Journal of Mammalogy, 87(6):1204–1217, 2006

KANGAROO MICE (*MICRODIPODOPS MEGACEPHALUS*) OF THE MONO BASIN: PHYLOGEOGRAPHY OF A PERIPHERAL ISOLATE

JOHN C. HAFNER,* EMILY REDDINGTON, AND MATTHEW T. CRAIG

Moore Laboratory of Zoology and Department of Biology, Occidental College, Los Angeles, CA 90041, USA (JCH, ER)
Hawaii Institute of Marine Biology, P.O. Box 1346, Kaneohe, HI 96744, USA (MTC)

Kangaroo mice (*Microdipodops*) inhabiting the Mono Basin and adjoining valley regions of California and Nevada represent a disjunct distributional isolate and have been considered as a distinct species or, more recently, as 2 subspecies of *M. megacephalus*. Analysis of patterns of geographic variation in 11 populations in the Mono Basin region shows that kangaroo mice inhabiting the northern portion of this peripheral isolate are relatively large and dark (referred to as *M. m. nasutus*), and those to the southern end are small and pale (termed *M. m. polionotus*), and a cline exists between the 2. Inasmuch as several morphological characters are correlated positively with environmental measures (e.g., hind-foot length and climatic severity; pelage color and soil color), it appears that variation in morphology is responding to a selection gradient. Mitochondrial DNA sequence data, together with chromosomal and protein information, reveal that the 2 subspecies are nearly identical genetically. Moreover, molecular phylogeographic analysis reveals that the Mono kangaroo mice belong to the southeastern geographic unit of *M. megacephalus* (a mean of 1.86% sequence divergence) and are genetically most close to animals from the San Antonio locality (more than 100 km to the east). It is hypothesized that distributional shifts in the geographic range of kangaroo mice in response to climatic fluctuation during the late-Pleistocene and Holocene times resulted in the westward expansion and eventual colonization and isolation of kangaroo mice in the Mono Basin region. Our vicariant biogeographical interpretation suggests a historical route through the Lahontan Trough and the physiographic discontinuity east of Mono Lake (i.e., between the Wassuk Range and the White Mountains) that may serve as a biogeographic model for other basin-dwelling organisms. Lastly, the systematic status of the 2 subspecies is evaluated; a single subspecies, *polionotus*, is recognized, with *nasutus* placed in synonymy.

Key words: biogeography, chromosomes, kangaroo mice, *Microdipodops*, mitochondrial DNA, Mono Lake, phylogeography, systematics

Kangaroo mice, genus *Microdipodops*, are sand-obligate desert rodents that inhabit stabilized sand dunes and sandy ridges and valleys in the Great Basin of western North America. These sandy environments were created by depositional and eolian processes during the Pleistocene and Holocene and are generally associated with pluvial lake basins (Bagnold 1941; Benson et al. 1990; Eissmann 1990; Morrison 1964, 1991; Smith 1982). The Mono Lake basin, located on the western edge of the Great Basin, is one such pluvial lake basin. Kangaroo mice from the Mono Basin region are of interest because they represent a distributional isolate and they have long been recognized to be morphologically and taxonomically distinct from other populations of kangaroo mice (Grinnell 1914; Hafner 1981; Hall 1941). Furthermore, the Mono Basin region provides a unique geological and biogeographical backdrop for the study of differentiation within kangaroo mice; late-Pleistocene lake-level chronologies of the pluvial lake in the Mono Basin (Lake Russell) were different from those of the large pluvial lakes of the Great Basin (Benson et al. 1990) and Brown (1973:784) considered the sand-dune rodent community in the Mono Basin to be ''depauperate in an evolutionary sense'' largely because of its harsh winter climate. Kangaroo mice are the only sand-obligate member of the sand-dune rodent community in the Mono Basin region and this study examines their morphologic variation over geography, historical biogeography, and systematic status.

Grinnell (1914) provided the 1st record of occurrence of kangaroo mice from this region: 10 specimens of kangaroo mice

* *Correspondent: hafner@oxy.edu*

collected from southeast of Mono Lake (near Benton, California; Fig. 1). Grinnell (1914:302) noted the distinctiveness and ''extreme pallor of coloration'' of these kangaroo mice and described them as a new species, *M. polionotus*. In his revision of the genus, Hall (1941) examined morphological characters and treated *M. polionotus* as a subspecies of *M. megacephalus*. At the same time, Hall (1941) described another subspecies, *M. m. nasutus*, from northeast of Mono Lake (Fletcher, Nevada) on the basis of 6 specimens. Hall (1941) considered *M. m. nasutus* to be larger and darker than *M. m. polionotus*. Hafner (1981) examined morphological, chromosomal, and protein data and affirmed Hall's (1941) assignment of kangaroo mice from the Mono Basin to *M. megacephalus*. Additionally, Hafner (1981) found that kangaroo mice from near the type localities of Benton and Fletcher had identical karyotypes, shared a high level of protein similarity, and suggested that only 1 subspecies, *polionotus*, should be recognized.

Despite an apparent high degree of variation in pelage color, all populations of kangaroo mice from the Mono Lake basin and adjoining valley regions of California and Nevada are now recognized to be those of the dark kangaroo mouse, *M. megacephalus* (Hafner 1981; Hall 1941). Importantly, kangaroo mice from the Mono region are separated from other populations of the species by more than 100 km of unsuitable habitat and represent a peripheral isolate on the western margin of the species' distribution (Hafner 1981; Hall 1941; Fig. 1). After his examination of patterns of protein, chromosomal, and morphological variation within the species, Hafner (1981) hypothesized that the kangaroo mice from the Mono region were derived from populations of *M. megacephalus* to the east (central Nevada). The present study tests this hypothesis of evolutionary affinity and expands on previous studies by evaluating additional morphometric, chromosomal, and mitochondrial DNA (mtDNA) sequence data.

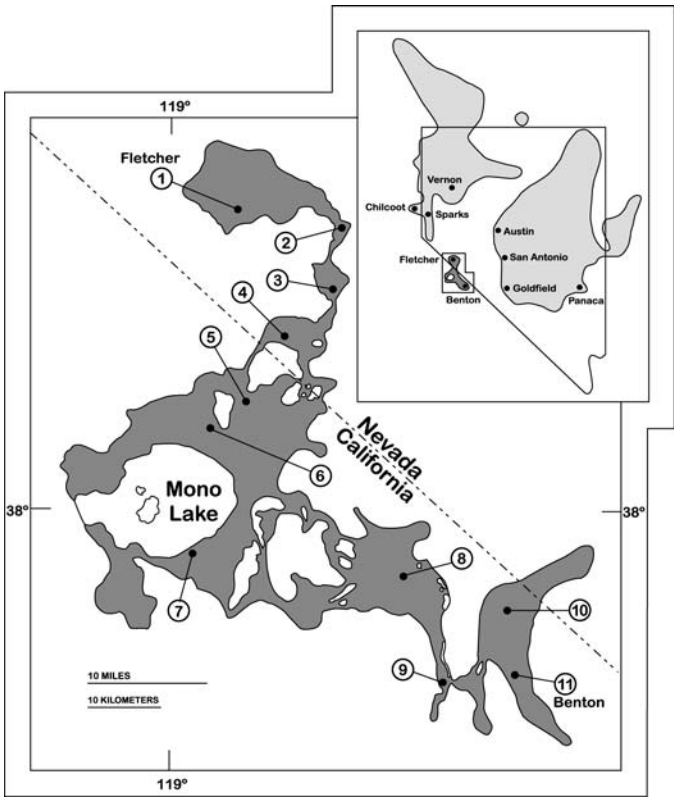

Fig. 1.—Geographic map of the Mono Basin and adjoining valley regions of California and Nevada showing locations of populations of *Microdipodops megacephalus* analyzed in this study. The shading around Mono Lake delineates areas of sandy basin, sagebrush (*Artemisia*) habitat across the study site and the encircled numbers refer to study localities. Inset map, with the outline of the state of Nevada for reference, shows the geographic distribution of *M. megacephalus* and identifies other populations sampled from the main northwestern and southeastern geographic units of the species.

Materials and Methods

Study area and specimens examined.—Before the initiation of this study, specimens of kangaroo mice from the Mono region were known only from near the type localities of *M. m. nasutus* (vicinity of Fletcher, Nevada) and *M. m. polionotus* (vicinity of Benton, California) and several records around Mono Lake (Hall 1941). For the ecomorphological portion of this study, effort was made to sample populations of kangaroo mice between the type localities for the named subspecies in the Mono Basin and adjoining valleys. Of the 176 total specimens used for the morphological portion of this study (Appendix I), 86 specimens were captured in the wild specifically for this study. An additional 21 specimens of *M. megacephalus* were collected and used in the mtDNA and chromosomal analyses, including 2 specimens collected near the type locality of *M. m. nasutus* and 3 specimens from near the type locality of *M. m. polionotus* (Appendix II). For the phylogeographic portion of this study, 3 populations of kangaroo mice were sampled from the northwestern distributional unit of *M. megacephalus* (Vernon, Chilcoot, and Sparks; Fig. 1) and 4 populations were sampled from the southeastern distributional unit of *M. megacephalus* (Austin, San Antonio, Goldfield, and Panaca). Selection of these localities was guided by 3 criteria: proximity to the Mono distributional isolate, populations that represented both the major northwestern and southeastern geographic units of *M. megacephalus*, and populations within the northwestern and southeastern units that represented both the 40-α and 40-β chromosomal forms of *M. megacephalus* (Hafner 1981). Initial outgroup taxa included the sister species, the pallid kangaroo mouse (*M. pallidus*); a representative kangaroo rat, the chisel-toothed kangaroo rat (*Dipodomys microps*); and a pocket mouse, the little pocket mouse (*Perognathus longimembris*; Appendix II). Final selection of outgroup taxa excluded *P. longimembris* because preliminary phylogenetic analyses showed it to be less closely related to *Microdipodops* than is *Dipodomys*. Our outgroup selection is also supported by other studies (Alexander and Riddle 2005; Hafner 1993; Hafner and Hafner 1983; Hafner 1982; Mantooth et al. 2000; Rogers 1990). Animals collected during the course of this study were treated in a humane manner following procedures approved by the American Society of Mammalogists (Animal Care and Use Committee 1998) and Occidental College's Institutional Animal Care and Use Committee.

Morphological analyses.—Morphological variation in kangaroo mice in the Mono Basin region was assessed by examining both pelage coloration and a battery of cranial and external morphometric variables for 176 adult specimens from 11 populations (Appendix I). Specimens were judged to be adult by the presence of wear on the 4th upper permanent premolar and the general transparency of the auditory bullae. Pelage color variation was assessed using the Munsell

system of color notation in combination with the Munsell soil color charts (Munsell Color Co., Inc. 1954; see Miller [1958] for discussion of technique). Munsell's (Munsell Color Co., Inc. 1954) system expresses color using 3 components: hue (refers to a range of frequencies on the color spectrum), value (determines how close as a color is to black or white), and chroma (expresses the purity of a color). The pelage color value in the terminal portion of the tail (indicative of overall dorsal pelage coloration) was measured and recorded for each specimen. A series of 5 reference specimens was chosen to encompass the total range of value variation. The reference specimens, ranked in order of increasing color value from black to gray, and their Munsell notation in parentheses (i.e., hue [e.g., 10YR], value [indicated in bold], and chroma [e.g., /2]) are as follows: MVZ 142264 (10YR **2**/2), MVZ 142256 (10YR **3**/l), MVZ 142236 (10YR **4**/2), MVZ 142274 (10YR **5**/2), and MVZ 142219 (10YR **6**/2). All other study specimens were compared with these reference specimens under a Macbeth Super Color Matching Skylight hood (GretagMacbeth LLC, New Windsor, New York) and assigned an appropriate color-value score.

Ten cranial and external characters were examined in the morphometric analysis. These characters, used and described in previous studies (e.g., DeBlase and Martin 1974; Hafner et al. 1979), included basal length, nasal length, greatest breadth of skull, maxillary breadth, least interorbital breadth, total length, tail length, hind-foot length, greatest length of skull, and mandibular length. Cranial characters were measured with dial calipers (read to the nearest 0.01 mm) and external dimensions were read directly from specimen labels. Univariate and multivariate statistical routines were performed using SYSTAT 9 software (SPSS, Inc. 1999), BMDP (Dixon 1983), and the Statistical Package for the Social Sciences (Nie et al. 1975). Although previous studies indicated a lack of sexual dimorphism in kangaroo mice (Hafner 1976, 1981; Hall 1941; Schitoskey 1968), the possible extent of sexual dimorphism in the morphological characters under study was examined for the Fletcher population (the largest sample size available, $n = 41$ [17 females and 24 males]) using analysis of variance (ANOVA). Our results support previous findings of no secondary sexual variation ($P > 0.05$ for all 11 characters); hence, sexes were pooled in our analyses.

Several ordination procedures were executed in an attempt to understand the multivariate morphological relationships among the 11 populations in the Mono Basin region and, specifically, to determine if the observed patterns of variation agreed with the a priori hypothesis of 2 principal groupings reflecting the present subspecific taxonomy. A discriminant function, canonical variate analysis was performed initially with each of the 11 localities entered as a separate group (character basal length was omitted because of individuals with missing data). In a subsequent analysis, a 2-group discriminant function analysis was performed with the Fletcher and Benton samples entered as known groups (i.e., the subspecies *nasutus* and *polionotus*, respectively), whereas individuals from the intermediate localities were entered as unknown groups. Assignment of individuals from the unknown group to either known group was based on posterior probability values. A summary of interlocality morphometric divergence among the 11 populations was obtained by constructing a taxonomic distance matrix using the NTSYSpc program (version 2.02j, Applied Biostatistics, Inc.; Exeter Software, Setauket, New York). All specimens used in the morphological portion of this study are deposited in the Museum of Vertebrate Zoology (MVZ) or The Museum, Texas Tech University (TTU) as voucher study preparations (Appendix I).

Geographic distances and environmental characters.—Pairwise interlocality geographic distances were estimated between the 11 sample localities by examination of topographic maps. A minimum-path geographic distance matrix (Sokal 1979) was produced that reflects likely distances traversed by kangaroo mice through appropriate habitat and not beeline or great-circle distances. To test for the significance of geographic variation patterns in the morphological data, the geographic distance matrix was compared with the taxonomic distance matrix using Mantel's test (Mantel 1967; Sokal 1979) and the program NTSYSpc.

Four environmental and climatological variables were recorded for the 11 study localities in the Mono Basin region: climatic severity, isophane, soil color, and percentage sand. The index of climatic severity is largely indicative of differential temperature regimes and was calculated according to the method of Findley and Traut (1970). Climatic severity, reported in thousands of ''adjusted'' meters, was calculated for a locality by adding the elevation to the product of latitude times 106.68. The rationale for this formula lies in the assumption that a 1° shift in latitude is equivalent to 106.68 m of elevation in causing climatic change in western North America. Isophane, similar to the index of climatic severity, is based on the Bioclimatic Law of Hopkins (1938) and describes the general retardation of growing seasons that occurs with increase in latitude, longitude, and elevation. Isophane values for all the Mono Basin localities were calculated following the procedures of Power (1970): from a base equal to 40.00, 1.00 is subtracted for each degree south of 40° north latitude, 1.00 is added to the base for every 5° west of l00° west longitude, and 0.25 is added to the base for each 30.48 m above sea level. Soil color value was determined for 11 study localities by using the Munsell system of color notation. Three surface soil samples were selected randomly at each locality and were scored by comparison with the Munsell soil color charts. The mean soil color value was chosen as representative of a locality and used in the climatological analyses. Soil samples were analyzed further to determine the percentage sand fraction (soil particles < 2 mm) as follows: soil samples were worked through a 2-mm sieve and the proportion of total soil sample (by weight) less than 2 mm was determined. Degree of association between the environmental and morphological characters was evaluated by use of the Pearson product-moment correlation coefficient.

Chromosomal analyses.—Nonpreferentially stained chromosomal preparations were made from 20 specimens of *M. megacephalus* following the postmortem technique described by Hafner and Sandquist (1989). Nine populations were sampled for the chromosomal analysis and the specimens used were the same as those employed in the mtDNA analysis except that only 3 specimens were karyotyped from the Austin locality (see Appendix II). Chromosomal variation for 6 of the 9 populations was characterized previously by Hafner (1981) but this study resampled those populations, as well as 3 other populations (Goldfield, Austin, and San Antonio) for which chromosomal information was lacking. Terminology and description of karyotypes used here follow Hafner (1981).

Mitochondrial DNA analyses.—Whole genomic DNA was extracted from liver or kidney tissues using the DNeasy Tissue Kit (QIAGEN Inc., Valencia, California). Standard protocol suggested by Qiagen was followed except at the following stages: 1) a $2 \times 2 \times$ 1-mm piece of liver or kidney tissue was used; 2) samples were incubated at 55°C for at least 24 h; and 3) the resuspension of DNA was performed in 2 steps (1st, 150 µl of Buffer AE was pipetted into the spin column and then incubated for 1 min at room temperature before spinning and, 2nd, an additional 50 µl of Buffer AE was added, incubated for 1 min, and then spun). Extracted DNA was stored at −70°C. Two mitochondrial genes, 16S rRNA and cytochrome *b* (*Cytb*), were chosen for analysis because their different rates of evolution (16S is more conservative than *Cytb*—Ferris et al. 1983;

Springer et al. 2001) should facilitate the resolution of clades at deeper and more shallow levels, respectively. Mitochondrial DNA was amplified using REDTaq Ready Mix (Sigma, St. Louis, Missouri). Each polymerase chain reaction amplification was performed using the PTC100 thermal cycler (MJ Research Inc., Waltham, Massachusetts) and included 2 μl (10–100 ng) of genomic DNA, 1 μl of each of 2 primers (10 μM), 21 μl double-distilled H_2O, and 25 μl of REDTaq to make a total reaction volume of 50 μl. Polymerase chain reaction and sequencing of 16S rRNA gene were performed using the 16Sar and 16Sbr human primers (Hillis et al. 1996). The *Cytb* gene was amplified and sequenced using primers MVZ05 and MVZ04 (Smith and Patton 1991). Polymerase chain reaction amplifications for 16S were completed using the following thermal profile: 30-s denaturation at 95°C, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 45 s, and extension at 72°C for 45 s. *Cytb* amplifications were performed following Lovejoy (2000), with the modification of a 52°C fixed annealing temperature.

The amplified DNA was purified using the QIAquick kit (QIAGEN Inc.) and standard protocol with purified products resuspended in double-distilled H_2O. Automated, fluorescently labeled, dideoxy, chain-terminator sequencing was performed for all samples at the DNA Sequencing Facility at California State University, Northridge, using the ABI Prism 377 DNA Sequencer (Applied Biosystems, Inc., Foster City, California) and the BigDye Version 3.1 sequencing cocktail (Applied Biosystems, Inc.).

Double-stranded sequences were edited and aligned in GeneTool 1.0 (Biotools, Inc. Edmonton, Alberta, Canada). All sequences were submitted to GenBank (GenBank accession numbers DQ422887–DQ422913 for 16S; DQ422914–DQ422940 for *Cytb*). Multiple sequence alignments were performed using Clustal_X (Thompson et al. 1997) with the default settings (gap opening = 10, gap extension = 0.20) for 16S, *Cytb*, and the combined data set. All alignments were visually examined and edited in MacClade (Maddison and Maddison 2000) without the use of structural models because the alignments lacked complicated insertions or deletions (Leaché and Reeder 2002).

Phylogenetic analyses were performed 1st on 16S (550 base pairs [bp]) and *Cytb* (468 bp) separately, thereby allowing us to identify any incongruence between them (Leaché and Reeder 2002; Weins 1998). Finding none, we then analyzed the combined alignment of 1,018 bp. MEGA (Kumar et al. 2000) was used to calculate transition : transversion ratios. PAUP*4.0b10 (Swofford 2003) was used to test for the presence of phylogenetic signal according to the procedures of Hillis and Huelsenbeck (1992), to calculate percent sequence divergence with uncorrected *p*, and to construct maximum-parsimony, neighbor-joining distance, and maximum-likelihood trees. The consistency index (CI) and the retention index (RI) also were calculated in PAUP during the maximum-parsimony analyses. Maximum-parsimony and neighbor-joining trees were built for each individual gene and all 4 trees were topologically identical. A partition homogeneity test was performed for 16S and *Cytb* and a nonsignificant result ($P = 1.0$) allowed us to combine the 2 genes.

Heuristic maximum-parsimony searches with equally weighted sites from the combined data set were performed using a full heuristic search, tree-bisection-reconnection branch swapping, simple sequence addition, and 1,000 bootstrap pseudoreplicates. A neighbor-joining tree was built using uncorrected *p* and 1,000 bootstrap pseudoreplicates. The most appropriate phylogenetic model for the combined data set suggested by ModelTest (Posada and Crandall 1998) was the general time-reversible model with invariant sites and among-site variation (GTR+I+Γ—Gu et al. 1995; Yang 1994). Maximum-likelihood phylogenetic analysis was conducted using a full heuristic search and parameters specified by ModelTest. The maximum-likelihood tree was then constructed using 200 bootstrap pseudoreplicates. Bayesian phylogenetic analysis was performed with MrBayes 2.01 (Huelsenbeck and Ronquist 2001) using GTR+I+Γ with uniform priors and with variables I and Γ estimated by each Bayesian analysis (Leaché and Reeder 2002). Four incrementally heated Monte Carlo Markov chains were run concurrently for 10,000,000 generations and were sampled every 1,000 generations. Two independent Bayesian analyses were conducted to make sure that the searches were not limited to local optima (Leaché and Reeder 2002). Convergence of the chain on a stable likelihood value was evaluated graphically by plotting log-likelihood values against generation time. Once stationarity was reached, the first 200 trees were eliminated as burn-in points. The remaining 9,800 trees (from each of the 2 analyses) were used to create a 50% majority rule consensus tree where each clade's posterior probability value is indicative of the percentage of samples that recover that particular clade (Huelsenbeck and Ronquist 2001).

Results

Geographic setting.—The 18 populations of kangaroo mice sampled in this study (Fig. 1) include a focal series of 11 populations from the distributional isolate around Mono Lake and a comparison set of populations from the main northwestern and southeastern distributional units of the species. Within the distributional isolate around Mono Lake, kangaroo mice are restricted to sandy basins that support a floral community dominated by sagebrush (*Artemisia*) and rabbit brush (*Chrysothamnus*). The physiography of the Mono Basin and adjoining valley regions of California and Nevada is rather complex, with mountains and ranges separating the sandy basins to differing degrees. Hence, kangaroo mouse populations here are distributed in a disjunct pattern that is coincident with the patchy distribution of the sandy basin habitat. Despite the vagarious distributional pattern, extensive field reconnaissance around the Mono Basin region did not reveal any geographic barrier or habitat hiatus existing between adjacent pairs of populations from the *nasutus* type locality in the north (Fletcher; locality 1) to the *polionotus* type locality in the south (Benton; locality 11).

Morphological variation.—Comparison of the geographic distance matrix with the taxonomic distance matrix shows that there is a significant linear association between the 2 matrices (raw Mantel statistic, $Z = 7{,}029.055$ and standardized Mantel coefficient, $r = 0.445$, $P = 0.006$ [with 10,000 random permutations] and $P = 0.001$ with the asymptotic approximation). Hence, spatial distance is correlated significantly with morphometric distance and we reject the hypothesis that the morphological patterns are random over geography.

Univariate examination for heterogeneity among the sample means (ANOVA) for the 11 populations from the Mono Basin region reveals significant differences ($P < 0.05$) for all characters except basal length and nasal length. The highest *F*-values (all with $P < 0.001$) are seen in pelage color value ($F = 7.812$); 2 cranial characters, greatest breadth of skull and greatest length of skull ($F = 5.007$ and 4.159, respectively); and 3 external characters, tail length, hind-foot length, and total length ($F = 101.069$, 4.917, and 4.022, respectively). In general, kangaroo mice from the northern populations are dark

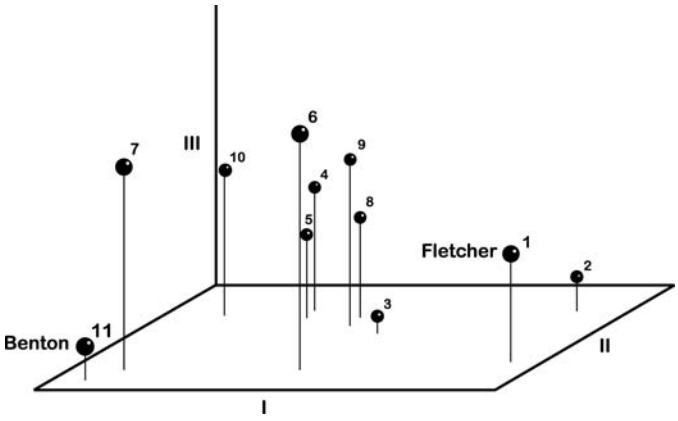

FIG. 2.—Projection of the 11 sample localities of *Microdipodops megacephalus* (shown in Fig. 1) onto the 1st 3 canonical variate axes. These 3 canonical axes account for 86.38% of the total morphological variation.

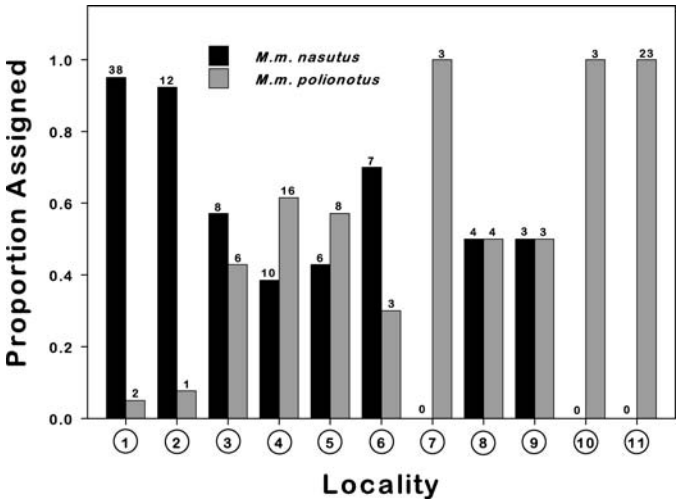

FIG. 3.—Frequency histograms (based on the posterior probability assignment from the discriminant function analysis) depicting the proportion of individuals of *Microdipodops megacephalus* from each sample that are classified as either *M. m. nasutus* or *M. m. polionotus*. Note the intergradation across the 11 transect populations from the northern (*nasutus*) to the southern (*polionotus*) morphology (geographic position of localities is shown in Fig. 1). Numbers above the bars represent number of individuals.

in pelage color (means for pelage color value for localites 1, 2, and 3 are 3.425, 3.077, and 3.821, respectively), whereas southern populations are light in color value (means for pelage color value for localities 9, 10, and 11 are 5.222, 5.250, and 4.733, respectively). The 4 morphometric variables, greatest breadth of skull, greatest length of skull, hind-foot length, and total length, reflect mainly differences in overall size of kangaroo mice and the magnitudes for these characters are large for several of the northern populations (e.g., means for greatest breadth of skull for localities 1 and 2 are 18.57 and 18.88 mm, respectively) and small for southern samples (e.g., means for greatest breadth of skull for localities 9 and 11 are 18.24 and 18.11 mm, respectively). Indeed, there seems to be a general clinal trend in size and color from the large and dark kangaroo mice from populations northeast of Mono Lake to the small and pale kangaroo mice from localities southeast of the lake.

Results of the discriminant function, canonical variate analysis (with each of the 11 populations from the peripheral isolate entered as a separate group) exhibit the polarization of localities 1 and 2 (extreme northern samples) from localities 10 and 11 (extreme southern groups) and locality 7 (central sample; Fig. 2). Except for locality 7 ($n = 3$; Appendix I), it appears that the 1st canonical axis achieves a north–south stratification of groups and accounts for 48.8% of total variance explained. The 5 characters most responsible for separation among the groups along the 1st canonical axis (standardized canonical coefficients in parentheses) are as follows: greatest length of skull (0.945), mandibular length (−0.776), pelage color value (0.450), maxillary breadth (−0.442), and total length (0.423). The kangaroo mice from northeast of the Mono Basin are dark colored and have generally large measurements for greatest length of skull and total length, but have small values for mandibular length and maxillary breadth. Comparison of the 3-dimensional canonical plot (Fig. 2) with the study-area map (Fig. 1) reveals that the midtransect samples are positioned largely in an intermediate fashion in the ordination analysis. Canonical variates II and III (expressing 22.2% and 15.4% of the total variation, respectively) seem to illustrate a minor degree of distinctness of the midtransect groups. Characters most responsible for explaining the separation along the 2nd axis include maxillary breadth (−0.882) and greatest breadth of skull (0.752), whereas tail length (−1.041) and total length (0.875) are weighted most heavily along the 3rd canonical axis.

The ordination analysis fails to partition the 11 populations into a northern group equivalent to *M. m. nasutus* (type locality: Fletcher) and a southern assemblage equivalent to *M. m. polionotus* (type locality: Benton). The question concerning the distinctness of the northern and southern nominal taxa is evaluated further in a 2-group discriminant function analysis (Fig. 3). In this analysis, the Fletcher and Benton populations (localities 1 and 11, respectively) are entered as known groups, whereas individuals comprising the other 9 populations are entered as unknowns into the discriminant analysis program. Assignment of individuals to either the *nasutus* or *polionotus* group is based on posterior probability values and Fig. 3 portrays, in histogram format, the posterior probability classification matrix for the 11 populations. Characters mainly responsible for separating the 2 groups are the same 5 as in the earlier discriminant function analysis. This analysis (Fig. 3) reveals that there is a character gradient over geography from the ''*nasutus*-like morphology'' to the ''*polionotus*-like morphology.'' Although Fletcher and Benton are significantly different in this multivariate analysis ($F = 14.86$, $P < 0.001$), the results of this analysis affirm those of the earlier analyses. That is, these statistical routines are not able to recognize unambiguously 2 distinct aggregations (equivalent to *nasutus* and *polionotus*) among the 11 populations. Moreover, nearly 20% of all individuals (10 of 63 specimens) from the type

localities Fletcher and Benton cannot be correctly classified at a posterior probability ≥ 0.95 and, indeed, 2 specimens are misclassified.

Environmental variation.—Indices of climatic severity and isophane for the 11 localities around the Mono Basin demonstrate that the more northern localities are climatologically harsh relative to the more southern localities. Values for climatic severity and isophane are at their maximal levels at locality 2 (6,255 adjusted meters and 59.85, respectively) and the indices generally taper off in magnitude from that region (e.g., locality 1 shows 5,953 adjusted meters and 57.40 for these 2 indices, respectively, and locality 11 has values of 5,648 adjusted meters and 54.74, respectively). Elevational information is used in the computation of both climatic severity and isophane, and it is the effect of elevation, doubtless, that explains the high overall association ($r = 0.999$, $P < 0.01$) between these 2 indices. The small and narrow basins to the north are surrounded by mountains and are almost 600 m higher than the more broad, southern basins.

Mean soil color value is darkest at the northern localities (i.e., localities 1, 2, and 3 show values of 5.83, 5.67, and 5.58, respectively), with the southern localities in particular being lighter in color (localities 10 and 11 both have values of 6.67). The darkness of the soils to the north is attributable largely to degradation products of low-density volcanic debris coupled with a high proportion of humus that necessarily accumulates in the small basins from the surrounding conifer-covered ridges. In contrast, the Mono Lake basin proper is a large enclosed basin holding the saline, remnant pluvial Lake Russell; localities 5–7 in the Mono Lake basin have color values of 6.50. Weathering of silicate rocks, the products of which become highly concentrated by evaporation, no doubt results in the lighter-colored soils in the Mono Lake basin and the other broad southern basins.

Mean percentage composition of sand (soil particles <2 mm) also varies across the Mono Basin transect region. Localities 4, 6, and 7 have the most sandy soils (99.54%, 99.54%, and 99.74%, respectively), whereas locality 1 has the lowest percentage sand (68.41%). The soil at the Fletcher site (locality 1) is characterized by the presence of a gravel overlay and is unlike the other localities, which have a high (>90%) proportion of sand.

Correspondence of morphological and ecological data.—Comparisons between the mean values of the 11 morphological characters and the 4 environmental variables reveal that 4 morphological characters are correlated significantly with 1 or more of the environmental (independent) variables. Hind-foot length is correlated highly with both climatic severity ($r = 0.868$, $P < 0.01$) and with isophane ($r = 0.867$, $P < 0.01$). In each case, these environmental characters statistically explain more than 75% of the total variation in hind-foot length (proportion of variance explained is obtained from the coefficient of determination [r^2]). Soil color value is significantly associated with pelage color value ($r = 0.786$, $P < 0.01$), and with greatest breadth of skull ($r = -0.658$, $P < 0.05$). Further, percentage sand is positively correlated with mandibular length ($r = 0.750$, $P = 0.01$).

The 4 morphological characters that are associated significantly with the environmental variables, namely hind-foot length, greatest breadth of skull, pelage color value, and mandibular length, also contribute to the interpopulational morphometric differentiation noted before. Specifically, hind-foot length, greatest breath of skull, and pelage color value are among those characters that show the greatest amount of heterogeneity among the sample means in the ANOVA comparisons. Mandibular length, pelage color value, and greatest breadth of skull are also characters that are important in distinguishing among the population samples in the discriminant function analyses.

Chromosomal analyses.—The kangaroo mice from both ends of the Mono Basin study area, Fletcher and Benton, have identical karyotypes. Specimens from near the type localities of both named subspecies possess a karyotype having 40 chromosomes and a fundamental number (FN) of 74. This karyotype, termed the *M. megacephalus* 40-α karyotype (Hafner 1981), is characterized by a pair of tiny acrocentric autosomes. No intrapopulational chromosomal variation was observed and these results agree with the chromosomal data of Hafner (1981) regarding populations of kangaroo mice in the Mono Basin region.

Comparative chromosomal information from other populations from the main northwestern and southeastern distributional units of the species (Fig. 1) may provide inferences regarding the ancestry of the Mono Basin peripheral isolate. Examination of chromosomal preparations reveals the presence of the 40-α karyotype at Vernon, whereas animals from the Chilcoot, Sparks, and Panaca populations possess the 40-β karyotype (the 40-β karyotype has 40 chromosomes, with all biarmed autosomes [FN = 76]); these results agree with the findings of Hafner (1981). New chromosomal information from 3 populations along the western margin of the southeastern unit of the species, Austin, San Antonio, and Goldfield, reveals that these populations show the 40-α karyotype. Because the Mono Basin populations share the same 40-α karyotype with kangaroo mice from 1 population (i.e., Vernon) from the northwestern unit and several populations (i.e., Austin, San Antonio, and Goldfield) from the southeastern unit, the chromosomal data are equivocal in identifying an ancestral source area for this peripheral isolate.

Mitochondrial DNA analyses.—The combined 16S and *Cytb* data set (1,018 bp) shows a total of 205 variable characters (83 and 122 variable characters 16S and *Cytb*, respectively) of which 193 characters are parsimony informative (76 and 117 parsimony-informative characters for 16S and *Cytb*, respectively). For the 10 samples (23 individuals) of *Microdipodops* studied, the mean base frequencies for A, C, G, and T are 0.330, 0.210, 0.192, and 0.268, respectively, for 16S, and 0.296, 0.282, 0.136, and 0.286, respectively, for *Cytb*. Chi-square tests for possible heterogeneity of base frequencies across the samples of kangaroo mice are not significant for each gene ($\chi^2 = 2.349$, $P = 1.000$ for 16S; $\chi^2 = 2.857$, $P = 1.000$ for *Cytb*) nor for codon position for *Cytb* ($\chi^2 = 0.508$, $P = 1.000$, $\chi^2 = 0.159$, $P = 1.000$, and $\chi^2 = 12.394$, $P = 1.000$ for the 1st, 2nd, and 3rd positions, respectively; base-frequency

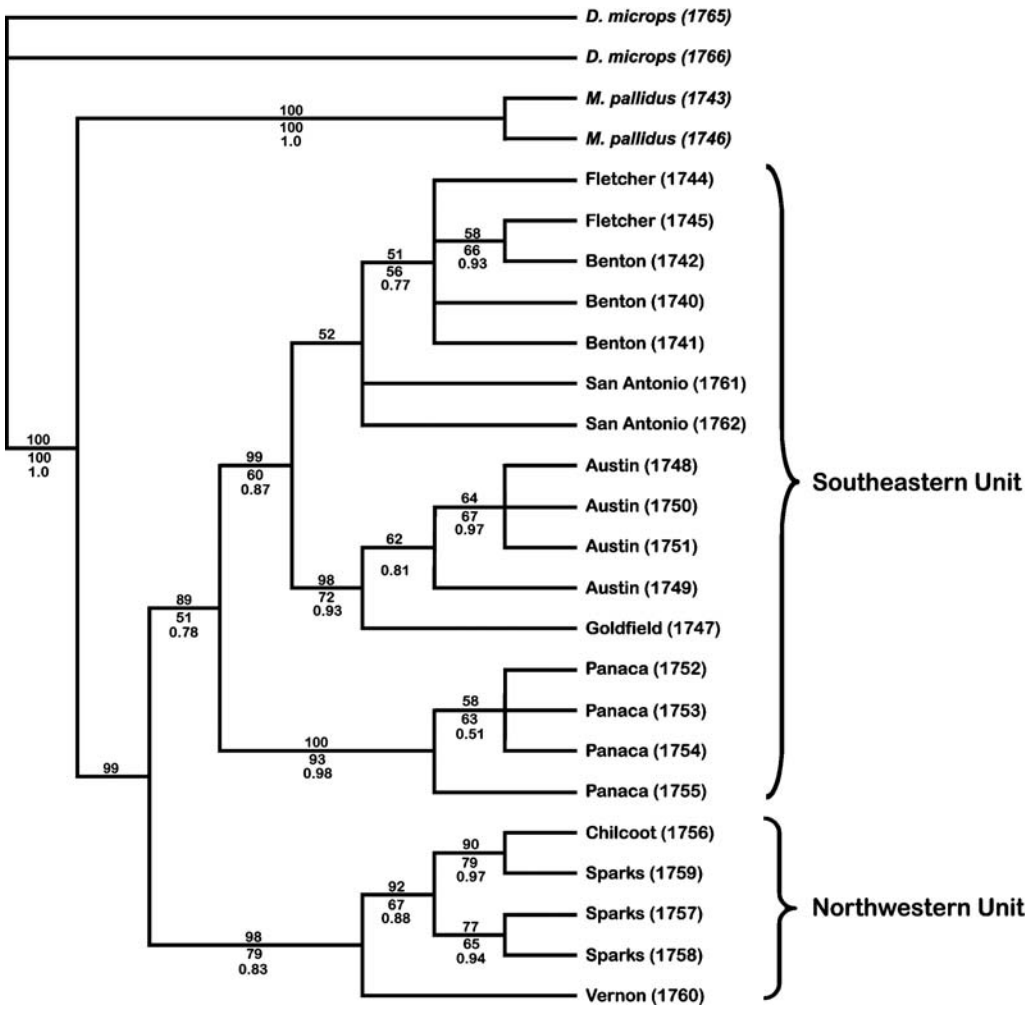

FIG. 4.—Maximum-parsimony tree based upon combined 16S and cytochrome-*b* DNA sequence data (CI = 0.816, RI = 0.918) for 9 ingroup populations of *Microdipodops megacephalus* and 2 outgroup taxa (*Dipodomys microps* and *Microdipodops pallidus*). The northwestern and southeastern cladistic units of *M. megacephalus* are described in text and population locations are shown in Fig. 1 (numbers in parentheses are MLZ specimen numbers [Appendix II]). Parsimony bootstrap support values are indicated above the nodes, with maximum-likelihood support values and Bayesian posterior probabilities below the nodes.

data are available on request). Thus, base composition is unlikely to cause phylogenetic bias. Transition : transversion ratios for 16S, *Cytb*, and the combined data set are 1.443, 7.600, and 3.938, respectively (over all positions, using uncorrected *p,* and without *D. microps*). Following the methods of Barker and Lanyon (2000), a plot of uncorrected-*p* distance versus transitions shows no evidence for saturation for 16S nor for *Cytb* for the 10 populations of kangaroo mice studied. However, 3rd-position transitions for *Cytb* show saturation when *D. microps* is included in the analysis. Tests for phylogenetic signal (nonrandom structure) in our data sets show significance for both genes studied (for 16S [83 characters], $g_1 = -1.344$, $P < 0.01$ and for *Cytb* [122 characters], $g_1 = -1.073$, $P < 0.01$).

Among the 21 individuals representing 9 ingroup populations of *M. megacephalus*, 95 polymorphic sites and 15 unique composite haplotypes are identified. For individual genes, there are 29 and 66 polymorphic sites and 8 and 14 unique haplotypes for 16S and *Cytb*, respectively. Interestingly, all individuals examined from the Fletcher and Benton populations from the Mono Basin region share the identical 16S haplotype and this same haplotype is represented in 1 individual from the San Antonio population to the east. For the *Cytb* gene, all 5 individuals examined from the Mono Basin region have unique haplotypes but all individuals share a base substitution (a synapomorphy) not seen in other populations of the species.

Analyses of our combined 16S and *Cytb* sequence data using maximum-parsimony, distance (neighbor-joining), maximum-likelihood, and Bayesian approaches show the same tree topology. The inclusion or exclusion of *D. microps* as a more-distant outgroup in the analyses did not alter tree structure within *M. megacephalus*. Similarly, inclusion or exclusion of *M. pallidus* in the defined outgroup did not affect tree structure of the ingroup. Maximum-parsimony analysis yields a single most-parsimonious tree (Fig. 4) of the combined sequence data (CI = 0.816, RI = 0.918). Two basal clades are recognizable within *M. megacephalus* and individuals from the populations

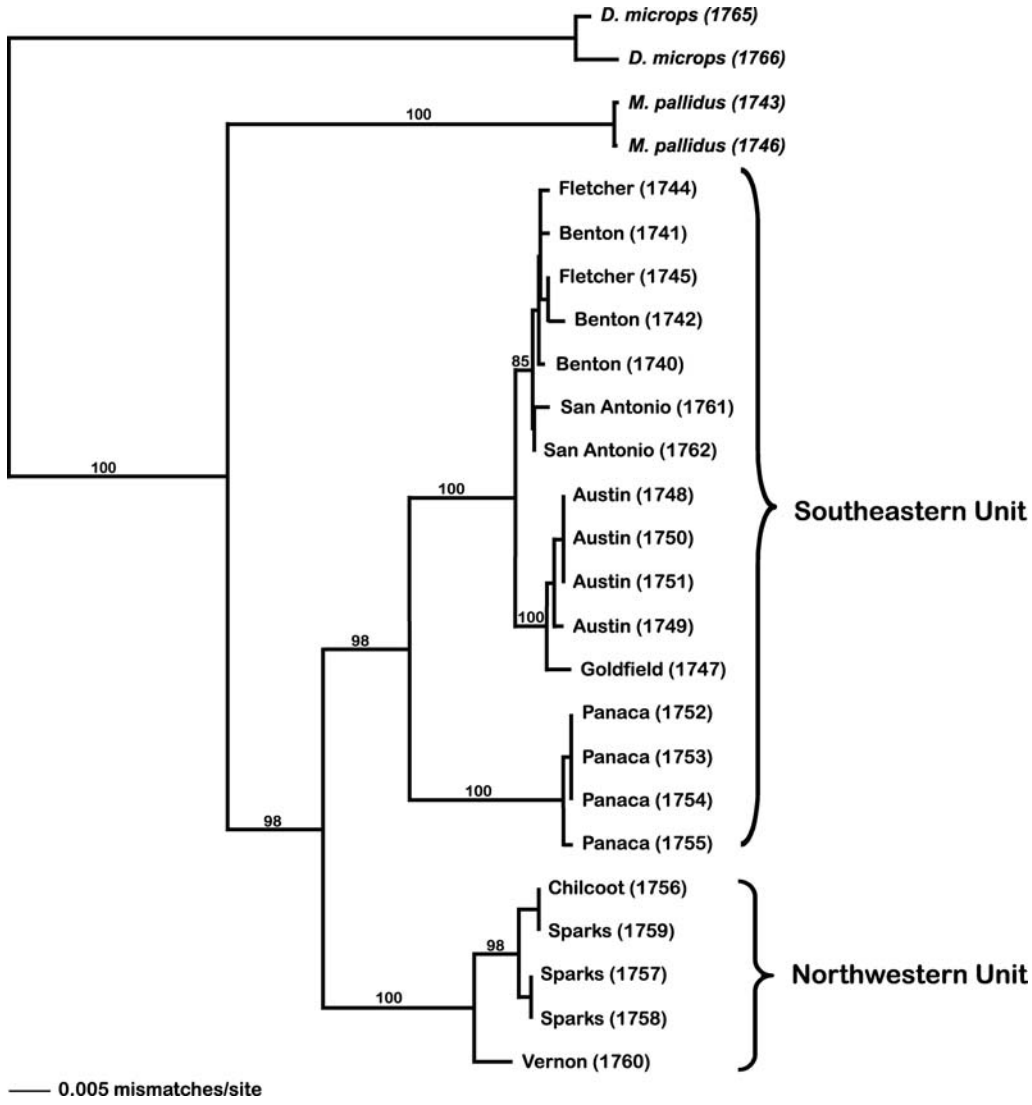

Fig. 5.—Distance (neighbor-joining) tree showing relationships among 21 individuals from 9 localities of *Microdipodops megacephalus* based upon combined 16S and cytochrome-*b* DNA sequences. Numbers at the nodes are bootstrap support values based on 1,000 replicates.

group on the basis of geography: a northwestern cladistic unit and a southeastern cladistic unit. Individuals from Fletcher and Benton, the 2 populations from the Mono Basin peripheral isolate, belong to the southeastern clade and cluster with individuals from San Antonio.

The neighbor-joining (distance) tree (Fig. 5) illustrates sequence divergence among the samples. Comparison of kangaroo mouse samples from the Mono Basin with samples from the southeastern geographic unit of the species shows a mean of 1.86% sequence divergence (range = 0.197–3.848%), whereas a comparison of the Mono Basin samples with samples from the northwestern geographic unit shows a mean of 5.09% sequence divergence (range = 4.736–5.230%) based on uncorrected-*p* distance. Mean sequence divergence between the northwestern and southeastern cladistic units of *M. megacephalus* and between all populations of *M. megacephalus* and *M. pallidus* is 5.45% (range = 4.736–6.111%) and 8.66% (range = 8.287–8.992%), respectively.

Discussion

Morphology and ecology.—Kangaroo mice from this distributional islet have been a rather problematic assemblage for systematists (Grinnell 1914; Hafner 1981; Hall 1941, 1946). Although once thought to represent a distinct species because of their pale pelage coloration (Grinnell 1914), the kangaroo mice from the Mono region are now recognizable as *M. megacephalus* by a variety of morphological features (Hafner 1981; Hall 1941). Hafner (1981) showed that the kangaroo mice from Mono Basin populations of Fletcher and Benton are large in maxillary breadth, and small in basal length, bullar length, and mandibular length compared to other populations of the species.

Results of this study show that the kangaroo mice from the Mono region are variable in pelage color. This variation in pelage color also was noted by Hall (1941) in his descriptions of the subspecies. In comparing *polionotus* with *nasutus*,

Hall (1941:252) noted that ''Most, but not all, specimens are lighter-colored on the upper parts than *nasutus*.'' Color variation among the Mono kangaroo mice also is reflected on their undersides. Although not quantified in this study, the lighter-colored individuals also have white underparts, with hairs pure white to the base; animals with darker-colored dorsal pelage typically have white underparts but the hairs are very light plumbeous basally. Although not all Mono kangaroo mice have belly hairs that are white to the base, it is interesting to note that the combination of having pure-white belly hairs and the 40-α karyotype seen in the Mono animals is unique among populations of *M. megacephalus* examined (see Hafner 1981). All other populations of the species that have pure-white belly hairs also have the 40-β karyotype (e.g., populations from Chilcoot, Sparks, and Panaca), and all other populations that have the underparts white but gray basally also show the 40-α karyotype (e.g., San Antonio, Austin, Goldfield, and Vernon; see also Hafner 1981).

Analysis of morphometric variation of the 11 populations of kangaroo mice from the Mono peripheral isolate shows a pattern of clinal variation, with kangaroo mice being generally larger in body size and darker in dorsal pelage color in the northern populations and smaller and more pale in the southern populations. Given the observed correlations between the morphological characters and environmental characters (e.g., soil color and pelage color; general size characters such as hindfoot length and climatic severity), it seems likely that natural selection played a predominant role in the formation and maintenance of these morphological character gradients. Although such pronounced geographic variation in a small peripheral isolate appears unusual, it must be remembered that there is about a 600-m elevational gradient coupled with marked changes in soil color across the region. In retrospect, it is understandable that earlier workers, being constrained by the limited specimens available to them, judged the kangaroo mice from the northern and southern ends of the cline as representing distinct taxa.

Phylogeographic considerations.—Genetic data of 2 kinds (mtDNA sequences and chromosomes) from specimens near the type localites of *M. m. nasutus* and *M. m. polionotus* (Fletcher and Benton, respectively) indicate near genetic identity for kangaroo mice in the Mono Basin region. These results are in agreement with chromosomal and protein electrophoretic analyses of Hafner (1981) that reported a high level of genetic similarity ($S = 0.94$) between populations of kangaroo mice from the northern and southern portions of this peripheral isolate. A similarity value of this magnitude is well within the bounds of intrapopulational variation for the genus (Hafner 1981).

Although having identical 16S sequences, all specimens examined from Fletcher and Benton show unique *Cytb* haplotypes. Examination of *Cytb* sequence data shows no fixed differences between the Fletcher and Benton populations and variation among the haplotypes is due to 2 polymorphic sites for Fletcher and 5 polymorphisms for the Benton population; combining these 2 populations, there is a total of 6 *Cytb* polymorphic sites for kangaroo mice from the Mono Basin region. Although our sample sizes are rather small and our geographic survey of populations is limited, the level of *Cytb* variation at Benton (5 variable sites) exceeds that of other populations of *M. megacephalus* (other populations show 0–3 polymorphisms; $\bar{X} = 1.00$ variable bases for the other 8 populations of the species).

The mtDNA results presented here show that the kangaroo mice from the Mono peripheral isolate belong to the southeastern clade of *M. megacephalus* and share a close genetic affinity with the populations immediately to the east, especially San Antonio. This finding is consistent with the chromosomal data, in that kangaroo mice from the Mono isolate share the 40-α karyotype with other populations on the western margin of the southeastern geographic unit of the species (namely, San Antonio, Austin, and Goldfield). These data are also consistent with the protein electrophoretic data presented by Hafner (1981) that showed that kangaroo mice from the Mono region have a higher overall genetic similarity value with populations of *M. m. megacephalus* to the east ($S = 0.89$) rather than with populations of *M. m. californicus* to the north ($S = 0.70$).

Historical biogeography.—The mitochondrial sequence data allow us to identify San Antonio as the probable source population for the Mono kangaroo mice. Not only does the San Antonio population share close ancestry with the Mono peripheral isolate, but its geographical position seems ideal for a reconstruction of past events. Although we are uncertain of the reliability of a molecular clock for mitochondrial genes for kangaroo mice, an average sequence divergence of 0.515% for *Cytb* between the Mono samples and the San Antonio samples suggests a divergence time in the late Pleistocene (assuming mtDNA divergence occurs at mean rate of approximately 2–4% per million years for mammals and other vertebrates—Arbogast et al. 2002; Brown et al. 1979, 1982; Ferris et al. 1983; Shields and Wilson 1987).

The Mono peripheral isolate of *M. megacephalus* lies within the geologically complex region known as the Walker Fault Zone (also termed the Walker Lane, or Walker Belt—Fiero 1986; Grayson 1993; Morrison 1991). To the east of Mono Lake, there is a region of northeast-trending hills (including the Anchorite Hills, Excelsior Mountains, and the Adobe Hills; Fig. 6) and, beyond that, the Lahontan Trough (Reveal 1979) that separates the kangaroo mice of the Mono peripheral isolate from other populations of the species farther to the east. The hills and basins immediately east of Mono Lake are aligned perpendicular to the tall (>3,000-m), northwest-trending mountains of the Wassuk Range and the White Mountains and represent a 45-km region of geologic and topographic discontinuity between these 2 great ranges (Fig. 6).

The Lahontan Trough is a low-elevational region that extends from the Columbia Plateau to the Mojave Desert on the western flank of the floristic Great Basin (see Epps et al. 1998; Grayson 1993; Reveal 1979). Reveal (1979) emphasized the importance of the Lahontan Trough as a corridor for the northward and elevationally upward expansion of plants and animals into the Great Basin since the Pleistocene. Grayson (1993) suggested that the trough served as a low-elevational

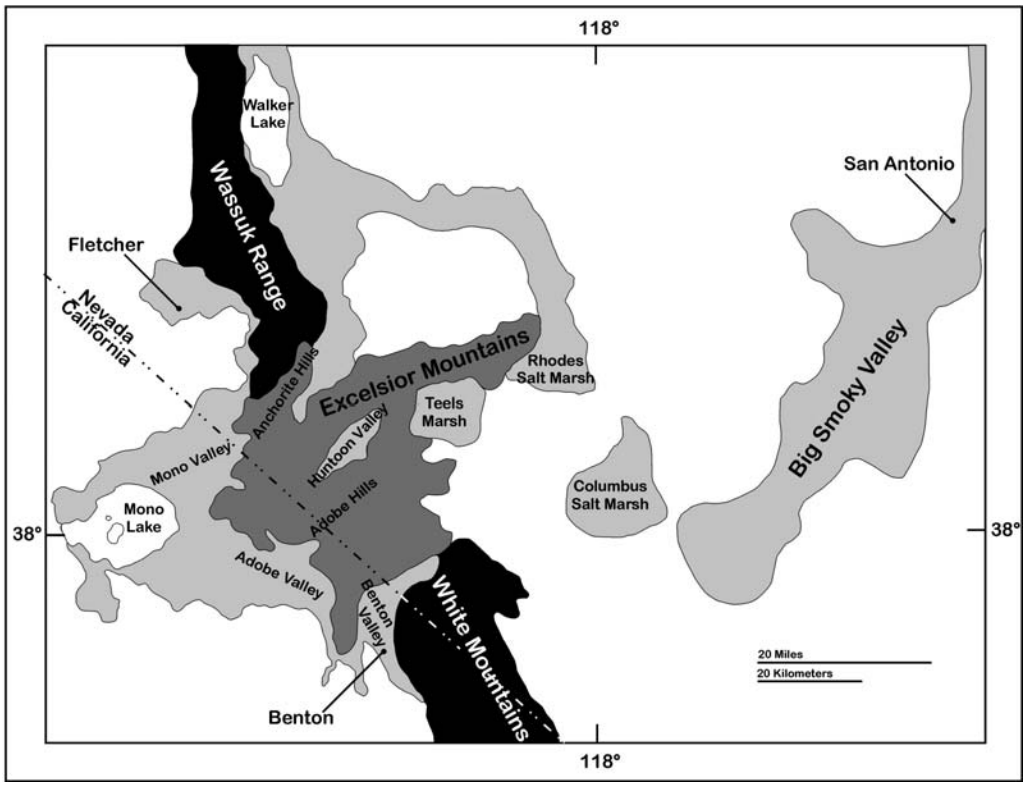

FIG. 6.—Geomorphic map of the central region of the Lahontan Trough showing physiographic features discussed in text. Kangaroo mice (*Microdipodops megacephalus*) may have gained entry into the Mono Basin region through the topographic discontinuity between the Wassuk Range and the White Mountains by traversing the string of nearly continuous east–west basins (including Big Smoky Valley, Columbus Salt Marsh, Rhodes Salt Marsh, Teels Marsh, and Huntoon Valley). Sandy basin habitats are shown in light gray, forested mountain ranges are shown in black, and the hilly region east of the Mono Basin is shown in medium gray.

barrier that reduced the rate at which Sierran plants were able to colonize the mountainous regions of the Great Basin.

For *M. megacephalus* and many other plants and animals in the western Great Basin today, the Lahontan Trough probably represents a low-elevational (approximately 1,350- to 1,700-m) barrier to disperal. In the region between the Mono distributional isolate and the San Antonio population, the Lahontan Trough is represented by a series of basins including (from west to east) Huntoon Valley, Teels Marsh, Rhodes Salt Marsh, Columbus Salt Marsh, and Big Smokey Valley (Fig. 6). These sandy, low-lying basins support floral communities that are unsuitable for *M. megacephalus*, being dominated by greasewood (*Sarcobatus*) and saltbush (*Atriplex*). Although the habitats are inappropriate for *M. megacephalus*, each of these basins today supports populations of its congener, *M. pallidus*.

It is well documented that the late-Pleistocene and Holocene times were dominated by large-scale climatic fluctuations in this region (e.g., Benson 1991; Benson et al. 1990; Fiero 1986; Grayson 1993; Mifflin and Wheat 1979; Morrison 1991; Reveal 1979). During the last lacustral of the Pleistocene, for example, plant communities of the Great Basin were depressed 500–600 m below their present levels (Mifflin and Wheat 1979; Reveal 1979). Rather than to postulate that *M. megacephalus* gained access to the Mono region by jump dispersal across barriers such as mountain ranges and the Lahontan Trough (or for that matter, river barriers such as the Carson, Walker, Truckee, and Humboldt rivers to the north and northeast of Mono Lake), it is more parsimonious to hypothesize that kangaroo mice gained access to the Mono region by adjusting their distributions in concert with changing climates and vegetational zones during the late-Pleistocene and Holocene times.

Following a vicariance biogeographical interpretation, we hypothesize that populations of *M. megacephalus* from near San Antonio (known today from 1,707 to 2,042 m) adjusted their distribution elevationally downward and followed the Big Smokey Valley southwestward into the Lahontan Trough (about 1,350–1,500 m) during cooling trends of the late Pleistocene and early Holocene such as the Eetza and Sehoo lacustrals of Lake Lahontan (350,000–130,000 and 35,000–8,000 years ago, respectively—Morrison 1991). Pollen data from the Carson Sink (located in the Lahontan Trough about 100 km to the northwest) suggest that late-Pleistocene vegetation of the Lahontan Trough was likely a sagebrush-dominated community (Grayson 1993; Wigand and Mehringer 1985). Simultaneous with the arrival of the sagebrush steppe, populations of *M. pallidus* likely vacated the Lahontan Trough, withdrew southward and elevationally downward, and were replaced by populations of *M. megacephalus* (*M. megacephalus* and *M. pallidus* are rarely found in sympatry—Hafner et al. 1979, 1996). Eventual colonizations of the Mono region may

have occurred during the warmer and drier times such as the Wyemaha–Churchill interlacustral (130,000–35,000 years ago), the nonlacustral period of the middle–middle Holocene (7,000–5,000 years ago—Morrison 1991), or both as populations of *M. megacephalus* adjusted their distributions elevationally upward and westward. Indeed, Huntoon Valley (1,737 m) likely provided a nice stepping stone from the lower basins of the Lahontan Trough, upward and westward through the low hills guarding the Mono Basin region (i.e., Mono Valley and Adobe Valley; Fig. 6). Huntoon Valley is located just 15 km east of Mono and Adobe valleys and today harbors *M. pallidus* near this species' recorded elevational extreme (Hall 1941).

The high level of nucleotide polymorphism noted at the Benton population may be explained by its location within the distributional isolate and the history of the region. Of all sandy basins throughout the Mono region, the population from Benton occurs at the lowest elevation (1,600 m) in Benton Valley and at the extreme southern end of the distributional isolate. Given its lower elevation and latitude, it may be hypothesized that the regions around Benton (and areas farther south at the head of Owens Valley) may have represented a refugium for the Mono Basin kangaroo mice during the climatic fluctuations of the late-Pleistocene and early-Holocene times; over time, it is likely that different haplotypes would accumulate in this area. Although this explanation is based on limited data, it is consistent with emerging patterns of molecular variation for the genus that show higher levels of haplotype variation and cladogenesis in the extreme southern portions of the ranges of both species of kangaroo mice (E. Reddington and J. C. Hafner, in litt.).

The historical biogeographical hypotheses proposed here are based on a single species but they stand, nevertheless, as a model for other basin-dwelling plants and animals of the western Great Basin, particularly the Mono Basin region. Although Reveal (1979) describes the Lahontan Trough as an important corridor for northern floral and faunal shifts after the Pleistocene, biologists should not overlook its role as a route for possible east-to-west (and west-to-east) distributional adjustments of plants and animals as they responded to climatic changes since the late Pleistocene. The Lahontan Trough, together with the low, physiographic discontinuity between the Wassuk Range and the White Mountains, seems to represent a previously unrecognized biogeographical avenue between the extreme western margin of the Great Basin (i.e., the Mono Basin region and possibly the Owens Valley) and the interior of the Great Basin. Comparative phylogeographic studies such as those employed by Riddle et al. (2000) and advocated by Arbogast and Kenagy (2001) may reveal a more general role of the Lahontan Trough as an east–west biogeographical route for basin-dwelling organisms.

Taxonomic conclusions.—Field collecting and reconnaissance shows that this Mono distributional isolate represents a concatenated array of populations; across the region, kangaroo mice are found in interconnected basins that are free of barriers to dispersal. In view of the intergradation seen in the morphological characters across the populations, the absence of a step cline, and a paucity of genetic differentiation, it is best to consider the kangaroo mice of the Mono Basin and adjoining valley regions of California and Nevada as representing but a single subspecies. This single subspecies, as here recognized, is a demonstrable phylogeographical subunit of the species. In addition to being a peripheral isolate of the species, kangaroo mice from the Mono region possess a *Cytb* nucleotide substitution unique to the species (this substitution not seen in *M. megacephalus* outside the Mono region although we have examined more than 100 *Cytb* sequences). Morphologically, the Mono kangaroo mice show marked clinal variation but the presence of pure-white belly fur (seen in many, but not all, individuals) combined with the 40-α karyotype is unique among populations of this species (Hafner 1981). This taxonomic conclusion, then, follows our systematic philosophy of not naming purely phenetic units (that are often distributed in chaotic patterns reflecting various environmental regimes), but recognizing independently evolving subdivisions of the species (Hafner 1981). In accordance with the rules of priority, these kangaroo mice are referable to *M. m. polionotus* with *nasutus* as a synonym. A synonymy for Mono Basin kangaroo mice follows.

Microdipodops Merriam, 1891

Microdipodops Merriam, 1891:115. Type species *Microdipodops megacephalus* Merriam.

The genus includes 2 species, *M. megacephalus* and *M. pallidus* (Hafner 1981; Williams et al. 1993).

Microdipodops megacephalus Merriam, 1891
Dark Kangaroo Mouse

Microdipodops megacephalus Merriam, 1891:116.

Before this study, *M. megacephalus* included 13 subspecies: *albiventer*, *ambiguus*, *atrirelictus*, *californicus*, *leucotis*, *medius*, *megacephalus*, *nasutus*, *nexus*, *oregonus*, *paululus*, *polionotus*, and *sabulonis* (Williams et al. 1993). This study reduces the number of subspecies to 12.

Microdipodops megacephalus polionotus (Grinnell, 1914)

Microdipodops polionotus Grinnell, 1914:302. Type locality "McKeever's Ranch, two miles south of Benton Station, 5,200 feet, Mono County, California." Type specimen adult male, skin and skull, Museum of Vertebrate Zoology number 17031, collected 10 July 1912 by C. D. Holliger, original number 184. Regarded as a subspecies of *M. megacephalus* by Hall (1941:251).

Microdipodops megacephalus polionotus Hall, 1941:251. First use of current name combination.

Microdipodops megacephalus nasutus Hall, 1941:251. Type locality "Fletcher, 6,098 feet, Mineral County, Nevada."

Geographic range.—Mono Basin and adjoining valley regions of California and Nevada. Occurs from the vicinity of Fletcher, Nevada, in the north, to the extreme head of Owens Valley (vicinity of Benton) in the south (Fig. 1).

Description.—Kangaroo mice of the subspecies *polionotus* have the 40-α karyotype (including 1 pair of tiny acrocentric autosomes). Dorsal pelage color is grayish and variable in brightness (color value); underparts white, but hairs of the venter vary in being pure white to base in many individuals to very light plumbeous basally in other individuals. In cranial measurements, these animals are large in maxillary breadth, and small in basal length, bullar length, and mandibular length compared to other members of the species.

As a peripheral isolate, *polionotus* is separated from other known taxa of this species by more than 100 km. The geographic distribution of subspecies of *M. megacephalus* nearest *polionotus* includes *californicus* and *medius* to the north and *megacephalus* and *sabulonis* to the east (Hafner 1981; Hall 1941). In comparisons with these subspecies, *polionotus* differs from *megacephalus* and *sabulonis* in having the basal portion of the belly hairs pure white or very faint gray, instead of distinct plumbeous bases to the ventral hairs. The subspecies *polionotus* differs from *californicus* in possessing the 40-α karyotype, whereas *californicus* has the 40-β karyotype (totally biarmed autosomes). Further, *polionotus* is distinguished from *californicus* and *medius* in having grayish rather than buffy or more reddish dorsal pelage. This Mono Basin taxon is unique among all *M. megacephalus* subspecies in possessing the following combination of characters: grayish dorsal pelage, ventral pelage that is pure white or nearly pure white basally, and the 40-α karyotype.

Acknowledgments

Appreciation is extended to J. L. Patton for encouragement to JCH in the early stages of this project. Fieldwork was accomplished with the assistance of D. J. Hafner, M. S. Hafner, P. M. Hafner, G. B. Lotz, and W. L. McNeil. D. J. Hafner provided assistance in scoring of specimens for the color analysis. S. Nemzer and N. S. Upham assisted in the preparation of the figures and J. P. C. Franck provided guidance during the initial stages of our molecular analyses. We thank N. S. Upham for assistance with library research on Great Basin pluvial history and D. J. Pondella II, J. A. Williams, and P. Lieb for helpful conversations. This research was supported in part by the Nevada Department of Wildlife (contract 05-21 to JCH) and by a grant (DEB-7822038, Doctoral Dissertation Research in Systematics) from the National Science Foundation (to J. L. Patton for JCH). Finally, we acknowledge the following 2 institutions and their curators for support during specimen examination: Museum of Vertebrate Zoology, University of California, Berkeley, and The Museum, Texas Tech University, Lubbock. We also are grateful to D. J. Hafner and M. S. Hafner for their comments on this paper.

Literature Cited

Alexander, L. F., and B. R. Riddle. 2005. Phylogenetics of the New World rodent family Heteromyidae. Journal of Mammalogy 86: 366–379.

Animal Care and Use Committee. 1998. Guidelines for the capture, handling, and care of mammals as approved by the American Society of Mammalogists. Journal of Mammalogy 79:1416–1431.

Arbogast, B., et al. 2002. Estimating divergence times from molecular data on phylogenetic and population genetic timescales. Annual Review of Ecology and Systematics 33:707–740.

Arbogast, B., and G. J. Kenagy. 2001. Comparative phylogeography as an intergrative approach to historical biogeography. Journal of Biogeography 28:819–825.

Bagnold, R. A. 1941. The physics of blown sand and desert dunes. Chapman & Hall, London, United Kingdom.

Barker, F. K., and S. M. Lanyon. 2000. The impact of parsimony weighting schemes on inferred relationships among toucans and neotropical barbets (Aves: Picaformes). Molecular Phylogenetics and Evolution 15:215–234.

Benson, L. V. 1991. Timing of the last highstand of Lake Lahontan. Journal of Paleolimnology 5:115–126.

Benson, L. V., et al. 1990. Chronology of expansion and contraction of four Great Basin lake systems during the past 35,000 years. Palaeogeography, Palaeoclimatology, Palaeoecology 78: 241–286.

Brown, J. H. 1973. Species diversity of seed-eating desert rodents in sand dune habitats. Ecology 54:775–787.

Brown, W. M., W. George, Jr., and A. C. Wilson. 1979. Rapid evolution of animal mitochondrial DNA. Proceedings of the National Academy of Sciences 76:1967–1971.

Brown, W. M., E. M. Prager, A. Wang, and A. C. Wilson. 1982. Mitochondrial DNA sequences of primates: tempo and mode of evolution. Journal of Molecular Evolution 18:225–239.

DeBlase, A. F., and R. E. Martin. 1974. A manual of mammalogy with keys to families of the world. Wm. C. Brown Company, Publishers, Dubuque, Iowa.

Dixon, W. J. (ed.). 1983. BMDP statistical software. University of California Press, Berkeley.

Eissmann, L. J. 1990. Eolian sand transport in western Nevada: applications of Landsat TM imagery. M.S. thesis, University of Nevada, Reno, Reno.

Epps, T. M., H. B. Britten, and R. W. Rust. 1998. Historical biogeography of *Eusattus muricatus* (Coleoptera: Tenebrionidae) within the Great Basin, western North America. Journal of Biogeography 25:957–968.

Ferris, S. D., R. D. Sage, E. M. Prager, U. Ritte, and A. C. Wilson. 1983. Mitochondrial DNA evolution in mice. Genetics 105: 681–721.

Fiero, B. 1986. Geology of the Great Basin. University of Nevada Press, Reno.

Findley, J. S., and G. L. Traut. 1970. Geographic variation in *Pipistrellus hesperus*. Journal of Mammalogy 51:741–765.

Grayson, D. K. 1993. The desert's past: a natural prehistory of the Great Basin. Smithsonian Institution Press, Washington, D.C.

Grinnell, J. 1914. A second species of the mammalian genus *Microdipodops* from California. University of California Publications in Zoology 12:301–304.

Gu, X., Y.-X. Fu, and W.-H. Li. 1995. Maximum likelihood estimation of the heterogeneity of substitution rate among nucleotide sites. Molecular Biology and Evolution 12:546–557.

Hafner, D. J., J. C. Hafner, and M. S. Hafner. 1979. Systematic status of kangaroo mice, genus *Microdipodops*: morphometric, chromosomal, and protein analyses. Journal of Mammalogy 60: 1–10.

Hafner, J. C. 1976. Specific and evolutionary relationships of kangaroo mice, genus *Microdipodops*. M.S. thesis, Texas Tech University, Lubbock.

HAFNER, J. C. 1981. Evolution, systematics, and historical biogeography of kangaroo mice, genus *Microdipodops*. Ph.D. dissertation, University of California, Berkeley.

HAFNER, J. C. 1993. Macroevolutionary diversification in heteromyid rodents: heterochrony and adaptation in phylogeny. Pp. 291–318 in Biology of the Heteromyidae (H. H. Genoways and J. H. Brown, eds.). Special Publication 10, American Society of Mammalogists.

HAFNER, J. C., D. J. HAFNER, AND M. S. HAFNER. 1996. Habitat selection and coexistence of species of kangaroo mice (*Microdipodops*). Pp. 249–259 in Contributions in mammalogy: a memorial volume honoring Dr. J. Knox Jones, Jr. (H. H. Genoways and R. J. Baker, eds.). Museum of Texas Tech University, Lubbock.

HAFNER, J. C., AND M. S. HAFNER. 1983. Evolutionary relationships of heteromyid rodents. Great Basin Naturalist Memoirs 7:3–29.

HAFNER, J. C., AND D. R. SANDQUIST. 1989. Postmortem field preparation of bird and mammal chromosomes: an evaluation involving the pocket gopher, *Thomomys bottae*. Southwestern Naturalist 34:330–337.

HAFNER, M. S. 1982. A biochemical investigation of geomyoid systematics (Mammalia: Rodentia). Zeitschrift für Zoologische Systematik und Evolutionsforschung 20:118–130.

HALL, E. R. 1941. Revision of the rodent genus *Microdipodops*. Field Museum of Natural History, Zoology Series 27:233–277.

HALL, E. R. 1946. Mammals of Nevada. University of California Press, Berkeley.

HILLIS, D. M., AND J. P. HUELSENBECK. 1992. Signal, noise, and reliability in molecular phylogenetic analyses. Journal of Heredity 83:189–195.

HILLIS, D. M., C. MORITZ, AND B. K. MABLE. 1996. Molecular systematics. 2nd ed. Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts.

HOPKINS, A. D. 1938. Bioclimatics. A science of life and climate relations. United States Department of Agriculture, Miscellaneous Publication 280:1–188.

HUELSENBECK, J. P., AND F. R. RONQUIST. 2001. MrBayes: Bayesian inference for phylogeny. Biometrics 17:754–756.

KUMAR, S., K. TAMURA, AND M. NEI. 2000. MEGA: molecular evolutionary genetics analysis, version 2.01. Pennsylvania State University, University Park.

LEACHÉ, A. D., AND T. W. REEDER. 2002. Molecular systematics of the eastern fence lizard (*Sceloporus undulatus*): a comparison of parsimony, likelihood, and Bayesian approaches. Systematic Biology 51:44–68.

LOVEJOY, N. R. 2000. Reinterpreting recapitulation: systematics of needlefishes and their allies (Teleostei: Beloniformes). Evolution 54:1349–1362.

MADDISON, D. R., AND W. P. MADDISON. 2000. MacClade 4: analysis of phylogeny and character evolution. Version 4.0. Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts.

MANTEL, N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Research 27:209–220.

MANTOOTH, S. J., C. JONES, AND R. D. BRADLEY. 2000. Molecular systematics of *Dipodomys elator* (Rodentia: Heteromyidae) and its phylogenetic implications. Journal of Mammalogy 81: 885–894.

MERRIAM, C. H. 1891. Description of a new genus and species of dwarf kangaroo rat from Nevada (*Microdipodops megacephalus*). North American Fauna 5:115–117.

MIFFLIN, M. D., AND M. M. WHEAT. 1979. Pluvial lakes and estimated pluvial climates of Nevada. Nevada Bureau of Mines and Geology, Bulletin 94:1–157.

MILLER, R. S. 1958. The Munsell system of color notation. Journal of Mammalogy 39:278–286.

MORRISON, R. B. 1964. Lake Lahontan: geology of southern Carson Desert, Nevada. United States Geological Survey Professional Paper 401. United States Government Printing Office, Washington, D.C.

MORRISON, R. B. 1991. Quaternary stratigraphic, hydrologic, and climatic history of the Great Basin, with emphasis on Lake Lahontan, Bonneville, and Tecopa. Pp. 283–320 in The geology of North America. Vol. K-2. Quaternary nonglacial geology: conterminous U.S. (R. B. Morrison, ed.). Geological Society of America, Boulder, Colorado.

MUNSELL COLOR CO., INC. 1954. Munsell soil color charts. 3rd ed. Munsell Color Co., Inc., Baltimore, Maryland.

NIE, N. H., C. H. HULL, J. G. JENKINS, K. STEINBRENNER, AND D. H. BENT. 1975. Statistical package for the social sciences. McGraw-Hill Book Co., New York.

POSADA, D., AND K. A. CRANDALL. 1998. ModelTest: testing the model of DNA substitution. Bioinformatics 14:817–818.

POWER, D. M. 1970. Geographic variation of red-winged blackbirds in central North America. University of Kansas Publications, Museum of Natural History 19:1–83.

REVEAL, J. L. 1979. Biogeography of the intermountain region: a speculative appraisal. Mentzelia 4:1–92.

RIDDLE, B. R., D. J. HAFNER, L. F. ALEXANDER, AND J. R. JAEGER. 2000. Cryptic vicariance in the historical assembly of a Baja California Peninsular Desert biota. Proceedings of the National Academy of Sciences 97:14438–14443.

ROGERS, D. S. 1990. Genic evolution, historical biogeography, and systematic relationships among spiny pocket mice (subfamily Heteromyinae). Journal of Mammalogy 71:668–685.

SCHITOSKEY, F., JR. 1968. Notes on the morphological variation in the dark kangaroo mouse. Southwestern Naturalist 13:243–248.

SHIELDS, G. F., AND A. C. WILSON. 1987. Calibration of mitochondrial DNA evolution in geese. Journal of Molecular Evolution 24:212–217.

SMITH, M. F., AND J. L. PATTON. 1991. Variation in mitochondrial cytochrome *b* sequence in natural populations of South American akodontine rodents (Muridae: Sigmodontinae). Molecular Biology and Evolution 8:85–103.

SMITH, R. S. U. 1982. Sand dunes in the North American deserts. Pp. 481–526 in Reference handbook on the deserts of North America (G. L. Bender, ed.). Greenwood Press, Westport, Connecticut.

SOKAL, R. R. 1979. Testing statistical significance of geographic variation patterns. Systematic Zoology 28:227–323.

SPRINGER, M. S., ET AL. 2001. Mitochondrial versus nuclear gene sequences in deep-level mammalian phylogeny reconstruction. Molecular Biology and Evolution 18:132–143.

SPSS, INC. 1999. SYSTAT 9 statistics. SPSS, Inc., Chicago, Illinois.

SWOFFORD, D. L. 2003. PAUP*: phylogenetic analysis using parsimony (*and other methods). Version 4.0b10. Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts.

THOMPSON, J. D., T. J. GIBSON, F. PLEWNIAK, F. JEANMOUGIN, AND D. G. HIGGINS. 1997. The Clustal_X Windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 25:4876–4882.

WEINS, J. 1998. Combining data sets with different phylogenetic histories. Systematic Biology 47:568–581.

WIGAND, P. E., AND P. J. MEHRINGER, JR. 1985. Pollen and seed analyses. Pp. 108–124 in The archaeology of Hidden Cave, Nevada (D. H. Thomas, ed.). Anthropological Papers of the American Museum of Natural History 61:1–430.

WILLIAMS, D. F., H. H. GENOWAYS, AND J. K. BRAUN. 1993. Taxonomy. Pp. 38–196 in Biology of the Heteromyidae (H. H. Genoways and J. H. Brown, eds.). Special Publication 10, The American Society of Mammalogists.

YANG, Z. 1994. Maximum likelihood phylogenetic estimation from DNA sequences with variable sites over sites: approximate methods. Journal of Molecular Evolution 39:306–314.

Submitted 1 March 2006. Accepted 5 May 2006.

Associate Editor was Carey Krajewski.

APPENDIX I

Specimens examined in the morphometric portion of this study are deposited in the Museum of Vertebrate Zoology (MVZ; University of California, Berkeley, California) or The Museum, Texas Tech University (TTU; Lubbock, Texas) as voucher study preparations (see Fig. 1 for locality numbers).

Microdipodops megacephalus polionotus (n = 176).—Locality 1 ($n = 41$): Fletcher, 6,100 feet, Mineral County, Nevada (MVZ 40435, 40436, 40440, 105488–105495, 107277–107279, 107281–107284, 107286–107290, 107293, 107294, 107296, 107297, 107300, 107301, 107303, 109699, 112005, 113398, 113399 [all topotypes of *M. m. nasutus*]); ¼ mile N Fletcher, 6,100 feet, Mineral County, Nevada (MVZ 142184–142187, 142272, 142274, 142275). Locality 2 ($n = 13$): 2.5 miles NW Powell Mountain, 7,100 feet, Mineral County, Nevada (MVZ 142258–142270). Locality 3 ($n = 14$): ½ mile SE Alkali Lake, Aurora Valley, 7,040 feet, Mineral County, Nevada (MVZ 142251–142257, 143765, 143766); ½ mile SE Alkali Lake, Aurora Valley, 17.5 miles S, 6.5 miles W Hawthorne, 7,040 feet, Mineral County, Nevada (TTU 24681–24685). Locality 4 ($n = 27$): 2.5 miles NE Larkin Lake, Alkali Valley, 6,860 feet, Mineral County, Nevada (MVZ 142245–142249); 2.5 miles NE Larkin Lake, Alkali Valley, 21.5 miles S, 10.5 miles W Hawthorne, 6,860 feet, Mineral County, Nevada (TTU 24646–24649, 24653, 24655, 24657, 24659, 24660, 24663–24666, 24669, 24670, 24673–24676, 24678, 24679, 24717). Locality 5 ($n = 14$): 1 mile N Highway 167, 6.5 miles W state line, NE Mono Lake, 6,780 feet, Mono County, California (MVZ 142211, 142213, 142214, 142216, 142218–142223); 1 mile S Highway 167, 6 miles W state line, NE Mono Lake, 6,780 feet, Mono County, California (MVZ 142209, 142212, 142215, 142217). Locality 6 ($n = 10$): 7 miles S, 4 miles E Bodie, 6,500 feet, Mono County, California (MVZ 124334, 124335, 125188–125192, 126502, 126504, 126505). Locality 7 ($n = 3$): Mono Lake, Salmon Ranch, Mono County, California (MVZ 24101, 24103); 1 mile S, 5 miles E Lee Vining, 6,500 feet, Mono County, California (MVZ 142244). Locality 8 ($n = 10$): 1.5 miles SW River Spring Lakes, Adobe Valley, 6,490 feet, Mono County, California (MVZ 142233, 142236, 142239, 143763, 143764, 158917–158919; TTU 24689, 24690). Locality 9 ($n = 10$): Dutch Pete's Ranch, 4 miles W Benton, 6,500 feet, Mono County, California (MVZ 26794, 26795, 26798, 26799); Benton, 5,639 feet, Mono County, California (MVZ 26787–26792). Locality 10 ($n = 4$): Pellisier Ranch, 5 miles N Benton, 5,600 feet, Mono County, California (MVZ 26800–26803). Locality 11 ($n = 30$): Taylor Ranch, 2 miles S Benton Station, 5,300 feet, Mono County, California (MVZ 26762–26766, 26769–26786); McKeever's Ranch, 2 miles S Benton Station, 5,200 feet, Mono County, California (MVZ 17031 [holotype of *M. m. polionotus*], 17034–17036, 17038–17040 [topotypes of *M. m. polionotus*]).

APPENDIX II

Localities and numbers of specimens examined in the genetic portions of this study (this set of specimens is exclusive to that listed in Appendix I). All specimens are deposited in the Moore Laboratory of Zoology (MLZ; Occidental College, Los Angeles, California) as voucher specimens. Specimens of *Microdipodops megacephalus* listed were used in both the molecular and chromosomal portions of this study except 1 specimen from Austin (MLZ 1751) that was not karyotyped. Localities are shown in Fig. 1.

Microdipodops megacephalus (n = 21).—Locality 1: ¼ mile N Fletcher, 6,100 feet, Mineral County, Nevada ($n = 2$, MLZ 1744, 1745). Locality 10: 5 miles N Benton, 5,600 feet, Mono County, California ($n = 3$, MLZ 1740–1742). Vernon: 0.5 miles S, 11.5 miles W Vernon, 4,450 feet, Pershing County, Nevada ($n = 1$, MLZ 1760). Chilcoot: 1.7 miles N Chilcoot, 5,100 feet, Plumas County, California ($n = 1$, MLZ 1756). Sparks: 6 miles N, 4 miles E Sparks, 4,600 feet, Washoe County, Nevada ($n = 3$, MLZ 1757–1759). Austin: 6.2 miles S, 19.6 miles W Austin, 6,150 feet, Lander County, Nevada ($n = 4$, MLZ 1748–1751). San Antonio: 3.7 miles N, 3.2 miles E San Antonio, 5,600 feet, Nye County, Nevada ($n = 2$, MLZ 1761, 1762). Goldfield: 12.0 miles N, 2.5 miles W Goldfield, 4,860 feet, Esmeralda County, Nevada ($n = 1$, MLZ 1747). Panaca: 24 miles W Panaca, 4,600 feet, Lincoln County, Nevada ($n = 4$, MLZ 1752–1755).

Microdipodops pallidus.—12.0 miles N, 2.5 miles W Goldfield, 4,860 feet, Esmeralda County, Nevada ($n = 2$, MLZ 1743, 1746).

Dipodomys microps.—6 miles N, 0.5 miles W Bishop, 4,200 feet, Inyo County, California ($n = 2$, MLZ 1765, 1766).

Perognathus longimembris.—6 miles N, 0.5 miles W Bishop, 4,200 feet, Inyo County, California ($n = 2$, MLZ 1763, 1764).