris-citrate I (electrode pH 6.7, gel pH 6.3, 200 V, 6 h)—isocitrate dehydrogenase (1.1.1.42; IDH-1, -2), lactate dehydrogenase (1.1.1.27; LDH-1,-2), malate dehydrogenase (1.1.1.37; MDH-1, -2), and phosphogluconate dehydrogenase (1.1.1.43; PGD); tris-citrate II (pH 8.0, 130 V, 6 h)—alcohol dehydrogenase (1.1.1.1; ADH), glutamate dehydrogenase (1.4.1.3; GLUD), glutamic-oxaloacetic transaminase (2.6.1.1; GOT-1, -2), glycerol-3-phosphate dehydrogenase (1.1.1.8; GPD) and sorbitol dehydrogenase (1.1.1.14; SDH); tris-hydrochloric acid (electrode pH 8.2, gel pH 8.5, 250 V, 3 h)—esterases (3.1.1.1; EST-1). Ridgeway et al. (1970; electrode pH 8.5, gel pH 8.0, 250 V, 3 h)—peptidases (3.4.11 or 3.4.13; PEP-A, -B, -C, -D). Using the buffer system of Ridgeway et al. (1970), four zones of peptidase activity were observed using di- and tripeptide substrates L-leucyl-L-alanine (PEP-A), L-leucyl-L-proline (PEP-B), L-leucylglycylglycine (PEP-C), and L-valine-L-proline (PEP-D).

Allozymic data were analyzed using BIOSYS-I (Swofford and Selander, 1989). Loci were considered polymorphic if the frequency of the most common allele was <0.99 in any population. Allelic variation in esterase (ES-1, the most anodal esterase; Selander et al., 1971), which has been shown to consist of an active ($ES\text{-}1^{100}$) and a "null" ($ES\text{-}1^{0}$) allele under assay conditions, was analyzed following Calhoun and Greenbaum (1991). Since ES-1 heterozygotes could not be distinguished, data for this locus were not used in the computation of average heterozygosity and fixation indices.

The parameters used to characterize genetic variability included average heterozygosity observed over all loci in a population (H_{obs}), and proportion of polymorphic loci per population (P). In addition, chi-square analysis was used to test for conformance to Hardy-Weinberg expectations within samples. *F*-statistics (Wright, 1978) were calculated following Nei and Chesser (1983), as modified by Van Den Bussche et al. (1986).

To address relationships among populations, allele frequencies generated in this study (Table 1) and by Calhoun and Greenbaum (1991) were analyzed using BIOSYS-I. Based on the recommendations of Rogers (1991), the distance Wagner procedure (Farris, 1972) using Cavalli-Sforza and Edwards (1967) chord distances was used to generate a phenogram rooted at the midpoint of the greatest branch length without branch-length optimization. The sequence of samples added to the developing Wagner network employed the multiple-addition-criterion algorithm (Swofford, 1981). To provide a basis of comparison to previous studies, a cluster analysis using the unweighted pair-group method with arithmetic average (UPGMA—Sneath and Sokal, 1973) of coefficients of genetic similarity (Rogers, 1972) also was computed.

Mitochondrial-DNA analysis.—The mtDNA analysis was performed for a subset of the animals for which chromosomal and allozymic data were available. Sample sizes for the mtDNA analysis were: *P. m. algidus* (locality 44, $n = 5$); *P. m. austerus* (localities 5 and 16, $n = 11$); *P. m. keeni* (localities 35, 36, and 39, $n = 10$); *P. m. macrorhinus* (localities 32–34, $n = 19$); *P. m. prevostensis* (locality 40, $n = 3$); *P. oreas isolatus* (locality 16, $n = 8$); *P. sitkensis* (locality 41, $n = 3$).

Mitochondrial-DNA procedures followed the methods described in Hillis and Davis (1986). All samples were cut with 11 restriction endonucleases with six-base recognition sites. Restriction enzymes used were *Bam*H I, *Bgl* II, *Dra* I, *Eco*R I, *Eco*R V, *Hin*d III, *Kpn* I, *Pst* I, *Pvu* II, *Sac* I, and *Stu* I. The restriction-fragment patterns were analyzed using the unmapped comparative approach in which each fragment produced by a particular enzyme was given a different (but arbitrary) numeric designation. The size of

each mtDNA fragment was estimated using the method of Schaffer and Sederoff (1981). *Peromyscus* with the same numeric designations for all restriction enzymes were considered to have the same haplotype. Haplotypes were identified by arbitrary alphabetic designations. Sequence divergence (p) was estimated by the method of Nei and Li (1979). A dendrogram reflecting the relationship of the haplotypes was produced from the matrix of divergence values using the Fitch-Margoliash option in the computer program PHYLIP (Felsenstein, 1987).

Results

Chromosomal analysis.—Deer mice from localities 31–44 had karyotypes with 2n = 48 and FNs ranging from 86 to 92. These karyotypes were identical to the high FN karyotypes described for *P. oreas* (Gunn, 1988; Gunn and Greenbaum, 1986) and *P. sitkensis* (Pengilly et al., 1983). Chromosomes 1–3, 9–14, and 16–23 were consistently biarmed. Of these, chromosomes 12 and 15–18 had heterochromatic short arms and chromosomes 11, 19, 21, and 23 had short arms including a C-band negative region proximal to the centromere, and distal heterochromatin. The remainder of these autosomes had heterochromatin restricted to the centromeric regions.

Chromosomal polymorphisms that effected FN variation included the presence or absence of heterochromatic short arms on chromosomes 4, 8, and 15, and a terminal pericentric inversion of both chromosomes 6 and 7. The presence or absence of an interstitial C-band in the short arm of chromosome 5 did not affect FN.

Allozymic analysis.—Of the 21 loci examined, five were polymorphic (Table 1). With the exception of ADH^{-120}, which was detected in low frequency at seven localities, and GPD^{110}, present in one individual from locality 33 (Table 1), electromorphs identified in this study were identical to those reported by Calhoun and Greenbaum (1991).

Within the populations sampled, deviations from expectations of Hardy-Weinberg equilibrium were nonsignificant ($P > 0.05$) with the following exceptions: 6PGD at localities 38 and 41, GOT-I at locality 31, and PEP-B at locality 43. Levels of heterozygosity within populations for each locus are given in Table 1. Average heterozygosity over all loci ranged from 0.006 to 0.060. Mean fixation indices for samples 31–44 are listed in Table 2 and were highly variable among loci. The overall fixation index (F_{st}) was 0.371.

Matrices of genetic distance (Cavalli-Sforza and Edwards, 1967) and similarity (Rogers, 1972) were calculated from allele frequencies for samples 31–44, and 1–30 (from Table 1 of Calhoun and Greenbaum, 1991). The distance Wagner phenogram (Fig. 2) generated from Cavalli-Sforza and Edwards (1967) chord distances separated the populations sampled into two clusters. One cluster corresponded to populations of *Peromyscus* characterized by low FNs, the other to populations characterized by high FNs. *Peromyscus* from localities 31 through 44 uniformly grouped with the populations in the high-FN cluster. UPGMA clustering of Rogers' similarity values resulted in the same grouping of low and high-FN samples. Differences between the two procedures occurred only in the topology of taxa within the two major clusters. Rogers' (1972) genetic similarity value between the two clusters was 0.87.

Mitochondrial-DNA analysis.—Eight of the 11 restriction endonucleases were informative and revealed a total of 42 restriction sites, resulting in eight composite mtDNA haplotypes (haplotypes A–H, Table 3). Haplotype B was present in 28 (47.4%) of the individuals and occurred in six of the 12 localities sampled. The matrix of *P*-values between haplotypes generated the phenogram presented in Fig. 3. Mitochondrial data revealed the same two major clusters obtained from karyotypic and electrophoretic data. The cluster of haplotypes A, B, C, D, and H represents populations characterized by high FNs, while the cluster of haplotypes E, F, and G includes only low-FN samples. With the exception of high-FN

TABLE 1.—*Allele frequencies and genetic variability in populations of* Peromyscus *from the Pacific Northwest:* H_{obs} = *average heterozygosity observed (direct count) for all loci in a population;* P = *proportion of polymorphic loci per population (0.99 criterion);* n = *sample size; locality numbers are given in Fig. 1 and Appendix I.*

Locality	n)	Allele frequencies													
		ADH			GOT-1		PEPB			6PGD			GPD			
		−120	−113	−100	100	73	108	100	90	129	112	100	110	100	H_{obs}	*P*
31	(8)		0.875	0.125	0.125	0.875	0.250	0.750				1.000		1.000	0.025	15.00
32	(7)		1.000			1.000		1.000			0.143	0.857		1.000	0.014	5.00
33	(19)	0.026	0.921	0.053	0.263	0.737		1.000			0.026	0.974	0.026	0.974	0.034	20.00
34	(27)	0.037	0.907	0.056	0.192	0.808	0.037	0.963		0.037	0.019	0.944		1.000	0.033	20.00
35	(25)	0.020	0.940	0.040	0.880	0.120		0.980	0.020	0.240		0.760		1.000	0.040	20.00
36	(12)		1.000		0.917	0.083		1.000			0.417	0.583		1.000	0.033	10.00
37	(24)	0.042	0.875	0.083	0.792	0.208		1.000		0.396		0.604		1.000	0.060	15.00
38	(39)	0.013	0.962	0.026	0.667	0.333		1.000		0.333	0.038	0.628		1.000	0.047	15.00
39	(45)		0.989	0.011	0.856	0.144		0.956	0.044	0.422	0.022	0.556		1.000	0.048	20.00
40	(23)		0.957	0.043	0.978	0.022		1.000			0.065	0.935		1.000	0.013	15.00
41	(31)	0.016	0.968	0.016	1.000			1.000		0.016	0.048	0.935		1.000	0.008	10.00
42	(8)		1.000		1.000			1.000		0.062		0.937		1.000	0.006	5.00
43	(14)		1.000		0.429	0.571		0.857	0.143			1.000		1.000	0.029	10.00
44	(10)	0.050	0.900	0.050		1.000		1.000		0.300		0.700		1.000	0.040	10.00

TABLE 2.—*Summary of fixation indices for the polymorphic loci in* Peromyscus.

Locus	F_{IS}	F_{IT}	F_{ST}
AAT-1	−0.001	0.586	0.586
PEP-B	0.444	0.529	0.153
ADH	−0.082	−0.042	0.037
GPH	−0.027	−0.002	0.024
PGD	−0.104	0.103	0.188
Mean	−0.014	0.362	0.371

specimens from locality 44 (which clustered with the rest of the high-FN group at a divergence of ca. 2%), percent sequence divergence within the high-FN and low-FN clusters was <1%. Repeated shuffling of the input data in PHYLIP resulted only in topological changes within the two major groups.

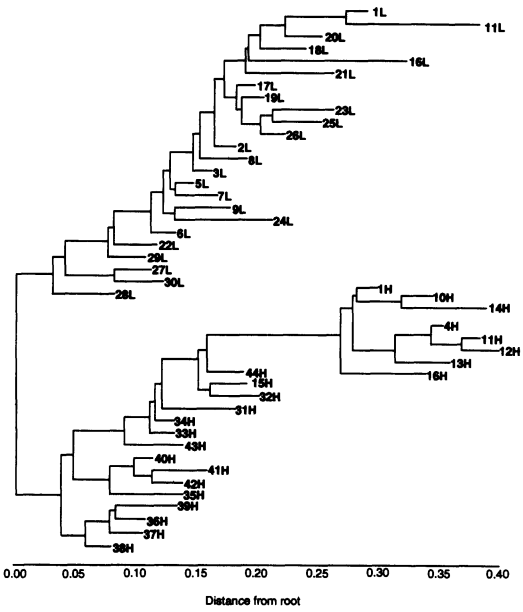

FIG. 2.—Distance-Wagner phenogram of the 44 samples of *Peromyscus* examined. Locality numbers are those shown in Fig. 1 and listed in Appendix I. In the phenogram, the sample localities are denoted either by "H" for high FN (85–92) or "L" for low FN (74–78). In instances where specimens with "high" FN and "low" FN occurred sympatrically, the subsamples were treated separately and are designated with an "L" or "H," respectively (e.g., 16H and 16L).

DISCUSSION

It is clear that the *Peromyscus* in the Pacific Northwest (from west of the Cascade and Coastal mountains and north of the Columbia River to southern Alaska) represent two distinct evolutionary lineages. Chromosomally, these two lineages are separable based on a dichotomy in the range of fundamental numbers. The distribution of heterochromatin and euchromatin observed in samples of *Peromyscus* from localities 31 through 44 are indistinguishable from those reported for *P. oreas* and *P. sitkensis* (Gunn, 1988; Gunn and Greenbaum, 1986; Pengilly et al., 1983). Homologous autosomes fixed in samples from localities 31–44, and those previously documented for *P. oreas* (Gunn, 1988; Gunn and Greenbaum, 1986) and *P. sitkensis* (Pengilly et al., 1983) include chromosomes 1–3, 5, and 9–22. Shared polymorphisms within these populations include heterochromatic short-arm variation at chromosomes 4 and 8, as well as pericentric-inversion heteromorphisms at chromosomes 6 and 7. Further, the high-FN deer mice exhibit a condition of chromosome 19 that distinguishes them from low-FN populations representing *P. m. austerus* (Gunn, 1988; Gunn and Greenbaum, 1986).

The allozymic data indicate a genic dichotomy identical to that of the high-FN versus low-FN divergence. Samples from localities 31–44 were monomorphic for ES-1^{0} (null allele), IDH-1^{100}, and IDH-2^{-100}; these loci are also monomorphic for these alleles in *P. oreas* (Calhoun and Greenbaum, 1991). These loci are polymorphic in low-FN populations referable to *P. m. austerus* (Calhoun and Greenbaum, 1991). While there are no fixed-allozymic differences between the high- and low-FN populations, there is a consistent dichotomy in allele frequencies between these two groups of deer mice.

Fixation indices (F_{ST}; Table 2) for local-

TABLE 3.—*Composite mitochondrial-DNA genotypes observed among samples of* Peromyscus. *Numbers describing mtDNA-fragment patterns are, from left to right,* BamH *I,* Pst *I,* Pvu *II,* EcoR *I,* EcoR *V,* Blg *I,* Dra *I,* Hind *III,* Kpn *II,* Sac *I, and* Stu *II. Letters refer to the composite clone for all 11 enzymes. Number of mice (*n*) sampled that have a particular clone are listed to the right.*

Clone	Haplotype											*n*
A	1	1	1	1	1	2	2	2	1	2	1	8
B	1	1	1	1	1	2	2	2	1	1	1	28
C	1	1	1	1	2	2	2	2	1	1	1	3
D	1	1	1	1	1	2	2	3	1	1	1	4
E	2	1	1	1	1	1	1	4	1	1	2	2
F	2	1	1	1	1	1	1	5	1	1	2	7
G	2	1	1	1	1	1	1	6	1	1	2	2
H	3	1	1	1	1	1	3	2	1	1	1	5

ities 31–44 suggest extensive genetic partitioning (F_{ST} = 0.371) between populations and are similar to the values previously reported for island populations of *P. oreas* (F_{ST} = 0.453; Calhoun and Greenbaum, 1991). The values are, however, considerably greater than mean values reported for *P. m. austerus* (F_{ST} = 0.156; Calhoun and Greenbaum, 1991). These data suggest a consistently greater degree of genetic subdivision within the high-FN group. In addition, mean-percent polymorphism (P = 13.6%) and average observed heterozygosity (H_{obs} = 0.031) for the populations sampled in this study were similar to values reported for populations of *P. oreas* (P = 12.5%, H_{obs} = 0.023) from Vancouver Island (Calhoun and Greenbaum, 1991). These values also are considerably smaller than values reported for sympatric populations of *P. m. austerus* (P = 24.5%, H_{obs} = 0.081; Calhoun and Greenbaum, 1991), suggesting a larger amount of genetic variability in the low-FN group.

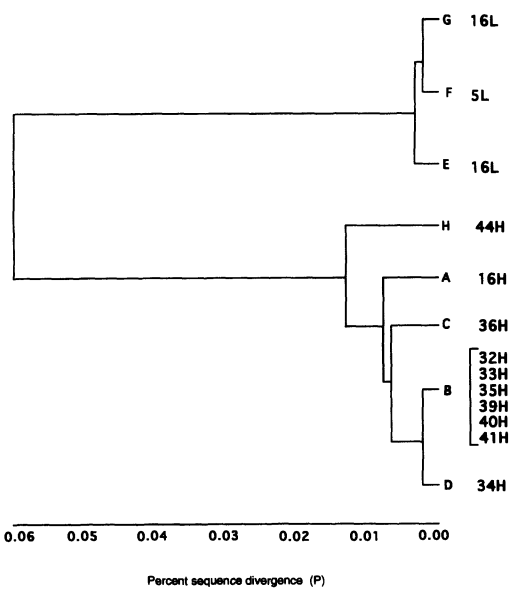

FIG. 3.—Fitch-Margoliash tree based on restriction-fragment-length-polymorphism data from the samples of *Peromyscus* examined. The sample localities are denoted as in Fig. 1.

Phenetic analysis of the allozymic data (Distance-Wagner phenogram, Fig. 2, and UPMGA, not shown) reflects the integrity of the high-FN group. The average Rogers' (1972) genetic similarity within the high-FN (S = 0.974) and low-FN (S = 0.94) groups was similar to values previously reported for *P. oreas* (S = 0.965) and *P. maniculatus* (S = 0.938) from Vancouver Island (Calhoun and Greenbaum, 1991). Between the high- and low-FN groups, the average genetic similarity of 0.87 is the same as that between *P. maniculatus* and *P. oreas* (Calhoun and Greenbaum, 1991), and is comparable to the genetic similarity between *P. maniculatus* and *P. polionotus* (Avise et al., 1979).

The dichotomy between high and low FNs also is reflected in the partitioning of

mtDNA variation. The high-FN group comprises five haplotypes of which the most common was found in currently recognized populations of both *P. maniculatus* (localities 32, 33, 35, 39, and 40) and *P. sitkensis* (locality 41). Percent-sequence divergence within the high-FN and low-FN groups was similar to values reported for within "assemblages" of *P. maniculatus* (Lansman et al., 1983). Between the high-FN and low-FN groups, the sequence divergence was ca. 6%. These data are consistent with divergence values observed between other species of *Peromyscus,* particularly *P. maniculatus* and *P. polionotus* (p = 4.5%; Avise et al., 1983).

Determining specific status can be straightforward when the populations in question are sympatric or continuously distributed. In these cases, reproductive isolation, or its correlates (i.e., maintenance of discrete character-state differences between populations in sympatry), can serve as the foundation for recognizing biological species. In the case of allopatric populations, however, efforts at designating species have focused on concepts that recognize species based on congruence and cohesion of characters (for review see Avise and Ball, 1990, and Templeton, 1989). In the case of the low- and high-FN forms of *Peromyscus* from the Pacific Northwest, the recognition of distinct species is warranted both on the grounds of reproductive isolation and genetic cohesion. Where the high-FN deer mice are sympatric with low-FN *P. maniculatus* in southern British Columbia and Washington, the taxa do not interbreed (Allard and Greenbaum, 1988; Calhoun and Greenbaum, 1991; Gunn and Greenbaum, 1986; Sheppe, 1961), and therefore, constitute biological species. When considering the allopatric portions of the distribution of these mice, all available datasets are concordant in suggesting the conspecificity of the high-FN deer mice of the Pacific Northwest.

Results of this and related studies indicate the need for a formal taxonomic revision of the *Peromyscus* in the Pacific Northwest. To this end, we formally recognize the high-FN *Peromyscus* from the Pacific Northwest as:

Peromyscus keeni (Rhoads, 1894)

Sitomys keeni Rhoads, 1894:258.
Sitomys macrorhinus Rhoads, 1894:259.
Peromyscus keeni Bangs, 1897:75.
Peromyscus sitkensis Merriam, 1897:223.
Peromyscus oreas Bangs, 1898:84.
Peromyscus prevostensis Osgood, 1901:29–30.
Peromyscus hylaeus Osgood, 1908:141–142.
Peromyscus maniculatus oreas Osgood, 1909:51–53.
Peromyscus maniculatus hylaeus Osgood, 1909:53–54.
Peromyscus maniculatus keeni Osgood, 1909:55.
Peromyscus maniculatus algidus Osgood, 1909:56.
Peromyscus maniculatus macrorhinus Osgood, 1909:57–58.

Holotype.—ANSP 7768; formally No. 768 from the collection of S. N. Rhoads. Type is an adult female collected from Masset, Graham Island, Queen Charlotte Islands, British Columbia, Canada, in 1892 by J.H. Keen.

Distribution.—From Washington north to southern Alaska, and west of the Coastal and Cascade mountain ranges. Also found on the Queen Charlotte Islands and islands along the coast of British Columbia and the Alexander Archipelago. Occurs sympatrically with *Peromyscus maniculatus austerus* in the southern portion of this distribution as well as Balaclava, Malcolm, and Vancouver Islands, British Columbia, Canada. In the southeastern portion of the range, *P. keeni* is parapatric with *P. m. artemisiae.*

Diagnosis.—*Peromyscus keeni* differs from *P. maniculatus* in size being significantly larger with length of tail typically >100 mm. The higher FN (85–92) of *P. keeni* makes it chromosomally diagnosable from sympatric populations of low-FN (74–78) *P. m. austerus.*

Etymology.—The name *keeni* is in rec-

TABLE 4.—*Locality designations, general localities, current taxonomy, and proposed taxonomy for* Peromyscus maniculatus, P. oreas, P. sitkensis, *and* P. keeni.

Sample[a]	Locality[b]	Current taxonomy[c]	Proposed taxonomy
1L	N. Vancouver Island	*P. m. austerus*	No change
1H	N. Vancouver Island	*P. o. interdictus*	*P. k. interdictus*
2L	Vancouver Island	*P. m. austerus*	No change
3L	Vancouver Island	*P. m. austerus*	No change
4H	Vancouver Island	*P. o. interdictus*	*P. k. interdictus*
5L	Vancouver Island	*P. m. austerus*	No change
6L	Vancouver Island	*P. m. austerus*	No change
7L	Vancouver Island	*P. m. austerus*	No change
8L	Vancouver Island	*P. m. austerus*	No change
9L	Vancouver Island	*P. m. austerus*	No change
10H	Nigei Island	*P. o. isolatus*	*P. k. isolatus*
11L	Balaklava Island	*P. m. austerus*	No change
11H	Balaklava Island	*P. o. isolatus*	*P. k. isolatus*
12H	Hurst Island	*P. o. isolatus*	*P. k. isolatus*
13H	Bell Island	*P. o. isolatus*	*P. k. isolatus*
14H	Heard Island	*P. o. isolatus*	*P. k. isolatus*
15H	Duncan Island	*P. o. isolatus*	*P. k. isolatus*
16L	Malcolm Island	*P. m. austerus*	No change
16H	Malcolm Island	*P. o. isolatus*	*P. k. isolatus*
17L	Quadra Island	*P. m. austerus*	No change
18L	Cortes Island	*P. m. austerus*	No change
19L	Denman Island	*P. m. austerus*	No change
20L	Hornby Island	*P. m. austerus*	No change
21L	Texada Island	*P. m. austerus*	No change
22L	Galiano Island	*P. m. austerus*	No change
23L	Mayne Island	*P. m. austerus*	No change
24L	Saltspring Island	*P. m. austerus*	No change
25L	N. Pender Island	*P. m. austerus*	No change
26L	Saturna Island	*P. m. austerus*	No change
27L	Orcas Island	*P. m. austerus*	No change
28L	Shaw Island	*P. m. austerus*	No change
29L	San Juan Island	*P. m. austerus*	No change
30L	Lopez Island	*P. m. austerus*	No change
31H	Hagensborg, B.C.	*P. m. macrorhinus*	*P. k. macrorhinus*
32H	Prince Rupert, B.C.	*P. m. macrorhinus*	*P. k. macrorhinus*
33H	Exchamsiks River, B.C.	*P. m. macrorhinus*	*P. k. macrorhinus*
34H	Kleanza Creek, B.C.	*P. m. macrorhinus*	*P. k. macrorhinus*
35H	Graham Island	*P. m. keeni*	*P. k. keeni*
36H	Graham Island	*P. m. keeni*	*P. k. keeni*
37H	Graham Island	*P. m. keeni*	*P. k. keeni*
38H	Graham Island	*P. m. keeni*	*P. k. keeni*
39H	Moresby Island	*P. m. keeni*	*P. k. keeni*
40H	Hotspring Island	*P. m. prevostensis*	*P. k. prevostensis*
41H	Baranof Island	*P. sitkensis*	*P. k. sitkensis*
42H	Barnaof Island	*P. s. sitkensis*	*P. k. sitkensis*
43H	Herbert River, B.C.	*P. m. hylaeus*	*P. k. hylaeus*
44H	Skagway	*P. m. algidus*	*P. k. algidus*

[a] H and L indicate samples comprising individuals with high or low fundamental numbers, respectively.

[b] Specific locality data are given in Appendix I.

[c] Current taxonomy follows Allard and Greenbaum (1988) and Hall (1981).

ognition of the contributions made by Rev. J. H. Keen to the natural history of the Queen Charlotte Islands.

Remarks.—Currently, little data exist to clarify the taxonomic affinity of insular taxa in the Pacific Northwest, i.e., *P. m. beresfordi, P. m. cancrivorus, P. m. carli, P. m. doylei, P. m. maritimus, P. m. pluvialis, P. m. rubriventer, P. m. sartinensis,* and *P. m. triangularis.* However, based on the karyotypic data presented by Thomas (1973) for *P. m. carli, P. m. doylei,* and *P. m. triangularis* (high FNs), and the morphological data (i.e., length of tails) presented by Cowan and Guiguet (1965), we suspect that these deer mice are representative of *P. keeni.* In the absence of data to positively identify these subspecies as belonging to *P. maniculatus,* we suggest these forms be referred to *P. keeni.* A summary of the proposed classification of *Peromyscus* from the Pacific Northwest is presented in Table 4.

Acknowledgments

The authors thank J. N. Derr for his help with analysis of the mitochondrial DNA. Special thanks to D. W. Hale, P. D. Sudman, and D. Nagorsen for assistance with the collection of specimens. We thank R. R. Hollander, F. Chavez-Ramirez, C. A. Stanvinhoa, D. R. Su, and L. R. Smith for helpful comments on this manuscript. The manuscript was greatly enhanced by comments from D. Nagorsen and an anonymous reviewer. This research was supported by National Institute of Health grant GM27014 to IFG.

Literature Cited

Allard, M. W., and I. F. Greenbaum. 1988. Morphological variation and taxonomy of chromosomally differentiated *Peromyscus* from the Pacific Northwest. Canadian Journal of Zoology, 66:2734–2739.

Allard, M. W., S. J. Gunn, and I. F. Greenbaum. 1987. Mensural discrimination of chromosomally characterized *Peromyscus oreas* and *P. maniculatus.* Journal of Mammalogy, 68:402–406.

Avise, J. C., and R. M. Ball. 1990. Principles of genealogical concordance in species concepts and biological taxonomy. Oxford Surveys in Evolutionary Biology, 7:45–67.

Avise, J.C., M. H. Smith, and R. K. Selander. 1979. Biochemical polymorphism and systematics in the genus *Peromyscus* VII. Geographic differentiation in members of the *truei* and *maniculatus* species groups. Journal of Mammalogy, 60:177–192.

Avise, J. C., J. F. Shapira, S. W. Daniel, C. F. Aquadro, and R. A. Lansman. 1983. Mitochondrial DNA differentiation during the speciation process in *Peromyscus.* Molecular Biology and Evolution, 1:38–56.

Baker, R. J., M. W. Haiduk, L. W. Robbins, A. Cadena, and B. F. Koop. 1982. Chromosomal studies of South American bats and their systematic implications. Pp. 303–327, *in* Mammalian biology in South America (M. A. Mares and H. H. Genoways, eds.). Special Publication Series, Pymatuning Laboratory of Ecology, University of Pittsburgh, 6:1–539.

Bangs, O. 1897. A new white-footed mouse from British Columbia. The American Naturalist 31:74–75.

———. 1898. Description of a new white-footed mouse from the Mount Baker Range, British Columbia. Proceedings of the Biological Society of Washington, 12:83–84.

Calhoun, S. W., and I. F. Greenbaum. 1991. Evolutionary implications of genic variation among insular populations of *Peromyscus maniculatus* and *Peromyscus oreas.* Journal of Mammalogy, 72:248–262.

Calhoun, S. W., I. F. Greenbaum, and K. Fuxa. 1988. Biochemical and karyotypic variation in *Peromyscus maniculatus* from western North America. Journal of Mammalogy, 69:34–45.

Cavalli-Sforza, L. L., and A. W. F. Edwards. 1967. Phylogenetic analysis: models and estimation procedures. Evolution, 21:550–570.

Committee for Standardization of Chromosomes of *Peromyscus.* 1977. Standardized karyotype of deer mice, *Peromyscus* (Rodentia). Cytogenetics and Cell Genetics, 19:38–43.

Cowan, I. M. 1935. A distributional study of the Peromyscus sitkensis group of white-footed mice. University of California Publication in Zoölogy, 40: 429–437.

Cowan, I. M. and C. J. Guiguet. 1965. The mammals of British Columbia. British Columbia Provincial Museum, Handbook, 11:1–414.

Dice, L. R. 1949. Variation of *Peromyscus maniculatus* in parts of western Washington and adjacent Oregon. Contributions from the Laboratory of Vertebrate Biology, University of Michigan, 44:1–34.

Farris, J. S. 1972. Estimating phylogenetic trees from distance matrices. The American Naturalist 106:645–668.

Felsenstein, J. 1987. PHYLIP (Phylogeny Inference Package), Version 3.0. Manual. University of Washington, [Seattle], 150 pp.

Foster, J. B. 1965. Evolution of mammals on the Queen Charlotte Islands, British Columbia. Occasional Publication, British Columbia Provincial Museum, 14:1–130.

Gunn, S. J. 1988. Chromosomal variation and differentiation among insular populations of *Peromyscus* from the Pacific Northwest. Canadian Journal of Zoology, 66:2726–2733.

Gunn, S. J., and I. F. Greenbaum. 1986. Systematic implications of karyotypic and morphologic variation in mainland *Peromyscus* from the Pacific Northwest. Journal of Mammalogy, 67:294–304.

HALL, E. R. 1981. The mammals of North America. Second ed. John Wiley & Sons, New York, 2:601-1181 + 90.

HARRIS, H., AND D. A. HOPKINSON. 1976. Handbook of enzyme electrophoresis in human genetics. North-Holland Publishing Company, Amsterdam (Looseleaf with Supplements in 1977 and 1978).

HILLIS, D. M., AND S. K. DAVIS. 1986. Evolution of ribosomal DNA: fifty million years of recorded history in the frog genus *Rana*. Evolution, 40:1275–1288.

JOHNSON, M. L., AND B. T. OSTENSON. 1959. Comments on the nomenclature of some mammals of the Pacific Northwest. Journal of Mammalogy, 40:571–577.

LANSMAN, R. A., J. C. AVISE, C. F. AQUADRO, J. F. SHAPIRA, AND S. W. DANIEL. 1983. Extensive genetic variation in mitochondrial DNA's among geographic populations of the deer mouse, *Peromyscus maniculatus*. Evolution, 37:1–16.

LIU, T. T. 1954. Hybridization between *Peromyscus maniculatus, P. oreas* and *P. m. gracilis*. Journal of Mammalogy, 35:448–449.

MCALPINE, P. J., N. VAN CONG, C. BOUCHEIX, A. J. PAKSTIS, R. C. DOUTE, AND T. B. SHOWS. 1987. The 1987 catalog of mapped genes and report of the nomenclature committee. Cytogenetics and Cell Genetics, 46:29–101.

MCCABE, T. T., AND I. M. COWAN. 1945. *Peromyscus maniculatus macrorhinus* and the problem of insularity. Transactions of the Royal Canadian Institute, 25:117–215.

MERRIAM, C. H. 1897. Descriptions of five new rodents from the coast region of Alaska. Proceedings of the Biological Society of Washington 11:221–223.

NAGORSEN, D. 1990. The mammals of British Columbia: a taxonomic catalogue. Royal British Columbia Museum Memoir, 4:1–140.

NEI, M., AND R. K. CHESSER. 1983. Estimation of fixation indices and gene diversities. Annals of Human Genetics, 47:253–259.

NEI, M., AND W-H. LI. 1979. Mathematical model for studying genetic variation in terms of restriction endonuclease. Proceedings of the National Academy of Sciences, 76:5269–5273.

OSGOOD, W. H. 1901. Natural history of the Queen Charlotte Islands, British Columbia. North American Fauna, 21:1–50.

———. 1908. A new white-footed mouse from Alaska. Proceedings of the Biological Society of Washington, 21:141–142.

———. 1909. Revision of the mice of the American genus *Peromyscus*. North American Fauna, 28:1–285.

PENGILLY, D., G. H. JARRELL, AND S. O. MACDONALD. 1983. Banded karyotypes of *Peromyscus sitkensis* from Baranof Island, Alaska. Journal of Mammalogy, 64:682–685.

RHOADS, S. N. 1894. Descriptions of four new species and two subspecies of white-footed mice from the United States and British Columbia. Proceedings of the Academy of Natural Sciences of Philadelphia, 46:253–261.

RICHARDSON, B. J., P. A. BAVERSTOCK, AND M. ADAMS. 1986. Allozyme electrophoresis. Academic Press, New York, 410 pp.

RIDGWAY, G. J., S. U. SHERBURNE, AND R. D. LEWIS. 1970. Polymorphism in the esterases of Atlantic herring. Transactions of the American Fisheries Society, 99:147–151.

ROGERS, J. S. 1972. Measures of genetic similarity and genetic distance. Studies in Genetics VII, The University of Texas Publication, 7213:145–153.

———. 1991. A comparison of the suitability of the Rogers, modified Rogers, Manhattan, and Cavalli-Sforza and Edwards distances for inferring phylogenetic trees from allele frequencies. Systematic Zoology, 40:63–73.

SCHAFFER, H. E., AND R. R. SEDEROFF. 1981. Improved estimation of DNA fragment lengths from agarose gels. Analytical Biochemistry, 115:113–122.

SEABRIGHT, M. 1971. A rapid banding technique for human chromosomes. The Lancet, 2:971–972.

SELANDER, R. K., M. H. SMITH, S. Y. YANG, W. E. JOHNSON, AND J. B. GENTRY. 1971. Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse (*Peromyscus polionotus*). Studies in Genetics VI, The University of Texas Publication, 7103:49–90.

SHEPPE, W., JR. 1961. Systematic and ecological relations of *Peromyscus oreas* and *P. maniculatus*. Proceedings of the American Philosophical Society, 105: 421–446.

SNEATH, P. H. A., AND R. R. SOKAL. 1973. Numerical taxonomy: the principles and practice of numerical classification. W. H. Freeman and Company, San Francisco, 573 pp.

SULLIVAN, R. M., S. W. CALHOUN, AND I. F. GREENBAUM. 1990. Geographic variation in genital morphology among insular and mainland populations of *Peromyscus maniculatus* and *Peromyscus oreas*. Journal of Mammalogy, 71:48–58.

SUMNER, A. T. 1972. A simple technique for demonstrating centromeric heterochromatin. Experimental Cell Research, 75:304–306.

SWARTH, H. S. 1911. Birds and mammals of the 1909 Alexander Alaska expedition. University of California Publication in Zoölogy 7:9–172.

SWOFFORD, D. L. 1981. On the utility of the distance Wagner procedure. Pp. 25–43, *in* Advances in cladistics: proceedings of the first meeting of the Willie Hennig Society (V. A. Funk and D. R. Brooks (eds.). New York Botanical Garden, Bronx, New York, 250 pp.

SWOFFORD, D. L., AND R. B. SELANDER. 1989. Biosys-1: a computer program for the analysis of allelic variation in population genetics and biochemical systematics. Release 1.7. Illinois Natural History Survey, Champaign, 43 pp.

TEMPLETON, A. R. 1989. The meaning of species and speciation: a genetic perspective. Pp. 3–27, *in* Speciation and its consequences (D. Otte and J. A. Endler, eds.). Sinauer Associates, Inc., Sunderland, Massachusetts, 679 pp.

THOMAS, B. 1973. Evolutionary implications of karyotypic variation in some insular *Peromyscus* from British Columbia, Canada. Cytologia, 38:483–493.

VAN DEN BUSSCHE, R. A., M. J. HAMILTON, AND R. K.

CHESSER. 1986. Problems of estimating gene diversity among populations. The Texas Journal of Science, 38:281–287.

WRIGHT, S. 1978. Evolution and the genetics of populations. Variability within and among natural populations. The University of Chicago Press, Chicago, 4:1–580.

Submitted 13 August 1992. Accepted 5 May 1993.

Associate Editor was Karen McBee.

APPENDIX I

Specimens Examined

Collection localities for specimens of *Peromyscus* listed in Fig. 1. Locality numbers correspond to numbering in Fig. 1. Taxonomic identity of specimens based on data from Allard and Greenbaum (1988), Gunn and Greenbaum (1986), and Hall (1981). Voucher specimens are deposited in the Texas Cooperative Wildlife Collection, Texas A&M University, College Station. Localities 1–30 are from Calhoun and Greenbaum (1991). *Peromyscus maniculatus algidus*: U.S.A.; ALASKA: *Locality 44*—2 km N Skagway, Liarsville Campground (n = 10). *Peromyscus maniculatus austerus*: CANADA; BRITISH COLUMBIA; VANCOUVER ISLAND: *Locality 1*—northern Vancouver Island (combined sample from 14.5 km W Holberg, San Josef Campsite; 1.1 km N Winter Harbour; 21.7 km W Port McNeill, Ruppert Inlet; Marble River Recreation Area; 7.1 km N or 4.7 km S Zeballos) (n = 48); *Locality 2*—Muchalat Lake Campsite (n = 19); *Locality 3*—SE shore Gooseneck Lake (n = 20); *Locality 5*—35.7 km W Port Alberni, Sproat Lake (n = 36); *Locality 6*—11.9 km W Honeymoon Bay, Lake Cowichan (n = 16); *Locality 7*—0.8 km S Ladysmith and Holland Creek (n = 9); *Locality 8*—3.2 km S Port Renfrew, Botanical Beach (n = 13); *Locality 9*—Matheson Lake Provincial Park (n = 20). CANADA; BRITISH COLUMBIA: *Locality 11*—Balaklava Island (n = 8); *Locality 16*—Malcolm Island, 4.8 km E Sointula (n = 11); *Locality 17*—Quadra Island, 9.2 km NW and 3.5 km SE Heriot Bay (n = 9); *Locality 18*—Cortes Island, Smelt Bay Provincial Park (n = 5); *Locality 19*—Denman Island, 6.1 km SE and 7.2 km SE Denman Island P.O. (n = 37); *Locality 20*—Hornby Island Co-operative Store and 0.5 km NW Co-op (n = 40); *Locality 21*—Texada Island, 3.2 km SSE Gillies, Harwood Point Park (n = 42); *Locality 22*—Galiano Island, Alcala Point (n = 26); *Locality 23*—Mayne Island, 1.1 km SW Mayne (n = 19); *Locality 24*—Saltspring Island, 2.3 km N and NE corner St. Mary Lake (n = 25); *Locality 25*—North Pender Island, Prior Centennial Provincial Park (n = 11); *Locality 26*—Saturna Island, 0.3 km W Eastpoint Lighthouse (n = 18). WASHINGTON; SAN JUAN CO.: *Locality 27*—Orcas Island, 4.3 km WSW Eastsound Village (n = 9); *Locality 28*—Shaw Island, Shaw Island Park (n = 31); *Locality 29*—San Juan Island, 4.8 km N 4.8 km W Friday Harbor and 2.4 km W Sportsman Lake (n = 27); *Locality 30*—Lopez Island, Spencer Spit State Park (n = 28). *Peromyscus maniculatus hylaeus*: U.S.A.; ALASKA; *Locality 43*:—21.2 km NW Auke Bay Ferry Terminal, Herbert River (n = 14). *Peromyscus maniculatus keeni*: CANADA; BRITISH COLUMBIA: *Locality 35*—Graham Island, 3.5 km E Masset (n = 25). *Locality 36*—Graham Island, 1.3 km N Masset (n = 12). *Locality 37*—Graham Island; Phantom Creek, 10 km marker (n = 24). *Locality 38*—Graham Island; Rennell Sound Recreation Site (n = 39). *Locality 39*—Moresby Island, Grey Bay Recreation Site (n = 45). *Peromyscus maniculatus macrorhinus*: CANADA; BRITISH COLUMBIA: *Locality 31*—16 km S, 12 km E Hagensborg (n = 8). *Locality 32*—32.4 km E Prince Rupert (n = 7). *Locality 33*—4.6 km W Exchamsiks River Provincial Park (n = 19). *Locality 34*—Kleanza Creek, Hwy 16 (n = 27). *Peromyscus maniculatus prevostensis*: CANADA; BRITISH COLUMBIA: *Locality 40*—Hotspring Island (n = 23). *Peromyscus oreas*: CANADA; BRITISH COLUMBIA; VANCOUVER ISLAND: *Locality 1*—Northern Vancouver Island (combined localities listed above) (n = 19); *Locality 4*—7.7 km S Tofino, Pacific Rim Campground (n = 20); CANADA; BRITISH COLUMBIA: *Locality 10*—Nigei Island, Port Alexander (n = 12); *Locality 11*—Balaklava Island (n = 7); *Locality 12*—Hurst Island (n = 10); *Locality 13*—Bell Island (n = 8); *Locality 14*—Heard Island (n = 28); *Locality 15*—Duncan Island (n = 13); *Locality 16*—Malcolm Island, 4.8 km E Sointula (n = 12). *Peromyscus sitkensis sitkensis*: U.S.A.; ALASKA: *Locality 41*—Baranof Island; 9.2 km N, 6.7 km E Sitka (n = 8). *Locality 42*—Baranof Island; 9.2 km N, 1.6 km W Sitka (n = 31).