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ZOOGEOGRAPHIC AND EVOLUTIONARY RELATIONSHIPS OF SELECTED POPULATIONS OF MICROTUS MEXICANUS

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Upper elevations of the higher mountains of the southwestern United States are inhabited by several species of boreal mammals that have been isolated on these mountain refugia for varying periods of time. These restricted populations provide an opportunity for investigating zoogeographic and evolutionary principles. One such mammal, the Mexican vole (*Microtus mexicanus*), chosen as the basis of this study, presently inhabits the Transition Zone on scattered mountain ranges from southwestern Colorado to the Mexican state of Veracruz. It typically is found in dry bunchgrass meadows and mesic grasslands scattered among yellow pine and fir at elevations generally above 2000 meters (m.). *M. mexicanus* is more tolerant of xeric conditions than are other microtines inhabiting this area and is often found considerable distances from any source of permanent water (Findley and Jones, 1962). The isolated nature of mountains in the Southwest and relatively small geographic ranges of the species have resulted in a series of small, isolated *Microtus mexicanus* populations.

This study concerns four populations of the Mexican vole located in the San Mateo, Manzano, Sacramento, and Guadalupe mountain ranges of New Mexico and Texas. These mountain ranges are separated from one another by variable expanses of dry lowlands, generally consisting of unsuitable habitat for microtine rodents. Three of the four populations have been referred previously to the subspecies *M. m. guadalupensis* (Manzano, Sacra-

mento, and Guadalupe mountain populations); the remaining San Mateo Mountain population was referred to *M. m. mogollonensis* (Hall and Kelson, 1959).

The primary objectives of this investigation were to reevaluate present subspecific assignments of populations of *M. mexicanus* in the region of study, to compare conclusions obtained by means of classical taxonomic methods with those obtained by using more recent systematic methods, to examine the zoogeographic history and evolutionary relationships of the populations concerned, and to attempt to place the evolutionary changes observed into a reasonable temporal framework. Additionally, this investigation was intended to explore aspects of the biology of *M. mexicanus* that previously have not been covered in the literature. These four populations of the Mexican vole were examined using protein electrophoresis, karyotypic analysis, sperm morphology, bacular morphology, and classical morphometrics.

Methods and Materials

Mexican voles were captured in Sherman live traps during the months indicated at the following localities: Texas: Culberson Co.: Upper Dog Canyon, Guadalupe Mountains National Park (April, August, December); The Bowl, Guadalupe Mountains National Park (August). New Mexico: Torrance Co.: 4th of July Campground, Cibola National Forest (September, October); Lincoln Co.: South Fork Campground, Lincoln National Forest (May, October, November); Soccoro Co.: Beartrap Canyon, Cibola National Forest (October). The collecting localities and the extent of the mountain ranges involved are illustrated in Fig. 1.

After collection, voles were transported from the field to the laboratory and were usually sacrificed within two weeks of capture. A total of 379 specimens of *M. mexicanus* were examined, including 121 that were live trapped and maintained in the laboratory for varying periods before being killed. All were prepared as standard museum skins and skulls and deposited in The Museum, Texas Tech University.

Karyotypes were prepared following the *in vivo* technique of Baker (1970). A minimum of five metaphase spreads were counted for each of 42 males and 49 females examined. Terminology describing chromosomal morphology and fundamental number follows that of Patton (1967).

Spermatozoa were obtained by removing the epididymis from freshly killed specimens, mincing it with scissors, and suspending

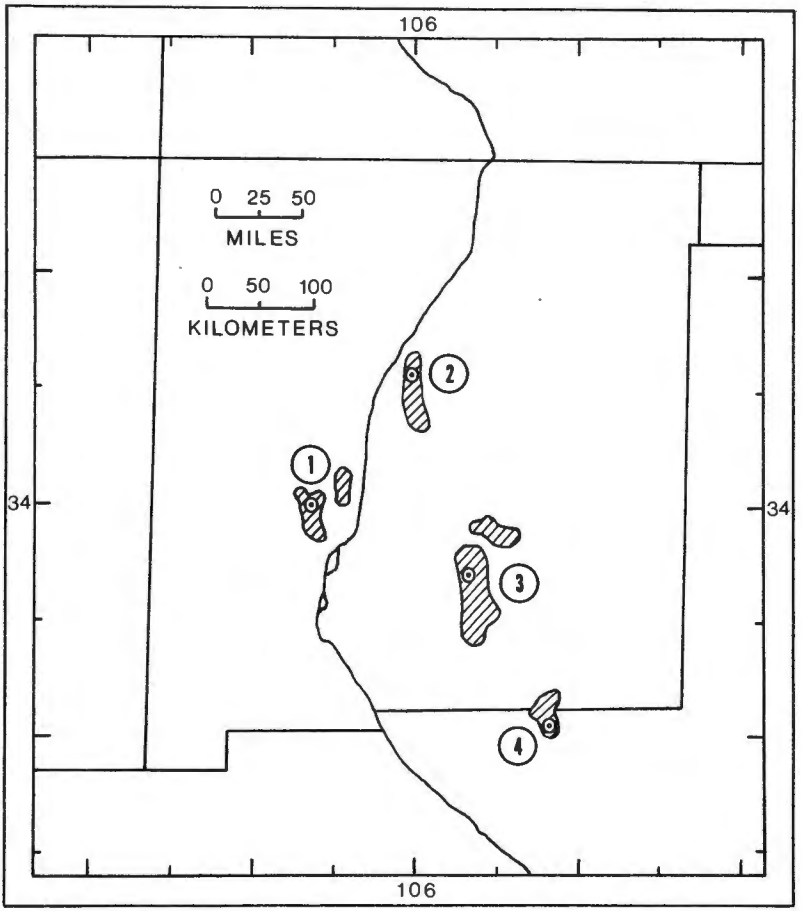

FIG. 1.—Geographic relationships and approximate extent of the mountain ranges containing collecting localities for populations of *Microtus mexicanus* examined. Localities are identified by numbers: 1) Beartrap Canyon (San Mateo Mountains, Soccoro County, New Mexico); 2) 4th of July Campground (Manzano Mountains, Torrance County, New Mexico); 3) South Fork Campground (Sacramento Mountains, Lincoln County, New Mexico); 4) Guadalupe Mountains (Culberson County, Texas). Circles indicate approximate collecting localities within each mountain range.

a small amount of fluid from the minced tissue in an isotonic solution of sodium citrate. Several drops of this solution were placed on a microscope slide and allowed to air dry. Slides were then fixed in a solution of one part acetic acid and four parts methanol prior to staining with a 0.15 per cent solution of Giemsa in hot water. Photomicrographs of spermatozoa were

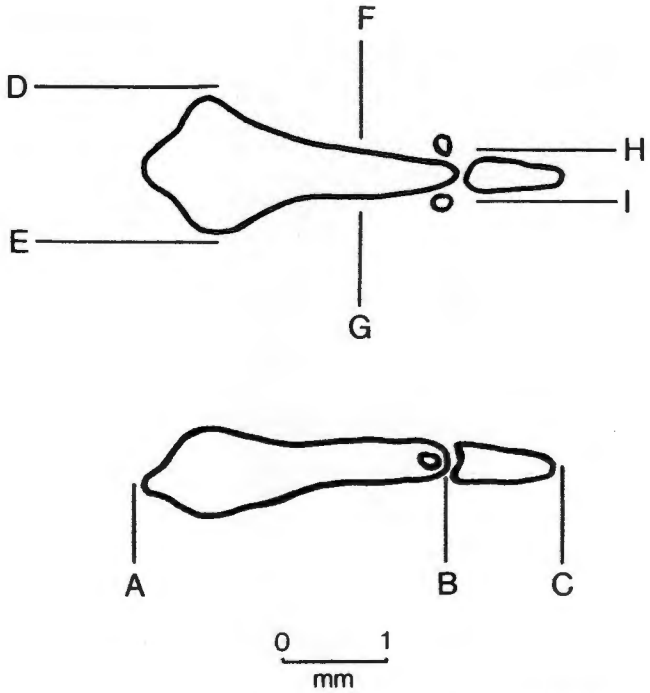

FIG. 2.—Baculum of *Microtus mexicanus guadalupensis* (TTU 27326) illustrating the dimensions described in the text: A-B, bacular length; D-E, width of base; F-G, width of shaft; B-C, length of median distal process; H-I, width of median distal process.

taken using a Leitz Wetzlar microscope at a magnification of 950×. Measurements in millimeters were taken directly from the four by five-inch negatives using Helios dial calipers and a Sakurai map measuring device. Measurements and terminology follow those of Linzey and Layne (1974). Ten spermatozoa were measured for each of the 28 specimens examined, and average values were used in the subsequent analyses.

Penes were removed from freshly killed specimens and stored in AFA; bacular preparation followed techniques outlined by Anderson (1960). After staining and destaining, penes were dissected away from the bacula, and the bacula were drawn in both dorsal and lateral views using a camera lucida attached to a Wild M5 microscope at a magnification of 12×. Measurements (in millimeters) of 64 specimens were taken directly from the drawings and included: bacular length, width at base, width of shaft, length of median distal process, and width of median distal process when an ossified distal process was present (Fig. 2).

The morphometric portion of the study was based on 379 specimens; only those specimens with a total skull length of 23 millimeters or more were included in the analysis. This corresponded to a minimum total body length of approximately 100 millimeters. Four standard external measurements were recorded from specimen labels and 14 cranial measurements were taken. Cranial measurements follow Goin (1943), Anderson (1954), and Snyder (1954).

Starch gel electrophoresis was used to assess allozymic variation for 80 *Microtus mexicanus* from the following localities: Upper Dog Canyon (11 males, 9 females); South Fork Campground (12 males, 8 females); 4th of July Campground (8 males, 12 females); and Beartrap Canyon (11 males, 9 females). Samples were prepared from liver, heart, and kidney extracts. Techniques of tissue preparation, electrophoresis, and biochemical staining were similar to those of Selander *et al.* (1971), but staining procedures for sorbitol dehydrogenase (SDH) and glutamate dehydrogenase (GDH) were modified after Shaw and Prasad (1970). A summary of electrophoretic methods used is given in Table 1.

Designation of alleles follows that of Smith *et al.* (1973). The allele occurring in the highest frequency at each locus was assigned the value of 100 for anodally migrating systems, or −100 for those migrating cathodally. Remaining alleles at a locus were described as percentages of the 100 allele by comparing relative migration distances. When more than one locus was present in a system, the most anodal locus in the system was designated "1" and more successively cathodal loci were assigned progressively higher numbers. Allozyme similarity was assumed if side-by-side comparisons failed to establish differences (see Smith *et al.*, 1973).

Statistical and clustering analyses were carried out using SAS·76 (Barr *et al.*, 1976), UNIVAR (Power, 1970), and NT-SYS programs. In all analyses, specimens were grouped into four aggregate populations representing the primary collecting localities; these aggregates were considered as Operational Taxonomic Units (OTU's).

SAS programs were used in univariate analyses to provide standard descriptive statistics and to perform both single classification and two-way analyses of variance in order to test for significant differences between or among means. When means were found to be significantly different, the Sums of Squares Simultaneous Test Procedure (SS-STP) was conducted using a UNIVAR program to determine maximally nonsignificant subsets. Multi-

variate analyses entailed calculation of Pearson product-moment correlation coefficients, again using SAS, to compare electrophoretic distance and correlation matrices with the corresponding morphometric and sperm morphology matrices. Cluster analyses were conducted on the correlation and distance matrices and a two-dimensional phenogram was generated for each using the NT-SYS program. This program utilizes the unweighted pair-group method using arithmetic averages (UPGMA). Phenograms were compared with their respective matrices and a coefficient of cophenetic correlation was computed for each comparison to assess the reliability of the phenogram. A matrix of correlation among the characters was computed, and the first principal components were extracted. A three-dimensional plot of the OTU's onto the first three principal components was then prepared. The percentage of the total variation accounted for by each principal component was calculated, as was the contribution of each character to each of the principal components. Additional data concerning methods and materials may be found in Wilhelm (1977).

Specimens Examined

In addition to specimens collected for this study, other specimens were borrowed from the following institutions for examination: Texas Cooperative Wildlife Collection, Texas A&M University (TCWC); The Museum, Texas Tech University (TTU); University of New Mexico (UNM); and University of Texas at El Paso (UTEP). Localities are not plotted separately on Fig. 1, but are grouped with the four primary collecting sites for purposes of analysis and discussion. The list below includes all specimens examined.

Microtus mexicanus guadalupensis (300).—Texas: *Culberson County*: Guadalupe Mountains National Park, The Bowl, 24 (6 TTU, 18 TCWC); Upper Dog Canyon, 33 (TTU); Guadalupe Peak, 1 (TTU). New Mexico: *Otero County*: Timberon, 2 (TTU); 8.5 mi. E, 4.5 mi. N Almo Peak, 2 (UNM); 1 mi. N Cloudcroft, 2 (UNM); 7 mi. E Cloudcroft, 2 (UNM); 7 mi. E, 2.5 mi. N Cloudcroft, 7 (UNM); 1 mi. S Cloudcroft, 1 (UNM); 10 mi. S Cloudcroft, 15 (UNM); 2 mi. W Cloudcroft, 1 (UNM); 2.4 mi. W Cloudcroft, 1 (UTEP); near Cloudcroft, 19 (8 UTEP, 11 TTU); Russian Canyon, 5 mi. S, 2.5 mi. E Cloudcroft, 2 (UTEP); north of Ruidoso, 3 (UTEP). *Lincoln County:* Padilla Point, 3 (UTEP); South Fork Campground, 27 (TTU); 5 mi. N, 9 mi. E Capitan, 2 (UNM); 1.5 mi. W Capitan, 3 (UNM); Capitan Mountains, 20 (UNM); Monjeau Peak, 10,000 ft., 6 (UNM); Lincoln County, 6 (TTU). *Torrance County*: 4th of July Campground, 36 (TTU); 0.5 mi. S Capillo Peak, 9000 ft., 1 (UNM); 5.5 mi. W Tajique, 8 (UNM); Red Canyon, 0.5 mi. S, 5 mi. W. Manzano, 34 (UNM); Red Canyon, 4 mi. W, 1 mi. S Manzano, 11 (UNM); 5 mi. W Manzano, 3 (UNM); Torrance County, 4 (TTU). *Bernalillo County*: Tree Springs, 8600 ft., 21 (UNM).

TABLE 1.—*Electrophoretic techniques utilized in this study. All gels were run for five hours.*

Gel type	tissue	milliamperage	stains
1. Continuous Tris-citrate I	Kidney	75	Lactate dehydrogenase (LDH)
	Kidney	75	Malate dehydrogenase (MDH)
	Kidney	75	Malate enzyme (ME)
	Kidney	75	Isocitrate dehydrogenase (IDH)
	Kidney	75	Acid phosphatase (ACP)
2. Continuous Tris-citrate II	Liver	85	Sorbitol dehydrogenase (SDH)
	Liver	85	Alcohol dehydrogenase (ADH)
	Liver	85	Glutamate oxalate transaminase (GOT)
	Liver	75	Leucine aminopeptidase (LAP)
3. Lithium Hydroxide	Kidney	80	Phosphoglucose isomerase (PGI)
	Kidney	90	Albumin (AB)
	Liver	75	Esterases (EST)
4. Phosphate	Liver	75	Phosphoglucomutase (PGM)
5. Discontinuous Tris-citrate	Kidney	85	Glucose-6-phosphate dehydrogenase (G-6-P)
(Poulik)	Kidney	85	Indophenol oxidase (IPO)
	Kidney	85	Alkaline phosphatase (AKP)
	Kidney	85	Xanthine dehydrogenase (XDH)
6. Tris-versene-borate	Kidney	85	Glutamate dehydrogenase (GDH)
	Kidney	85	αGlycerophosphate dehydrogenase (αGPDH)

Microtus mexicanus mogollonensis (79)—New Mexico: *Socorro County*: Beartrap Canyon, 78 (46 TTU, 28 UNM, 4 UTEP); 1.6 mi. from Water Canyon, 1 (UTEP).

Results and Discussion

Karyology

The karyotype of *Microtus mexicanus* was described from a single female specimen by Matthey (1957), who established the diploid number as 44. Being unable to identify the sex chromosomes, he was uncertain of the fundamental number and listed it as a minimum of 54 and perhaps 56.

The karyotype of *Microtus mexicanus* given in Fig. 3 shows a diploid number of 44 and a fundamental number of 54. No chromosomal polymorphism was noted among specimens examined. The autosomes consist of three pairs of large submetacentrics, one pair of medium submetacentrics, two pairs of small metacentrics, one pair of large acrocentrics, and 14 pairs of small acrocentrics. The X chromosome is a medium-sized submetacentric, and the Y is a small acrocentric.

Although no chromosomal polymorphism was found in the karyotype of the Mexican vole in this study, two separate chromosomal polymorphisms were described in Mexican voles from the states of Jalisco and Durango in México (Lee and Elder, 1977), and involved differences in both the diploid and fundamental numbers. Thus, *M. mexicanus* is the only exclusively Nearctic species of microtine from which infraspecific differences in chromosome morphology or number have been reported.

b Infraspecific variation in chromosome number is more common in Palearctic representatives of the genus, however. *Microtus hyperboreus* and *M. middendorfi* (Gileva, 1972) and *M. juldaschi* (Bol'shakov *et al.*, 1975) have been reported as polymorphic. Other genera of microtines that exhibit chromosomal polymorphism include *Dicrostonyx torquatus* (Rausch and Rausch, 1972; Kozlovskii, 1974), *Clethrionomys rutilus* (Rausch and Rausch, 1975), and *Pitimys subterraneus* (Meylan, 1972). The relatively few number of karyotypic studies of Nearctic microtines and the apparent uniformity of infraspecific chromosome complements might reflect a lack of data rather than extreme chromosomal conservatism of the group.

Sperm Morphology

Spermatozoa of *M. mexicanus* from the four populations studied were similar in all respects. The sperm head, widest just above

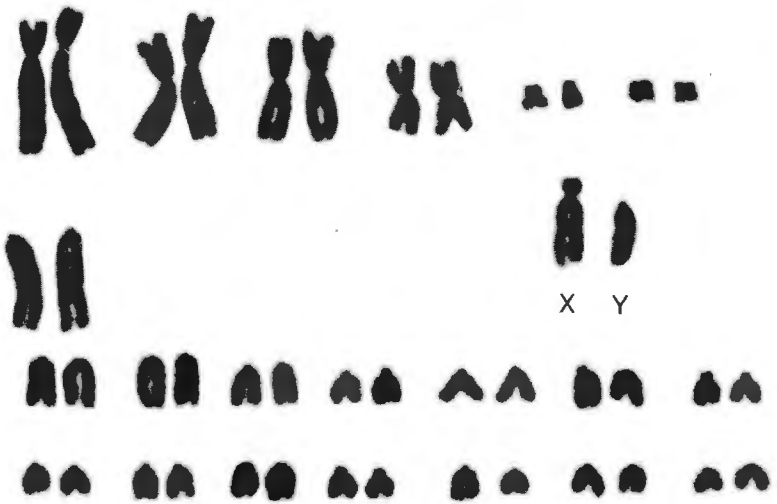

FIG. 3.—Karyotype of a male (TTU 27356) *Microtus mexicanus guadalupensis.*

the base, is asymmetrical with one margin convex and the other nearly straight. The base is smoothly convex with a notch on one side. Almost one-half of the head is enveloped by the acrosome, which is elongated into a recurved hook that lies on the side of the head having the straighter margin. Attachment of the midpiece is to the basal notch and is therefore eccentric. The tailpiece tapers gradually toward the tip and is sometimes difficult to distinguish from the midpiece. However, with the staining procedure used in this study, the tailpiece is generally of uniform appearance, whereas the midpiece is nearly always granular or mottled in appearance.

Mean values, followed by range in parentheses and sample size, for selected sperm measurements of *Microtus mexicanus* follow (measurements are in microns and localities are given in the order Beartrap, 4th of July, South Fork, and Guadalupe): *length of head*, 7.77(7.44-8.00) 5, 8.02(7.51-8.54) 5, 8.34(7.60-9.14) 10, 8.32(8.01-8.78) 8; *width of head*, 4.86(4.82-4.93) 5, 4.82(4.47-5.35) 5, 5.08(4.54-5.35) 10, 5.04(4.81-5.35) 8; *length of midpiece*, 18.45(17.12-20.32) 5, 18.10(16.80-18.80) 5, 18.32(16.72-19.36) 10, 18.46(15.84-20.72) 8; *length of tailpiece*, 66.02(60.16-71.36) 5, 70.21(65.84-74.32) 5, 72.02(68.48-76.08) 10, 70.51(65.92-73.04) 8.

Univariate analysis of sperm data showed a significant difference in the head width of spermatozoa ($P<0.05$) between some localities and in the length of the tailpiece ($P<0.01$) between oth-

ers. The following nonsignificant subsets were generated by SS-STP tests: *width of head*—Guadalupe, 4th of July, Beartrap; *length of tailpiece*—South Fork, Guadalupe, 4th of July; Guadalupe, 4th of July, Beartrap. In *M. m. mogollonensis*, the sperm head is slightly shorter but just as broad as that of *M. m. guadalupensis*. The tailpiece of *M. m. mogollonensis* is considerably shorter than that of the other subspecies.

The four sperm measurements also were analyzed using the NT-SYS multivariate program. Both correlation and distance matrices were computed and phenograms representing the phenetic relationships were plotted. A Pearson product-moment correlation matrix was computed comparing these two matrices with the corresponding electrophoretic matrices. Correlation coefficients for neither the distance matrices nor the correlation matrices were significant. The distance phenogram showed that the Beartrap and 4th of July specimens had smaller sperm than did those from the other two localities. Although on the basis of sperm data, the Beartrap (*M. m. mogollonensis*) and the 4th of July (*M. m. guadalupensis*) populations more closely resembled each other than either resembled the other two populations, their relationship to each other was not of the same magnitude as was that of populations from the South Fork and Guadalupe localities.

In the principal components analysis, the amount of phenetic variation expressed in the first principal component was 65.58; in the second, 33.60; and in the third, 0.80. The percentage contribution of each sperm character to each principal component, given in the order Component I, II, and III were *length of head*, 10.51, 0.71, 32.05; *width of head*, 5.87, 3.81, 22.89; *length of tailpiece*, 81.70, 59.85, 7.38; *length of midpiece*, 1.91, 35.62, 37.65. Length of tailpiece accounts for most of the phenetic variation. A three-dimensional perspective of the projection of the four OTU's onto the first three components, based on a matrix of correlation among the four sperm measurements, is given in Fig. 4. Essentially the same pattern was seen in the distance phenogram. South Fork and Guadalupe OTU's were clustered close to one another; Beartrap and 4th of July OTU's were loosely grouped. This grouping of the populations sampled agreed in part with the results from multivariate analyses of cranial morphometric data. Small sample sizes, however, render these results of limited value.

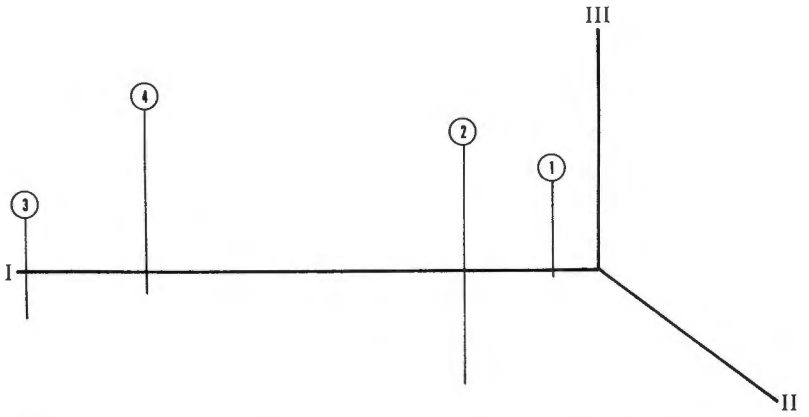

FIG. 4.—Three-dimensional projection of the four study populations of *Microtus mexicanus* onto the first three principal components based on a matrix of correlation among four sperm measurements. Localities are coded as in Fig. 1.

Bacular Morphology

Anderson (1960) described the baculum of *Microtus mexicanus*, and I noted no major deviations from his description. Of the 64 bacula examined, 20 possessed neither medial nor lateral processes; 32 possessed only the medial process; and 12 possessed both. Accordingly, of the five bacular measurements taken, only three (bacular length, width of base, and width of shaft) were consistently available for all specimens.

Mean values, followed by range in parentheses and sample sizes for selected bacular measurements were (measurements are in microns and localities are given in the order Beartrap, 4th of July, South Fork, and Guadalupe): *bacular length*, 2.81(2.37-3.76) 18; 2.98(2.07-3.53) 13; 2.69(2.05-3.21) 15; 2.90(2.52-3.23) 16; *width of base*, 1.35(0.98-1.85) 18; 1.36(0.55-1.87) 13; 1.25(0.71-1.77) 15; 1.39(0.97-1.71) 16; *width of shaft*, 0.27(0.16-0.37) 18; 0.28(0.17-0.38) 13; 0.27(0.17-0.38) 15; 0.30(0.22-0.40) 16. Univariate analysis of these three measurements revealed no significant differences among specimens from the four localities. The large amount of individual variation (CV's, 7.3-30.2) evident in bacular morphology, even among individuals of similar size (and presumably similar age), makes statistical treatment of bacular measurements difficult. Bacula enlarge and change somewhat in shape throughout life. Although dividing the bacula into age classes would reduce variation due to age, estimating the age of individual microtines

is difficult, and the small number of bacula available rendered an attempt to age specimens unfeasable at this time.

Although there have been several surveys of microtine bacula, few have treated bacular measurements statistically. Dearden (1958) performed an analysis of variance and reported standard errors for some bacular characters in several microtines. His results indicated that there were subspecific differences in the length of the bacular shaft in several subspecies of *Lagurus curtatus*, although the standard errors were nearly twice those that I found in the bacular shaft length of *Microtus mexicanus*. Coefficients of variation for bacular characters in the Mexican vole were large, compared to the corresponding statistic for any cranial measurement. This high degree of variation within a population, coupled with small sample sizes, makes interpopulational comparisons difficult. As a result, bacular morphology offered no information concerning relationships among the populations examined in this study.

Morphometric Analysis

Fourteen cranial and four external measurements were analyzed using the SAS univariate program. Descriptive statistics and results of the SS-STP tests for these measurements are given in Table 2. Coefficients of variation obtained for the cranial characters (3.26-8.95) agree with those values reported by Long (1968) for rodents.

A two-way analysis of variance was conducted to detect possible sexual dimorphism and to determine if differences existed among localities for cranial and external characters. Differences were found between sexes for depth of skull, total length, and length of hind foot below the probability level of 0.05; for interorbital breadth, 0.01. Total length of females was consistently greater than that of males, and the interorbital breadth was always larger in males than it was in females. Within the Beartrap population, females had deeper skulls and longer hind feet than did males; in the other three populations, males were larger than females in these two characters.

For diastema length, condylozygomatic length, lambdoidal breadth, and length of hind foot, there was no significant difference among localities. Nonoverlapping subsets were found in only two characters: the incisive foramen in specimens from the Guadalupe Mountains was significantly longer than in those from the other three populations; interorbital breadth was larger

TABLE 2.—*Descriptive statistics derived from two-way analysis of variance for external and cranial measurements of* Microtus mexicanus. *Groups of means found to be significantly different at P>0.05 were tested with the sums of squares simultaneous testing procedure to find the nonoverlapping subsets. Groups of means that were found to be not significantly different at P>0.05 are marked ns. Localities are coded as in Fig. 1.*

Locality	Results SS-STP	Mean	Range	SE	CV
		Total length of skull			
2		25.5	23.0-27.6	.10	4.29
1		25.4	23.1-27.6	.13	4.02
4		25.4	23.0-27.2	.16	4.51
3		25.1	22.8-27.4	.09	3.68
		Diastema length			
1		7.8	6.7-9.1	.06	5.72
2		7.7	6.7-8.7	.04	5.41
3	ns	7.7	6.7-8.8	.04	5.36
4		7.6	6.9-8.6	.05	5.26
		Length of incisive foramen			
4		4.7	4.1-5.3	.05	7.27
1		4.5	3.5-5.4	.03	6.22
3		4.5	3.8-5.3	.03	6.38
2		4.4	3.4-5.3	.04	8.59
		Palatilar length			
1		13.0	11.5-14.3	.07	4.28
2		13.0	11.7-14.2	.05	4.25
3		12.8	11.3-14.1	.05	3.92
4		12.8	11.5-14.2	.08	4.82
		Condylozygomatic length			
1		19.2	17.5-20.7	.03	3.49
2		19.2	17.6-20.8	.07	3.87
3	ns	19.2	17.6-12.1	.06	3.26
4		19.4	17.7-20.8	.10	3.95
		Length of nasals			
2		7.7	6.1-9.0	.04	5.71
1		7.6	6.0-8.4	.07	6.97
3		7.5	5.9-8.6	.04	6.19
4		7.3	6.2-8.1	.06	6.24
		Rostral breadth			
4		4.1	3.7-4.5	.03	4.80
2		4.1	3.7-4.5	.02	4.44
1		4.1	3.7-4.6	.02	4.78
3		4.0	3.6-4.8	.02	4.32

TABLE 2.—*Continued.*

		Interorbital breadth (males)			
2		3.4	2.9-3.9	.03	7.26
1		3.4	3.0-3.6	.03	4.76
4		3.2	2.8-3.6	.04	6.07
3		3.2	2.7-3.9	.03	6.66
		Interorbital breadth (females)			
1		3.4	2.9-3.8	.04	7.58
2		3.3	2.9-3.8	.03	6.87
4		3.2	2.7-3.7	.04	6.56
3		3.1	2.4-3.6	.03	6.76
		Zygomatic breadth			
4		15.0	13.3-16.7	.10	5.00
1		14.7	13.1-16.0	.08	4.57
3		14.7	13.4-16.4	.06	4.04
2		14.7	12.8-16.1	.06	4.60
		Prelamboidal breadth			
2		10.5	9.5-11.7	.03	3.54
4		10.4	9.4-11.2	.05	3.53
1		10.3	9.4-11.0	.04	3.34
3		10.3	9.5-11.8	.03	3.56
		Lambdoidal breadth			
1		11.7	10.6-12.6	.06	4.03
2		11.7	10.5-12.6	.04	3.90
3	ns	11.5	10.1-12.4	.04	3.85
4		11.5	10.2-12.4	.07	4.70
		Height of skull			
2		10.0	9.2-11.0	.03	3.29
1		9.9	9.0-11.2	.05	4.10
4		9.9	9.2-10.9	.06	4.10
3		9.8	9.0-10.6	.03	3.46
		Depth of braincase (males)			
2		7.8	7.3-9.2	.04	4.21
3		7.7	7.0-8.8	.05	4.75
4		7.6	7.1-8.2	.07	4.03
1		7.4	6.3-8.1	.08	5.42
		Depth of braincase (females)			
2		7.8	7.1-8.5	.04	3.68
1		7.7	6.9-8.5	.06	4.87
3		7.6	6.9-8.6	.05	4.64
4		7.4	6.7-8.3	.07	5.13

TABLE 2.—*Continued.*

		Rostral length			
2		6.3	4.9-7.3	.05	8.23
1		6.2	4.9-7.5	.07	8.91
4		6.0	4.7-7.3	.07	8.95
3		5.9	4.7-7.1	.04	7.68
		Total length (males)			
1		127.1	111.0-148.0	2.01	8.23
2		131.2	107.0-150.0	1.18	6.87
3	ns	131.2	119.0-148.0	1.05	5.80
4		127.4	114.0-140.0	1.75	6.30
		Total length (females)			
1		130.4	95.0-151.0	1.91	8.90
2		134.3	109.0-173.0	1.63	9.17
3	ns	134.1	110.0-158.0	1.38	7.84
4		129.2	111.0-152.0	1.82	8.09
		Length of tail			
4		30.9	22.0-42.0	.48	11.34
2		29.5	22.0-58.0	.42	15.13
1		28.7	15.0-42.0	.55	15.30
3		28.3	18.0-39.0	.37	13.58
		Length of hind foot (males)			
1		17.3	15.0-20.0	.21	6.35
2		17.8	15.0-22.0	.19	8.15
3	ns	17.5	16.0-19.0	.14	5.79
4		17.7	15.0-20.0	.37	9.53
		Length of hind foot (females)			
1		17.5	15.0-20.0	.19	6.70
2		17.2	12.0-23.0	.22	9.85
3	ns	17.2	16.0-20.0	.12	5.36
4		17.5	15.0-21.0	.29	9.37
		Length of ear			
4		12.7	10.0-18.0	.25	14.19
2		12.4	9.0-19.0	.14	12.28
1		12.1	10.0-19.0	.17	11.55
3		12.0	9.0-15.0	.10	8.32

in individuals from Beartrap and 4th of July populations than those from South Fork and Guadalupe localities. The remaining characters showed from two to three overlapping subsets but provided no distinct groupings.

Characters with high coefficients of variation (length of tail and length of ear) and the four characters which displayed sexual dimorphism (depth of skull, total length, length of hind foot, and

interorbital breadth) were eliminated from the subsequent multivariate analyses. The remaining 12 cranial measurements were then analyzed using the NT-SYS multivariate analysis program. Correlation and distance matrices were computed and phenograms representing the phenetic relationships of the four OTU's were plotted. In addition, a Pearson product-moment correlation was computed comparing these two morphometric matrices with the corresponding electrophoretic matrices. The correlation coefficients for the two distance matrices and for the two correlation matrices were not significant. The distance phenogram for morphometric characters differed from that produced for sperm measurements presented earlier, with two clusters: Beartrap, 4th of July, and South Fork forming the first, and Guadalupe forming the second.

The first three principal components were extracted and plotted, yielding the relationships illustrated in Fig. 5. Phenetic variation expressed by the first principal component was 54.78 per cent; second, 37.75; third, 7.47. The major contributing characters for each component and their percentage contribution were: first principal component: total length of skull (14.48), palatilar length (13.27), condylozygomatic length (11.34), zygomatic breadth (12.95), and lambdoidal breadth (12.40); second component: total length of skull (27.42) and condylozygomatic length (20.62); third component: total length of skull (15.55) and prelambdoidal breadth (18.74). Thus, characterization of the OTU's by principal components is, to a large degree, dependent on total length of skull. The three-dimensional plot arranged the OTU's into two groups that corresponded to those of the distance phenogram. The Beartrap and 4th of July samples were again grouped much closer to each other than to the South Fork and Guadalupe samples. The latter two populations are separated somewhat along axis II.

Thus, morphometric analyses yielded no conclusive evidence concerning relationships of the four populations studied. The four populations examined generally fell into two groups, corresponding to the same two groups produced by analysis of sperm morphology. There is some evidence from the phenogram based on morphometric characters and the results of the SS-STP test that the Guadalupe population is more distinct than the other three populations. The Guadalupe population has a significantly longer incisive foramen. However, for interorbital breadth, the Beartrap and 4th of July populations have relatively greater interorbital breadth than the South Fork and Guadalupe samples.

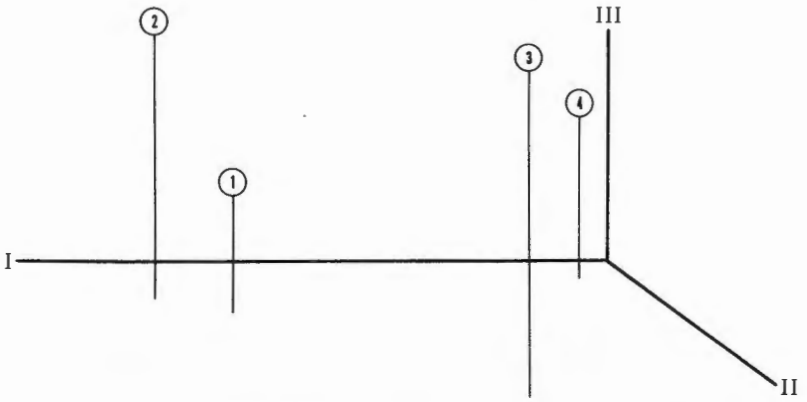

Fig. 5.—Three-dimensional projection of the four study populations of *Microtus mexicanus* onto the first three principal components based on a matrix of correlation among 12 cranial measurements. Localities are coded as in Fig. 1.

Principal component analyses separated the four populations into the same two groups identified in the sperm analysis and in the morphometric phenogram.

Microtine subspecies generally have been delimited by size differences as well as qualitiative characteristics, such as pelage color. *Microtus mexicanus guadalupensis* was distinguished by Bailey (1902) from *M. m. mogollonensis* primarily on the basis of cranial characters. Bailey (1902) reported measurements for the male holotype of *M. m. guadalupensis*, six of which may be compared with those taken in this study: total length, 152; length of tail, 34; length of hind foot, 20; basal length of skull, 24.5; length of nasals, 7.5; and zygomatic breadth, 16.0. A comparison of these measurements with those in Table 2 reveals that the total length of the holotype exceeds that of any Guadalupe male examined, and equals that of the largest Guadalupe female examined. The tail of the holotype was longer than the average length of tail found in this study, and the length of hind foot equals the largest value reported herein. The three skull measurements of the holotype all are near the average for those measurements in the Guadalupe specimens examined.

Electrophoretic Analysis

Nineteen protein systems were investigated, but only 16 were scored with confidence. These systems contained 24 scorable loci, which are listed in Table 3 along with the frequencies of each in the population. In the total sample, 14 loci (58 per cent) were polymorphic, and five (LDH-2, ADH, G-6-P, MDH-1, and EST-7)

TABLE 3.—*Alleles and frequencies (in parentheses) at 24 loci in* Microtus mexicanus *and the mean proportion of individuals heterozygous at each locus ($\bar{h}$); $\bar{h}$ is averaged over all four populations (N=80).*

Locus	Beartrap	4th of July	South Fork	Guadalupe	$\bar{h}$
LDH-1	100(1.00)	100(1.00)	100(1.00)	100(1.00)	0.00
LDH-2	100(.975)	100(1.00)	100(1.00)	100(1.00)	0.01
	72(.025)				
MDH-1	100(.975)	100(1.00)	100(1.00)	100(1.00)	0.01
	70(.025)				
MDH-2	−100(1.00)	−100(1.00)	−100(1.00)	−100(1.00)	0.00
ME	100(1.00)	100(1.00)	100(1.00)	100(1.00)	0.00
IDH	100(1.00)	100(1.00)	100(.925)	100(.650)	0.19
			85(.075)	85(.350)	
SDH	100(1.00)	100(1.00)	100(.950)	100(1.00)	0.03
			116(.050)		
ADH	−100(1.00))	−100(.825)	−100(.950)	−100(1.00)	0.11
		−86(.175)	−86(.050)		
LAP	100(1.00)	100(1.00)	100(1.00)	100(1.00)	0.00
PGI	100(.750)	100(.800)	100(.800)	100(.250)	0.40
	54(.250)		54(.200)	54(.750)	
		33(.200)			
PGM	100(.825)	100(1.00)	100(1.00)	100(1.00)	0.06
	160(.175)				
AB	100(1.00)	100(1.00)	100(1.00)	100(1.00)	0.00
IPO	100(1.00)	100(1.00)	100(1.00)	100(1.00)	0.00
GOT-1	100(1.00)	100(1.00)	100(1.00)	100(1.00)	0.00
GOT-2	−100(1.00)	−100(.900)	−100(.700)	−100(1.00)	0.15
		−41(.100)	−41(.300)		
G-6-P	100(1.00)	100(1.00)	100(1.00)	100(.975)	0.01
				88(.025)	
GDH-1	100(1.00)	100(1.00)	100(1.00)	100(1.00)	0.00
GDH-2	100(1.00)	100(1.00)	100(1.00)	100(1.00)	0.00
GDH-3	100(1.00)	100(1.00)	100(1.00)	100(1.00)	0.00
αGPDH-1	100(1.00)	100(.950)	100(1.00)	100(1.00)	0.03
		115(.050)			
αGPDH-2	100(.925)	100(.975)	100(.950)	100(.975)	0.09
	129(.050)	129(.025)	129(.050)	129(.025)	
	90(.025)				
αGPDH-3	100(.925)	100(1.00)	100(.950)	100(1.00)	0.04
	142(.075)		142(.050)		
EST-1	100(.475)	100(.800)	100(.850)	100(.925)	0.43
	93(.525)	93(.200)	93(.150)	93(.075)	
EST-7	100(.975)	100(.975)	100(1.00)	100(1.00)	0.03
	65(.025)	65(.025)			

were present in a frequency of only five per cent. Acid phosphatase (ACP), alkaline phosphatase (AKP), and xanthine dehydrogenase (XDH) were incompletely scored, but did exhibit some

polymorphism. The major features of the allozyme variations observed in *Microtus mexicanus* are listed below.

Malate dehydrogenase (MDH).—Two MDH loci were observed; the cathodally migrating MDH-2 was monomorphic for all populations. MDH-1 exhibited two alleles; the MDH-1^{70} allele was present in a single specimen from Beartrap.

Isocitrate dehydrogenase (IDH).—This system was represented by two alleles. The IDH^{85} allele was found in three individuals from South Fork and in 13 individuals from the Guadalupe sample.

Sorbitol dehydrogenase (SDH).—Two SDH alleles were identified; the SDH^{116} allele was found in two individuals from South Fork.

Alcohol dehydrogenase (ADH).—Two ADH alleles were observed in this cathodally migrating system. The ADH^{-86} allele was present in seven individuals from 4th of July and in two individuals from South Fork.

Phosphoglucose isomerase (PGI).—Three alleles were represented in this system. The PGI^{100} allele occurred in all samples. PGI^{54} was identified in eight individuals from Beartrap, eight from South Fork, and in all individuals from Guadalupe. PGI^{33} was present in eight specimens from the 4th of July Campground.

Phosphoglucomutase (PGM).—This locus was represented by two alleles. All populations exhibited the PGM^{100} allele; PGM^{160} was identified in six individuals from Beartrap.

Glutamate oxalate transaminase (GOT).—Two loci were observed; GOT-1 was monomorphic for all populations, and the polymorphic GOT-2 allele migrated cathodally. Four individuals from the 4th of July sample and 10 from South Fork exhibited the GOT-2^{-41} allele.

Glucose-6-phosphate dehydrogenase (G-6-P).—Two alleles were observed; the G-6-P^{100} allele was represented in all populations; the G-6-P^{88} allele was found in one individual from the Guadalupe sample.

αGlycerophosphate dehydrogenase (αGPDH).—Three loci were identified and all exhibited polymorphism. The αGPDH−1^{115} allele was found in two individuals from 4th of July. αGPDH−2 was represented by three alleles; all populations exhibited the α GPDH-2^{100} and αGPDH-2^{129} alleles, and the αGPDH−2^{90} allele was present in only one specimen from Beartrap. αGPDH−3^{142} was detected in two specimens from Beartrap and in two from South Fork.

TABLE 4.—*Proportion of individuals heterozygous per locus per population (h). P is the proportion of 24 loci polymorphic in each population (loci with commonest allele at a frequency of >0.95 were considered monomorphic);* $\bar{H}$*, mean proportion of loci heterozygous per individual, N = 20.*

	Populations sampled			
Locus	Beartrap	4th of July	South Fork	Guadalupe
LDH-2	.05			
MDH-1	.05			
IDH			.15	.60
SDH			.10	
ADH		.35	.10	
PGI	.30	.40	.40	.50
PGM	.25			
GOT-2		.20	.40	
G-6-P				.05
αGPDH-1		.10		
αGPDH-2	.15	.05	.10	.05
αGPDH-3	.05		.10	
EST-1	.45	.40	.20	.15
EST-7	.05	.05		
P	.166	.208	.353	.125
$\bar{H}$	.054	.065	.065	.056

Esterases (EST).—A total of seven esterase systems were identified, but only two could be scored with confidence. EST-1 contained two alleles found in all four populations. EST-7 also was composed of two alleles; EST-7^{65} was found in one specimen from Beartrap and in one from 4th of July Campground. Of the five remaining esterases identified, EST-2 and EST-3 exhibited polymorphism in all populations. EST-4 and EST-5 were polymorphic in all populations except that from the Guadalupe Mountains, and EST-6 was monomorphic in all populations.

Monomorphic proteins.—The following proteins were scored as monomorphic in all populations sampled: lactate dehydrogenase (LDH); malate dehydrogenase-2 (MDH-2); malate enzyme (ME); leucine aminopeptidase (LAP); albumin (AB); indolphenol oxidase (IPO); glutamate oxalate transaminase-1 (GOT-1); and glutamate dehydrogenase-1, −2, −3 (GDH-1, GDH-2, GDH-3).

Genic variability.—Table 4 gives the proportion of polymorphic loci in each population, the proportion of heterozygous loci per individual, and the proportion of heterozygous individuals per locus per population. Genic heterozygosity (H) is based on the analysis of 24 loci (14 polymoprhic, 10 monomorphic).

TABLE 5.—*Coefficients of genic similarity (Rogers' S), genic identity (Nei's I), and genetic distance, (Nei's D), respectively, for four populations of* Microtus mexicanus.

	Beartrap	4th of July	South Fork	Guadalupe
Beartrap	1.0000	0.9535	0.9401	0.9279
	0.0000	0.9883	0.9847	0.9526
	0.0000	0.0117	0.0155	0.0486
4th of July		1.0000	0.9572	0.9142
		0.0000	0.9933	0.9543
		0.0000	0.0067	0.0468
South Fork			1.0000	0.9119
			0.0000	0.9566
			0.0000	0.0444
Guadalupe				1.0000
				0.0000
				0.0000

The loci that contributed most to the heterozygosity values varied among populations. Phosphoglucose isomerase (PGI) was a major contributor in all populations, whereas EST-1 was responsible for most of the individual heterozygosity in the Beartrap and 4th of July populations. Other loci contributing significant amounts of heterozygosity were PGM in Beartrap, ADH in 4th of July, GOT-2 in South Fork, and IDH in the Guadalupe sample.

The Beartrap population exhibited four unique alleles (LDH-2^{72}, MDH-1^{70}, PGM^{160}, and αGPDH-2^{90}), whereas the 4th of July population possessed only two unique alleles (PGI^{33} and αGPDH-1^{115}). The remaining two populations had one unique allele each; South Fork (SDH^{116}) and Guadalupe (G-6-P^{88}).

Genic similarity.—Coefficients of genic similarity between populations were calculated, using Rodgers' similarity, S (Rodgers, 1972) and Nei's identity, *I* (Nei, 1972). Values for *I* are generally slightly higher than those for *S*, but both measures give comparable results. Both are reported in Table 5.

Electrophoretic studies generally show high levels of polymorphism in natural populations, with reduced levels of heterozygosity in small, isolated populations. Selander *et al.* (1971) demonstrated low levels of genic variability ($\overline{H}$ = 0.018) in insular populations of *Peromyscus polionotus* compared to the larger mainland populations ($\overline{H}$ = 0.054-0.088). Similar results were obtained for several species of *Peromyscus* (Avise *et al.*, 1974*b*), in which insular subspecies had an average of less than one per cent heterozygous loci. Reduced levels of heterozygosity in small, isolated populations are thought to be due to genetic drift.

The level of genetic variability is relatively uniform for the four populations of Mexican voles studied, ranging from 0.054 to 0.065 (mean, 0.060). These values are consistent with heterozygosity values for other mainland vertebrate populations ($\overline{H}$ = 0.01-0.09) as reported by Selander and Johnson (1973). The proportion of the 24 loci examined that were polymorphic (P) ranged from 0.125 to 0.353 (mean, 0.213). This figure also agrees with the value of 0.202 that Selander (1976) listed as an average value for rodents.

Rodgers' coefficient of genic similarity (S) between the Guadalupe sample and the other three populations studied is less than 0.93, whereas those between the other three all are more than 0.94. This relationship is more pronounced in Nei's identity coefficient, where the values separating the Guadalupe sample and the remaining populations are less than 0.96; those between the other three are greater than 0.98. Coefficients of similarity generally range from 0.90 to 1.00 for conspecific populations of rodents, although in strongly divergent populations of *Peromyscus polionotus* on Florida's barrier islands, the average similarity value drops to 0.84 (Selander and Johnson, 1973).

Other biochemical studies comparing subspecies have yielded Rodgers' similarity values of 0.769 in *Mus musculus* (Selander *et al.*, 1969), 0.89 to 0.95 in *Peromyscus boylii*, and 0.75 to 0.79 in *P. polionotus* (Avise *et al.*, 1974*a*), and 0.86 in *P. eremicus* (Avise et al., 1974*b*). The low values for *Mus* in reality could be due to the fact that the two subspecies studied are incipient species, whereas those reported for *P. polionotus* result from comparison of a subspecies occurring on Florida's barrier islands to a mainland population. The values obtained in this study ranged from 0.9119 to 0.9572, and, because these are well within the range of values reported for other rodent subspecies, would seem to indicate that the populations examined are no more differentiated than would be expected on the basis of their current taxonomic rank. Unfortunately, no data from other microtines are available for comparison.

Electrophoretic analysis revealed a close association among all four populations studied, but also indicated that there could be some slight differences between the Guadalupe population and the remaining three.

Inconsistencies in the morphometric and electrophoretic analyses result from two different approaches to the same problem and are to be expected. Electrophoretic analysis frequently fails to distinguish between or among subspecies that were described

initially on the basis of classical systematic criteria. Whether many subspecies are arbitrary units that do not reflect major gene differences or whether the resolution resulting from electrophoretic techniques is insufficient to detect the differences is not known at present, but both elements probably are involved (Avise, 1975). Contrasts between genic similarity and organismic similarity are not unknown, and it might be a general rule that organismic evolution and stuctural gene evolution proceed at virtually independent rates, as suggested by Wilson (1976). Therefore, in this study, electrophoretic evidence was accorded less consideration than were other data in determining systematic placement of the four populations.

Chronology

Findley (1969) explained many of the present mammalian distributional patterns in the southwestern United States on the basis of a series of boreal expansions and contractions in the late Pleistocene, the contractions leaving isolated boreal habitats and populations of certain mammals on scattered mountain ranges. The Mexican vole was probably widespread over the Mexican and Colorado plateaus during the cooler, more mesic pluvial periods of the late Pleistocene. The aridity of interpluvial periods caused fragmentation of the distribution of that vole, and with increasing aridity, those fragments became restricted to relatively mesic mountaintops (see also Smartt, 1977).

The chronology of late Pleistocene events in the southwestern United States was estimated by Wendorf (1975), based on pollen profiles from the Llano Estacado of West Texas. Evidence of a continuous boreal forest on the Llano as low as 1000 meters during the Early Tahoka Pluvial (17,000 BP) corresponds to a depression of vegetative zones of from 1300 to 1500 meters below their current levels. Because intermontane altitudes in the area under study range from about 1250 to 2050 meters, the presence of more or less continuous boreal forest there in the Early Tahoka seems probable.

The Late Tahoka Pluvial, dated approximately 11,500 BP, evidently was the most recent period of extensive pine-spruce forest in this area (Wendorf, 1975). This rather open forest covered much of the Southwest, as evidenced by the fact that the Llano Estacado was at least 50 per cent covered by spruce-pine forest (Wendorf, 1961). During this pluvial period, then, the Mexican vole was probably still widely distributed over Arizona, New Mex-

ico, and western Texas. *M. mexicanus* was present on the Llano Estacado at this time in extreme eastern New Mexico, at an elevation of 1350 meters in what is now Roosevelt County (Slaughter, 1975).

Fragmentation in the distribution of *Microtus mexicanus* probably began with the Scharbauer Interval (10,500 BP), which marked the end of the Late Tahoka Pluvial. This was a time of increasing aridity and was marked by a decline of the pine-spruce forest in the Southwest, although subsequent periods of resurgence of cooler and more moist conditions could have allowed temporary expansion of boreal habitats. The boreal extensions, both in time and geographic scope, since the late Tahokan are not presently known, but in all likelihood they were not of sufficient magnitude to connect previously isolated montane habitats. The climate of the Southwest has been one of increasing aridity since the Lubbock Subpluvial (9500 BP) and has undoubtedly resulted in the progressive geographic isolation of boreal elements on mountain tops.

The subspecies of *Microtus mexicanus* in this study almost certainly result from 9000 to 10,000 years of isolation (Slaughter placed the isolation of *M. mexicanus* at post-10,000 BP). The degree of differentiation observed between the two nominal taxa provides, therefore, some idea of the gross rate of evolution in this species, and, in a general way, underscores the relationship between geographic isolation and evolution in a small mammal.

Relative divergence times (T) for paired combinations of all populations were calculated following Nei's (1971*a*) technique using the expected number of amino acid differences (D) per protein that can be detected by electrophoresis. Nei (1971*b*) renamed the function D, calling it "genetic distance," and later presented a detailed description (Nei, 1972). Genetic distance is defined as $D = -\log_e I$, where I is Nei's identity value. The values of Nei's genetic distance are given in Table 5. Relative divergence times were then estimated using the formula $T = D_1/D_2$, where D_1 and D_2 are the genetic distance values for the population pairs in question, and are presented in Table 6. Using this table, it is possible to postulate the order of divergence of the four populations by listing the population pairs from one column in order of increasing divergence times. This suggests the following sequence of events. Isolation of the Guadalupe population (4) occurred first. It initially was separated from the Beartrap population (1), followed quickly by separation from the 4th of July (2) and South Fork (3) popula-

TABLE 6.—*Matrix of relative divergence times (T). Parentheses indicate exceptions from the general order discussed in the text. Pairs of populations compared on both the ordinate and abcissa are the same as coded in Fig. 1.*

D_1	1,2	1,3	1,4	2,3	2,4	3,4
D_2						
1,2	1.00	1.32	4.15	0.57	4.00	3.79
1,3	0.75	1.00	3.14	0.43	3.02	2.86
1,4	0.24	0.32	1.00	0.137	(0.80)	(0.91)
2,3	1.75	2.31	7.25	1.00	(6.08)	(6.63)
2,4	0.25	0.33	1.04	0.143	1.00	0.95
3,4	0.26	0.35	1.09	0.15	1.05	1.00

tions. The next population to be isolated was that at Beartrap. It was first separated from South Fork, then from 4th of July. The last separation isolated the 4th of July and the South Fork populations. It can be seen from the geographic relationships of the four study areas (Fig. 1), that the isolation of Guadalupe from South Fork probably occurred at about the same time as separation of the Guadalupe-4th of July and of the Guadalupe-Beartrap populations.

Inasmuch as the calculation of relative time of divergence is intimately related to Nei's identity value (I), it is not surprising that these values correlate perfectly with the hypothetical sequence (derived from observed divergence of characters) of isolation of the four populations studied. The highest identity values are found between those populations theoretically in contact for the longest period of time. Rodgers' similarity values (S) indicate the same situation, except that they suggest that the Guadalupe population was in contact with the Beartrap population for a slightly longer period than it was with either the South Fork or the 4th of July populations.

Although the calculation of relative divergence times can offer only gross estimates of temporal isolation, the sequence of events indicated by these data agrees with what would be expected from examination of the geologic evidence. Other recent studies on mammals have shown that Nei's evolutionary divergence time does correlate well with both fossil and morphological evidence (Nevo *et al.*, 1974; Zimmerman *et al.*, 1975; Kilpatrick and Zimmerman, 1976). As the Pleistocene glaciers retreated northward and the climate of the Southwest became warmer and drier, boreal taxa such as the Mexican vole were forced into isolation on montane refugia. Such restrictions evidently first took place on the southernmost Guadalupe Mountains, was followed by isolation of

the Beartrap population west of the Rio Grande on the San Mateo Mountains, and ended with isolation of the 4th of July and South Fork populations on the Manzano and Sacramento mountains, respectively. It is significant to note that the population thought to have been isolated for the longest period of time is the one showing the greatest degree of genic and morphological differentiation.

Results of this study indicate that *Microtus mexicanus* is an evolutionarily conservative microtine. Only slight change evidently has taken place in isolated populations of the species over a time span in which several European microtines have developed well-marked karyotypic and morphologic differences. No other North American microtine has been the subject of a detailed systematic investigation, however, and the Mexican vole could prove to be an exceptionally conservative species.

Taxonomic Conclusions

Subspecific differentiation of *Microtus mexicanus* in the region of study undoubtedly has occurred since the isolation of populations on mountain ranges in the late Pleistocene. The current subspecific boundries were established by Bailey (1932), with the Rio Grande designated as the line of demarcation between *M. m. mogollonensis* (occurring in the mountains to the west of the river) and *M. m. guadalupensis* (inhabiting montane areas to the east). This arrangement seems logical in a geographic sense, and places the Beartrap sample (San Mateo Mountains) in the subspecies *mogollonensis* and the remaining three samples (4th of July, Manzano Mountains; South Fork, Sacramento Mountains; and Guadalupe, Guadalupe Mountains) in the subspecies *guadalupensis*.

Results of the sperm and morphometric analyses demonstrate, however, that the 4th of July sample bears a closer relationship to the Beartrap sample than it does to either of the other two populations. Therefore, the 4th of July population is here transferred to the subspecies *M. m. mogollonensis*. The South Fork and Guadalupe populations remain referrable to *M. m. guadalupensis*.

There exists, however, a certain degree of divergence between the Guadalupe and South Fork samples, as evidenced by both cranial characters and values of electrophoretic similarity. Considering the isolated nature of these two populations and the degree of divergence exhibited, I conclude that they constitute an example of incipient subspeciation. The Guadalupe Mountains are

lower in altitude and considerably drier than are the other three mountain masses. This results in extremely restricted areas of habitat for Mexican voles and severely limits the population size. The Guadalupe sample thus represents a population with several of the attributes typically thought to contribute to evolutionary divergence. Geographic isolation, small population size, occupancy of a heterogenous habitat dividing this population into several smaller demes, and a harsh or stringent environment relative to other conspecific populations, might be reflected in the degree of divergence found in the Guadalupe population of *Microtus mexicanus.*

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