Syst. Zool., 35(3):311–324, 1986

CHROMOSOMAL, GENIC, AND MORPHOLOGIC VARIATION IN THE AGILE KANGAROO RAT, *DIPODOMYS AGILIS* (RODENTIA: HETEROMYIDAE)

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Abstract.—Chromosomal, genic, and morphologic variation was examined among populations of *Dipodomys agilis* from southern California and Baja California, including populations previously recognized as *D. antiquarius, D. paralius,* and *D. peninsularis.* Populations with a diploid chromosomal number of 62 and a fundamental number of 110 were found north of San Gorgonio Pass, and those possessing a diploid number of 60 and a fundamental number of 116 were found to the south. Genic data also indicated a similar separation of northern and southern populations. Analyses of morphologic data revealed that populations in the north were significantly larger than those in the south for several characters. Considering the three data sets, there is considerable differentiation of populations north of San Gorgonio Pass from those to the south. Apparently, the terrain at San Gorgonio Pass, and possibly human habitation, has prevented genetic exchange between these populations for a sufficient period for differences between them to accumulate. [Karyotypic variation; electrophoresis; morphologic variation; kangaroo rat; *Dipodomys*; Heteromyidae.]

Morphologic variation and the taxonomic relationships of the *heermanni* group of kangaroo rats have received considerable attention (Huey, 1951, 1962; Lackey, 1967; Csuti, 1971; Best and Schnell, 1974; Stock, 1974; Best, 1978, 1981a, b, 1983a, b; Schnell et al., 1978). Best (1983b) summarized these studies, assessed morphologic variation of the agile kangaroo rat (*Dipodomys agilis*), and emphasized differences in ecology, morphology, and chromosomes within this species. His analysis revealed that chromosomal differences (2N = 62, FN = 110 in northern populations; and 2N = 60, FN = 116 in southern populations) reported by Csuti (1971) and Stock (1974) were not concordant with morphologic differences among populations. Best (1983b) noted, however, that localities in California where Csuti and Stock collected specimens were geographically close to the populations that showed the greatest morphologic differentiation among those studied. He suggested that there may be chromosomal differences associated with some of the morphologic variation, but there was a paucity of specimens with karyotypic data, especially from Los Angeles, California, southward to near San Quintin and south of San Agustin, Baja California.

Thus, the distribution of the two chromosomal forms and the morphologic relationships between northern and southern populations of *D. agilis* have not been fully evaluated. Additional chromosomal data for several populations, including an analysis of the genic (electrophoretic) relationships among these populations, should provide a more thorough assessment of the patterns of differentiation in populations of this species. Therefore, our primary objective was to determine if patterns of chromosomal, genic, and morphologic variation are evolutionarily concordant or discordant within *D. agilis.* Because genic data have not been available for *D. antiquarius, D. paralius,* and *D. peninsularis* (considered to be conspecific with *D. agilis* by Best, 1978), we also examined relationships among these taxa.

MATERIALS AND METHODS

Chromosomal analysis.—Specimens were collected from 19 localities in California and Baja California (Appendix). The 84 animals used were live-trapped and processed in the field. Nonpreferentially stained karyotypes were prepared using a modification of the in vivo bone-marrow technique described by Lee and Elder (1980). Diploid determinations were based on examination of a minimum of 10 mitotic spreads from each specimen. Nomenclature of chromosome morphology follows Patton (1967).

Electromorphic analysis.—Genic variation of 170 individuals from 19 localities (Appendix) was assayed using standard starch-gel electromorphic methods (Selander et al., 1971). Gel types, tissues, electrical parameters, and staining procedures were as described by Yates and Greenbaum (1982) with the following modifications: glucose-6-phosphate dehydrogenase (Gd) was run on a tris-maleate gel (100 V for 6 h); sorbitol dehydrogenase (Sordh) and glycerol-3-phosphate dehydrogenase (Gpd) were run on a potassium phosphate gel (100 V for 7 h); and esterases (Es), tetrazolium oxidase (Sod), and albumin (Alb) were run on a lithium hydroxide gel (350 V for 5 h). Live-trapped specimens were processed in the field with tissue samples (heart, liver, kidney) preserved in liquid nitrogen. Sixteen presumptive genetic loci were assayed for animals from each population; abbreviations and Enzyme Commission numbers (Harris and Hopkinson, 1976) are: Es (3.1.1.1); Got-1, 2 (2.6.1.1); Gpd (1.1.1.8); Icd-1, 2 (1.1.1.42); Ldh-1, 2 (1.1.1.27); Mdh-1, 2 (1.1.1.37); Pgd (1.1.1.44); Pgm-1, 2 (2.7.5.1); Sordh (1.1.1.14); Sod (1.15.11); and albumin (Alb). Matrices of Nei's (1972) and Rogers' (1972) genetic distance values (D_N and D_R, respectively) were generated from allele frequencies for the various loci. Cluster analysis of the matrix of genetic distance values was performed using the UPGMA option of BIOSYS-1 (Swofford and Selander, 1981). Confidence limits (1 SE around branch point) placed on the UPGMA dendrogram follow the method of Nei et al. (1985). Clinal variation in electromorphic data was assessed by the correlation (Spearman rank correlation; Siegel, 1965) of allelic frequency, percent polymorphism (P), and mean heterozygosity (H) against latitude and sample size. Because *D. gravipes* is presumed to be a close relative of *D. agilis* (Lidicker, 1960), the former species also was included in our genetic survey along with *D. merriami*. A dendrogram depicting the hypothesized phylogenetic affinities between taxa was constructed using the distance-Wagner procedure (BIOSYS-1) produced by the outgroup method (Watrous and Wheeler, 1981; Farris, 1982; Rogers, 1984). A matrix of Rogers' genetic distance values (D_R) was used to construct the tree because the properties of this statistic conform to those of a metric (Sneath and Sokal, 1973; Swofford and Selander, 1981).

Morphologic variation.—Five external and 14 cranial measurements were examined for 1,355 adult specimens of *D. agilis* (718 males, 637 females). Characters used, methods of measuring, and aging techniques follow Best (1978, 1983b). Specimens used in this study were added to 12 of the OTUs studied by Best (1983b). OTU 7 of Best (1983b) corresponds to population 1 herein, his OTU 8 to population 2, OTU 11 to 3, OTU 13 to 4, the southern part of OTU 12 to 5, OTU 17 to 6, OTU 21 to 7, OTU 22 to 8, OTU 23 to 9, OTU 27 to 10 (previously *D. paralius*), OTU 30 to 11 (previously *D. antiquarius*), and OTU 29 to our population 12 (previously *D. peninsularis*; Appendix).

Character heterogeneity (between sexes and among the 12 populations) was tested using a single classification analysis of variance, and a sums-of-squares simultaneous test procedure (SS-STP) was used to determine maximally-nonsignificant subsets (Gabriel and Sokal, 1969). Mean measurements of each character for each population were used in the multivariate procedure (except discriminant function analyses). These characters were standardized (mean of 0 and a standard deviation of 1) across populations, and distance matrices (Sneath and Sokal, 1973) were calculated. Clusters of populations were obtained with the unweighted pair-group method using arithmetic averages (UPGMA). Principal components were calculated from a correlation matrix among characters, and

TABLE 1. Allele frequencies and genetic variability at 11 polymorphic loci in 12 populations of *D. agilis*, *D. gravipes*, and *D. merriami*.[a]

Locus	Wrightwood (17)[b]	Devore (4)	Big Bear City (7)	Banning (4)	Hemet (34)	Warner Springs (7)	Laguna Hanson (7)	Valle de Trinidad (38)	Colonia Guerrero (2)	Santa Catarina (41)	San Borja (7)	Rosarito (11)	*D. gravipes* (2)	*D. merriami* (10)
Sordh-1	a	a	a (.93)	a	a	a	a (.93)	a (.97)	a	a	a	a	a	
			b (.07)				b (.07)							b
								c (.03)						
Gpd-1	a	a	a	a	a	a	a	a	a	a	a (.86)	a (.86)	a	a
											b (.14)	b (.14)		
Icd-2	a	a	a (.86)		a (.04)		a (.86)	a (.80)	a	a	a	a	a	a
			b (.14)	b	b (.96)	b	b (.14)	b (.20)						
Ldh-1	a (.94)	a	a	a	a (.91)	a	a	a	a	a	a	a	a	a
	b (.06)				b (.09)									
Ldh-2	a (.94)	a (.75)	a	a	a	a	a (.93)	a (.98)	a	a	a	a	a	a
	b (.06)	b (.25)					b (.07)	b (.01)						
								c (.01)						
Es-1	a (.12)				a (.18)	a (.29)	a	a (.50)		a (.27)	a (.57)	a (.09)		
	b (.18)		b (.86)	b (.75)	b (.57)	b (.29)		b (.26)	b	b (.73)	b (.43)	b (.91)	b	
	c (.70)	c	c (.14)	c (.25)	c (.25)	c (.42)		c (.18)						
														d
								e (.06)						
Es-2		a (.25)		a (.75)	a (.54)	a	a	a (.97)	a (.50)	a (.98)	a	a		
		b (.25)			b (.10)			b (.03)						
									c (.50)					
										d (.02)				
	e (.71)	e (.25)	e	e (.25)	e (.21)									
	f (.29)	f (.25)			f (.15)									
													g	g
Pgm-1	a (.82)	a	a	a	a	a	a	a (.97)	a (.50)	a (.98)	a	a		
								b (.03)		b (.02)				b
	c (.18)								c (.50)					
													d	
Sod-1	a	a	a	a	a	a	a	a	a	a	a	a	a	
														b (.10)
														c (.90)
Pgd-1	a (.29)			a (.25)	a (.65)	a (.71)	a (.71)	a (.73)		a (.27)				
		b (.25)					b (.29)	b (0.1)						
	c (.71)	c (.75)	c	c (.75)	c (.35)	c (.29)		c (.26)	c	c (.71)	c	c	c	
										d (.02)				d
Alb-1	a	a	a (.86)	a	a	a	a	a	a	a (.98)	a	a (.91)	a	a
										b (.02)		b (.09)		
			c (.14)											
A	1.4	1.3	1.3	1.2	1.5	1.2	1.3	1.7	1.1	1.4	1.1	1.2	1.0	1.0
P	37.5	18.8	25.0	18.8	31.3	12.5	25.0	43.8	12.5	31.3	12.5	18.0	0.0	6.3
H	0.0	0.03	0.01	0.0	0.02	0.0	0.02	0.01	0.0	0.0	0.02	0.02	0.0	0.0

[a] *A* = number of alleles per locus; *H* = mean proportion of 16 loci heterozygous per individual; *P* = proportion of 16 loci polymorphic per population.

[b] Numbers in parentheses below localities are sample sizes.

projections of the populations were plotted on the first three components. A shortest minimally-connected network was computed from the original matrix of distances between populations. Correlation matrices and dendrograms were used to elucidate correlations among characters. Discriminant function analysis was used to characterize the extent of morphologic divergence among the 12 populations, and between the three northern (populations 1–3) and eight southern (populations 4–12) populations. This grouping was based upon the occurrence of 2N = 62 individuals in the north, and 2N = 60 individuals in the south. Geographic variation was assessed using discriminant function analysis (SPSS-X; SPSS Inc., 1983) by constructing isophene contour maps of overall similarity in cranial morphology based on *P*-values values of intergroup multivariate *F*-statistics (Hafner et al., 1983).

Interspecific comparisons were made among the 12 populations of *D. agilis, D. merriami,* and *D. gravipes.* Distance matrices were calculated using standardized data and phenograms were constructed. Data for *D. merriami* were obtained from 849 adult specimens from throughout the range of the species, whereas data for *D. gravipes* were taken from means of 110 adults presented by Best (1983a).

All specimens used in the chromosomal and genic studies were prepared as vouchers and are housed in The University of New Mexico Museum of Southwestern Biology. Statistical analyses were performed using the IBM computer system at The University of New Mexico Computation Center and the following programs: SAS (SAS Institute Inc., 1982), UNIVAR (written by D. M. Power), NT-SYS (Rohlf et al., 1974), and SPSS-X (SPSS Inc., 1983).

RESULTS

Chromosomal variation.—Karyotypic analysis revealed a consistent 2N and FN difference between populations of *D. agilis* north of San Gorgonio Pass (Wrightwood, Big Bear City, Devore; 2N = 62, FN = 110), and specimens from the nine populations south of this geographic discontinuity (2N = 60 and FN = 116). This is in agreement with the previous findings of Csuti (1971) and Stock (1974). The 2N = 60 populations had karyotypes composed of 58 biarmed autosomes, a metacentric *X* chromosome, and an acrocentric *Y* chromosome. The 2N = 62 populations had 56 biarmed autosomes, four uniarmed autosomes, a metacentric *X* chromosome, and an acrocentric *Y* chromosome. No intrapopulation karyotypic polymorphisms were found, and no indication of chromosomal intergradation was evident.

Interspecific genic variation and differentiation.—Allelic frequencies for the 11 loci (69%, $n = 16$) that exhibited variation within or among species of kangaroo rats are given in Table 1. Five (31%) loci were monomorphic and fixed for the same allele in all samples (Icd-1, Got-1, Got-2, Mdh-1, Mdh-2). No polytypic loci were found in any population of *D. agilis.* Although four populations of *D. agilis* had unique alleles at one or more loci, none of these electromorphs were fixed (Big Bear City, Alb-1; Valle de Trinidad, Sordh-1, Ldh-2, Es-1; Colonia Guerrero, Es-2; Santa Catarina, Es-2). Several of these unique alleles occurred at a frequency of less than 0.10 and, therefore, their occurrence is primarily sample-size dependent. There was no clinal variation in any of the polymorphic loci sampled as indicated by the lack of a statistically significant correlation between the frequency of various alleles and geographic position (latitude) of sampled populations ($r_s \leq 0.50$, $P > 0.05$, $n = 12$).

Dipodomys agilis had one unique allele at the Pgm-1 locus that occurred in every population in high frequency ($\bar{x} = 0.94$, range = 0.50–1.00). *Dipodomys gravipes* also had a unique allele at the Pgm-1 locus that was fixed. *Dipodomys merriami* was characterized by three unique alleles, one at the Es-1 locus (fixed) and two at the Sod-1 locus.

A matrix of genetic distance values (D_N and D_R) for the 12 populations of *D. agilis, D. gravipes,* and *D. merriami* at 16 loci is shown in Table 2. Average genetic distance (D_N) between populations of *D. agilis* was 0.089, and ranged from 0.009 (Rosarito versus Santa Catarina) to 0.197 (Laguna Hanson versus Big Bear City), compared to an average of 0.190 (range = 0.101–0.290) between populations of *D. agilis* and *D. gravipes,* and 0.463 (range = 0.417–0.535) between populations of *D. agilis* and *D. merriami.* The genetic distance (D_N) coefficient between *D. gravipes* and *D. merriami* was 0.369. *Dipodomys gravipes* was genetically most similar to *D. agilis* from Colonia Guerrero ($D_N = 0.101$). These two taxa are sympatric at this locality; although sample sizes are small (Table 1), no evidence of hybridization was detected.

A phenogram based on Nei's genetic distance values (D_N) suggests two primary genetic units within the range of *D. agilis* (Fig. 1). These groupings consist of a northern unit (Wrightwood, Devore, Big

TABLE 2. Matrices of Rogers' genetic distance values (above the diagonal) and Nei's genetic distance coefficients (below) for 12 populations of *D. agilis, D. gravipes,* and *D. merriami.*

Population	1	2	3	4	5	6	7	8	9	10	11	12	*D. gravipes*	*D. merriami*
1 Wrightwood	—	.085	.117	.157	.159	.178	.174	.141	.140	.116	.140	.152	.187	.353
2 Devore	.027	—	.145	.168	.174	.187	.171	.151	.156	.128	.133	.144	.206	.368
3 Big Bear City	.045	.090	—	.136	.169	.205	.190	.161	.117	.117	.126	.100	.156	.382
4 Banning	.128	.136	.098	—	.056	.070	.175	.131	.152	.099	.134	.122	.213	.416
5 Hemet	.116	.136	.119	.016	—	.052	.155	.105	.185	.128	.160	.162	.243	.409
6 Warner Springs	.153	.152	.183	.030	.015	—	.119	.072	.209	.122	.140	.156	.271	.417
7 Laguna Hanson	.137	.143	.197	.147	.105	.084	—	.057	.199	.107	.109	.145	.261	.374
8 Ville de Trinidad	.091	.098	.133	.084	.059	.049	.018	—	.162	.071	.085	.113	.225	.365
9 Colonia Guerrero	.096	.117	.073	.110	.139	.175	.168	.105	—	.097	.107	.082	.108	.355
10 Santa Catarina	.087	.091	.080	.078	.088	.097	.066	.027	.043	—	.050	.044	.159	.357
11 San Borja	.093	.089	.092	.097	.116	.117	.070	.043	.058	.013	—	.036	.170	.373
12 Rosarito	.103	.101	.073	.082	.114	.129	.117	.062	.036	.009	.016	—	.145	.384
D. gravipes	.166	.198	.142	.210	.234	.290	.282	.213	.101	.145	.162	.139	—	.310
D. merriami	.417	.440	.458	.533	.511	.535	.449	.433	.432	.431	.455	.469	.369	—

Bear City) distributed through the Petran Montane Coniferous Forest (Brown and Lowe, 1980) and chaparral habitats associated with the San Gabriel and San Bernardino mountains as well as adjacent foothills of the Los Angeles Basin north of San Gorgonio Pass. The second relatively undifferentiated genetic assemblage (containing taxa previously recognized as *D. agilis, D. antiquarius,* and *D. peninsularis*) extends throughout the chaparral and coastal scrub habitats of the Los Angeles Basin in southern California and northern Baja California, and inland into the chaparral-covered foothills of the Sierra San Pedro Martir.

Electrophoretic differentiation among these two inferred genetic units is due not to fixation for alternative alleles, but to the presence of unique alleles and several major shifts in the frequency of allelomorphs at the geographic borders of these units. For example, the frequency of Icd-2a in populations north of San Gorgonio Pass ranges from 0.86 to 1.00. In populations south of the pass and north of the Sierra de Juarez, the frequency of this allele ranges from zero to 0.04; however, in populations south of Warner Springs the frequency of Icd-2a is high (range = 0.80–1.00). Conversely, in populations north of San Gorgonio Pass and south of Warner Springs, the frequency of Icd-2b is low (range = 0–0.14 and range = 0–0.20 for the two populations, respectively), yet the frequency of this allele in populations associated with the coastal lowlands of the Los Angeles Basin is high (range 0.96–1.00). Moreover, there is an abrupt change in the frequency of Es-2a between the northern unit (range = 0–0.25) and the southern unit (range = 0.50–1.00, Table 1).

Intralocality differentiation.—Average number of alleles per locus (A) in populations of *D. agilis* ranged from 1.1 to 1.7; the average was 1.3 (±0.14 SE; Table 1). These values are similar to those reported for most species of kangaroo rats (Johnson and Selander, 1971), which ranged from

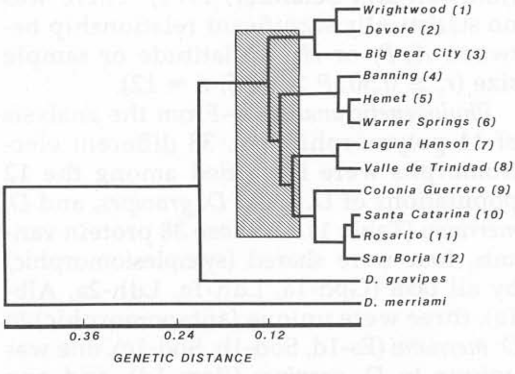

FIG. 1. Phenogram produced by UPGMA clustering of Nei's genetic distance coefficients (D_N). Shaded area represents one standard error around branch point of northern and southern karyotypic groups. F-value (Farris, 1972) is 2.528, percent standard deviation (Fitch and Margoliash, 1967) 37.064, and cophenetic correlation 0.962.

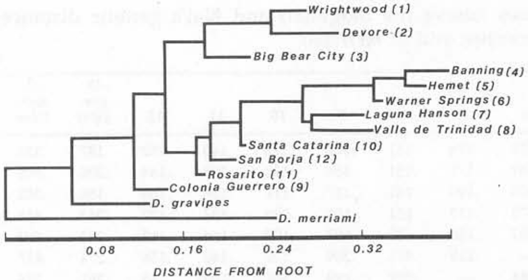

FIG. 2. Distance-Wagner tree rooted using outgroup method. *F*-value is 4.867 (Farris, 1972), percent standard deviation (Fitch and Margoliash, 1967) 45.4, and cophenetic correlation coefficient 0.906. Units are in Rogers' D_R-values (Rogers, 1972).

1.0 (*D. panamintinus*) to 1.2 (*D. nitratoides*), and averaged 1.1. The percentage of polymorphic loci per population of *D. agilis* ranged from 12.5% to 43.8%; the average over all populations was 24% (Table 1). Estimates of polymorphism in the present study differed considerably from those reported by Johnson and Selander (1971). Based on 17 loci (esterases not included), Johnson and Selander (1971) reported a P estimate of 8% per population, and a range of P from zero (*D. panamintinus, D. elator*) to 22% (*D. nitratoides*). Mean heterozygosity ranged from zero in five populations of *D. agilis* to 0.03 in the Devore sample ($\bar{x} = 0.01$). These values are similar to those reported for other species of *Dipodomys* (Johnson and Selander, 1971). There was no statistically significant relationship between A, P, or H and latitude or sample size ($r_s \leq 0.50, P > 0.05, n = 12$).

Phylogenetic analysis.—From the analysis of 11 polymorphic loci, 38 different electromorphs were identified among the 12 populations of *D. agilis, D. gravipes*, and *D. merriami* (Table 1). Of these 38 protein variants, four were shared (symplesiomorphic) by all taxa (Gpd-1a, Ldh-1a, Ldh-2a, Alb-1a), three were unique (autapomorphic) to *D. merriami* (Es-1d, Sod-1b, Sod-1c), one was unique to *D. gravipes* (Pgm-1d), and one was unique to all populations of *D. agilis* (Pgm-1a). Although two synapomorphic alleles group *D. gravipes* and all populations of *D. agilis* (Sordh-1a, Sod-1a), only one allele was shared exclusively between *D. gravipes* and *D. merriami* (Es-2g). Six alleles were unique to some, but not all, populations of *D. agilis* (Big Bear City, Alb-1c; Valle de Trinidad, Sordh-1c, Ldh-2c, Es-1e; Colonia Guerrero, Es-2c; Santa Catarina, Es-2d). Three alleles were symplesiomorphic between *D. merriami* and one or more populations of *D. agilis* (Sordh-1b, Pgm-1d, Pgd-1d); and two alleles were synapomorphic between *D. gravipes* and one or more populations of *D. agilis* (Es-1b, Pgd-1c). The remaining 14 alleles were shared by one or more populations of *D. agilis* (Gpd-1b, Icd-2b, Ldh-1b, Ldh-2b, Es-1a, Es-1c, Es-2a, Es-2b, Es-2e, Es-2f, Pgm-1c, Pgd-1a, Pgd-1b, Alb-1b). From these data a matrix of Rogers' distance coefficients (D_R-values) and a dendrogram were constructed using the distance-Wagner parsimony procedure (Fig. 2). This dendrogram reveals a closer phylogenetic relationship between *D. gravipes* and *D. agilis* than either taxon is to *D. merriami*. Except for the Colonia Guerrero sample ($n = 2$), the populations of *D. agilis* form two lineages. First, a group composed entirely of 2N = 62 chromosomal forms restricted in distribution to the chaparral and coniferous forest vegetation north of San Gorgonio Pass, California (Wrightwood, Big Bear City, Devore); and a second group composed of 2N = 60 chromosomal forms distributed south of San Gorgonio Pass and throughout northern Baja California. A distance-Wagner tree rooted midway between the two terminal taxa separated by the greatest patristic distance also was examined; however, this tree did not alter the conclusions reached from consideration of Figure 2. However, while the UPGMA phenogram does not suggest relationships different from those depicted by the distance-Wagner tree, except that the Colonia Guerrero sample clusters with adjacent populations of *D. agilis* rather than individually, confidence limits (Fig. 1) associated with the branch point delineating the northern and southern groups precludes a definitive statement regarding species-level differentiation.

Morphologic variation.—Results of the analyses of sexual dimorphism were sim-

TABLE 3. Character loadings of the first three principal components based on population means of 19 morphologic characters.

Character (abbreviation)	Sex	Principal components I	II	III
External				
Total length (TOT)	♂	.951	−.279	.004
	♀	.969	−.182	.125
Body length (BL)	♂	.885	−.345	−.243
	♀	.966	−.026	−.019
Tail length (TL)	♂	.942	−.229	.103
	♀	.915	−.265	.200
Hind foot length (HFL)	♂	.958	−.074	.151
	♀	.957	−.221	.037
Ear length (EL)	♂	.756	−.336	.063
	♀	.824	−.170	−.001
Cranium				
Basal length (BLS)	♂	.981	−.046	.102
	♀	.952	−.047	−.085
Greatest length (GLS)	♂	.893	.294	.229
	♀	.881	.286	.173
Maxillary arch spread (MAS)	♂	.829	.412	−.077
	♀	.804	.441	−.169
Interorbital width (IOW)	♂	−.083	.398	.790
	♀	−.535	.703	.200
Nasal length (NL)	♂	.961	−.142	−.001
	♀	.962	−.045	.008
Intermaxillary width (IMW)	♂	.446	.561	−.227
	♀	.608	.700	−.049
Alveolar length (AL)	♂	.342	.569	−.686
	♀	.089	.871	−.304
Lacrimal length (LAC)	♂	.687	.250	−.575
	♀	.446	.502	−.715
Maxillary arch width (MAW)	♂	−.010	.724	.052
	♀	.333	.729	.082
Basioccipital length (BOL)	♂	.559	.131	.206
	♀	.726	−.177	.367
Greatest depth (GDS)	♂	.254	.823	.204
	♀	.019	.867	.355
Greatest width (GWS)	♂	−.160	.810	.186
	♀	−.149	.860	.395
Zygomatic width (ZW)	♂	.941	.153	−.003
	♀	.902	.067	−.114
Nasal width (NW)	♂	.825	−.225	.415
	♀	.893	−.152	.090
Percent of total variance	♂	53.7	18.2	10.2
	♀	56.3	23.9	6.4

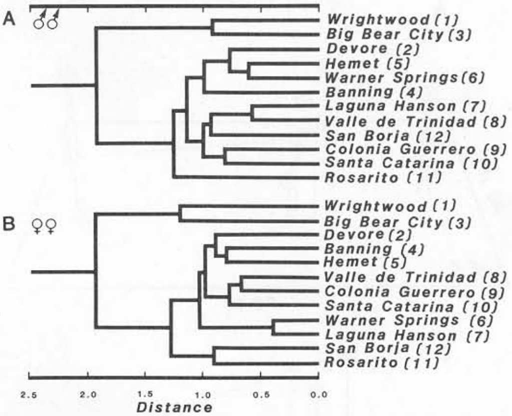

FIG. 3. Phenograms depicting overall morphologic relationships among populations of kangaroo rats. UPGMA phenograms constructed from distance matrices for (A) males and (B) females. Cophenetic correlation coefficients are 0.898 and 0.823 for A and B, respectively.

ilar to those previously observed (Best, 1983b); all characters except alveolar length and lacrimal length were dimorphic. All dimorphic characters were significantly different ($P \leq 0.001$) and correlations among characters were similar to those observed by Best (1983b). Interorbital width, intermaxillary width, maxillary arch width, greatest depth of cranium, and greatest width of cranium were the least correlated with other characters for males, and were separated from the remaining characters. The same was true for females, except alveolar length and lacrimal length were among the group of least correlated characters.

All characters, except interorbital width for both sexes and alveolar length for females, exhibited significant interlocality heterogeneity for both sexes. As indicated by the *F*-ratios, greatest length of cranium, maxillary arch spread, intermaxillary width, maxillary arch width, basioccipital length, and greatest depth of cranium for both sexes, and basal length of cranium, alveolar length, and lacrimal length for males exhibited the least interlocality variation (*F*-ratio < 8), whereas hind foot length, ear length, and basal length of cranium exhibited the greatest amount of interlocality heterogeneity for both sexes (*F*-ratio > 20). *F*-ratios for most characters were approximately the same for each sex, but males had values about twice that of females for tail length and greatest length of cranium, and females had considerably larger values for body length, basal length of cranium, and lacrimal length. With some exceptions (intermaxillary width, maxillary arch width, basioccipital length, greatest depth of cranium, and greatest

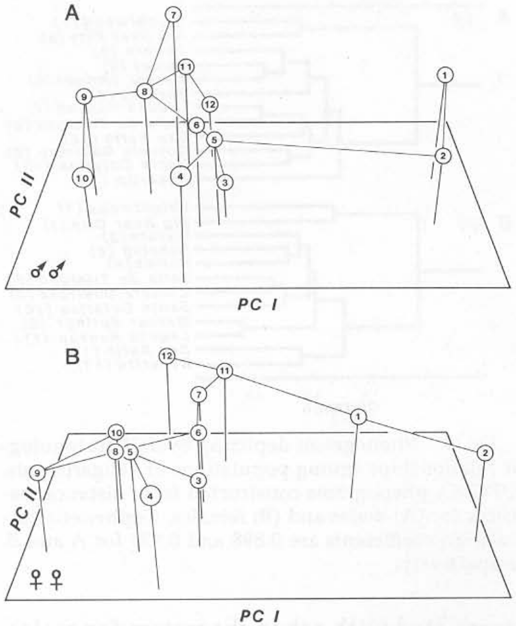

FIG. 4. Three-dimensional projections of populations onto first three principal component axes based on correlations of 19 morphologic characters for (A) male and (B) female *Dipodomys agilis*. Shortest simply-connected networks derived from matrix of distance coefficients.

width of cranium for both sexes and lacrimal length for females), the two northern populations (Wrightwood and Big Bear City) averaged larger for each character, whereas San Borja and Rosarito were among the largest means for intermaxillary width, greatest depth of cranium, and greatest width of cranium for both sexes and for maxillary arch width for females.

Geographic variation in several characters was significantly correlated with latitude for both sexes (TL, BL, HFL, EL, BOL, NL, GWS, NW; see Table 3 for character abbreviations), and with body length and ear length for males ($P \leq 0.05$, $n = 12$). All variables except greatest width of cranium exhibited a strong decrease in size with decreasing latitude. This north-to-south latitudinal pattern was especially pronounced in nasal width for both sexes ($r = 0.86$, $P \leq 0.01$; $r = 0.77$, $P \leq 0.01$, respectively). In all variables, except ear length and greatest width of cranium, abrupt changes in characters occurred between the two northern-high elevation populations (Wrightwood, Big Bear City) and the southern-lower elevation populations.

Phenograms for both sexes, constructed from distance matrices of the 19 characters, are presented in Figure 3. These phenograms for both sexes form two distinct clusters at about 1.9, with Wrightwood and Big Bear City well separated from the other populations. Distance phenograms that included *D. merriami* and *D. gravipes* showed populations 1 and 2 well separated from the rest of the *D. agilis* populations for both sexes. *D. gravipes* and *D. merriami* were widely divergent from the *D. agilis* populations and from each other. Because the relationships among the *D. agilis* populations were changed by including *D. gravipes* and *D. merriami,* only dendrograms without them are included as figures. Populations 1 and 2 were separated from the rest of the *D. agilis* populations at about 1.2 for both sexes. All *D. agilis* were separated from *D. gravipes* at about 2.1 for both sexes, and *D. gravipes* was separated from *D. merriami* at 2.5 for both sexes. Thus, there was not as much difference between the two clusters of *D. agilis* as between them and either *D. gravipes* or *D. merriami,* although all were well separated.

Loadings of characters on the first three principal components are presented in Table 3, and plots of populations on these components are depicted in Figure 4. Character correlations with component I are greater than 0.800 for basioccipital length in males, ear length in females, and 10 characters in both males and females (Table 3). Component I represents overall size in both sexes and accounts for most of the covariation among characters (Best, 1983b). For both sexes, populations 1 and 2 have the highest loadings along component I (Fig. 4). This component accounts for more than half of the phenetic variation (Table 3). The largest animals are in the northern portion of the range of *D. agilis.*

Principal component II has correlations greater than 0.500 with five characters for

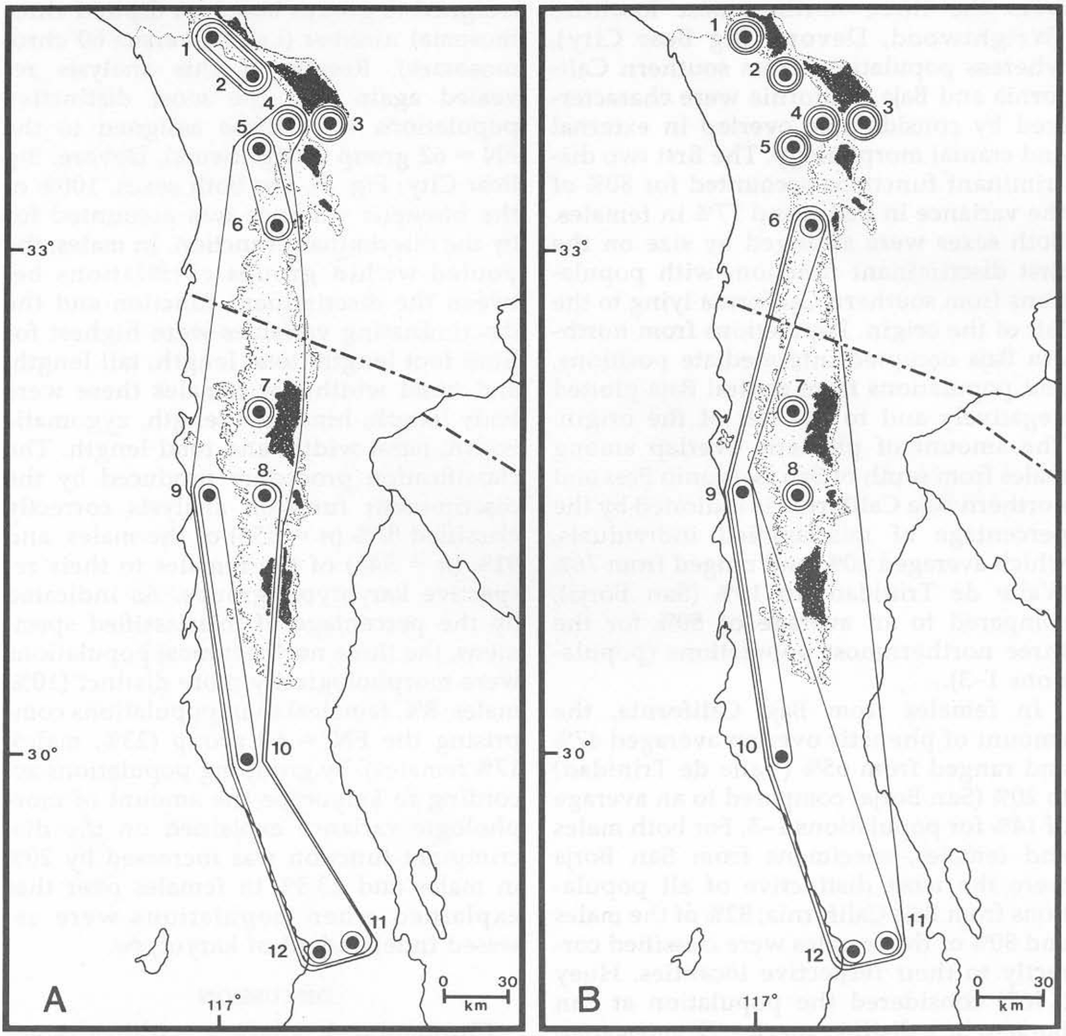

FIG. 5. Isophene contours of overall morphologic similarity among populations of *Dipodomys agilis* based on *P*-values of intergroup multivariate *F*-statistics (A = males, B = females). Contour lines refer to $P = 0.10$ (inside), $P = 0.05$ (middle), and $P = 0.01$ (outside).

both sexes and two additional characters for females (Table 3). The 18% of the variance accounted for by this component for males and 24% for females (Table 3) is shown in a rather uniform spread of populations across the component (Fig. 4).

Component III for males has loadings greater than 0.400 for interorbital width, alveolar length, lacrimal length, and nasal width for males, and lacrimal length for females. Only 10% of the phenetic variance is explained by this component for males and 6% for females (Table 3); no major pattern of ordination was evident along component III (Fig. 4). The three components explain more than 80% of the total character variation for each sex (Table 3). Thus, distortion of the phenetic distances between populations is relatively minor when the character space is reduced from 19 dimensions to 3.

Discriminant function analysis of both male and female kangaroo rats revealed that the most distinctive populations were from the three northernmost localities (Wrightwood, Devore, Big Bear City), whereas populations from southern California and Baja California were characterized by considerable overlap in external and cranial morphology. The first two discriminant functions accounted for 80% of the variance in males and 77% in females. Both sexes were arranged by size on the first discriminant function, with populations from southern California lying to the left of the origin. Populations from northern Baja occupied intermediate positions, and populations from central Baja plotted negatively and to the left of the origin. The amount of phenetic overlap among males from south of San Gorgonio Pass and northern Baja California is indicated by the percentage of misclassified individuals, which averaged 30%, and ranged from 76% (Valle de Trinidad) to 18% (San Borja), compared to an average of 50% for the three northernmost populations (populations 1–3).

In females from Baja California, the amount of phenetic overlap averaged 47% and ranged from 65% (Valle de Trinidad) to 20% (San Borja) compared to an average of 14% for populations 1–3. For both males and females, specimens from San Borja were the most distinctive of all populations from Baja California; 82% of the males and 80% of the females were classified correctly to their respective localities. Huey (1962) considered the population at San Borja to be a distinct species, *D. antiquarius*, but Best (1978) did not find sufficient difference between this taxon and adjacent populations to warrant specific-level recognition. For southern California, the most distinctive population of kangaroo rats was Devore (78% of the males and 100% of the females correctly classified). Morphologic relationships among populations are depicted in Figure 5. These geographic patterns of morphologic variation are in close agreement with the karyotypic and genic data sets.

To characterize the extent of morphologic divergence between the two karyotypic forms of *D. agilis*, discriminant function analysis was repeated on populations assigned to groups based on diploid chromosomal number (i.e., 62 versus 60 chromosomes). Results of this analysis revealed again that the most distinctive populations were those assigned to the FN = 62 group (Wrightwood, Devore, Big Bear City; Fig. 6). For both sexes, 100% of the phenetic variance was accounted for by the discriminant function. In males, the pooled-within groups correlations between the discriminant function and the discriminating variables were highest for hind foot length, total length, tail length, and nasal width; for females these were body length, hind foot length, zygomatic width, nasal width, and total length. The classification procedure produced by the discriminant function analysis correctly classified 87% ($n = 556$) of the males, and 91% ($n = 543$) of the females to their respective karyotypic groups. As indicated by the percentage of misclassified specimens, the three northernmost populations were morphologically more distinct (10%, males; 8%, females) than populations comprising the FN = 60 group (23%, males; 17% females). By grouping populations according to karyotype the amount of morphologic variance explained on the discriminant function was increased by 20% in males and 23.5% in females over that explained when populations were assessed independent of karyotype.

DISCUSSION

Chromosomal polytypy within the genus *Dipodomys* is rare (Stock, 1974; Csuti, 1979). Two karyotypes have been identified within *D. agilis* (Csuti, 1971; Stock, 1974): a northern karyotypic form (2N = 62, FN = 110) found north of San Gorgonio Pass in southern California, and a southern form (2N = 60, FN = 116) occurring south of San Gorgonio Pass into Baja California. Stock (1974) suggested that the southern California–Baja California populations were isolated from the parental northern populations of *D. agilis* only since the Wisconsin Pluvial (ca. 13,000 years BP) and have achieved their karyotypic and morphologic distinctness recently.

The 2N = 60 chromosomal form of *D.*

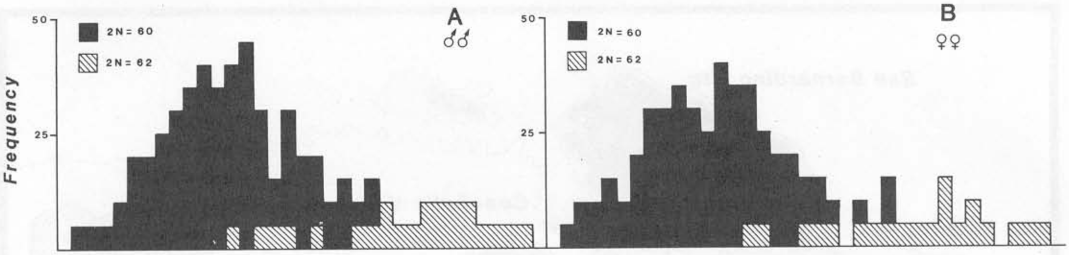

FIG. 6. Stacked histogram of distribution of (A) male and (B) female kangaroo rats along discriminant function based on morphologic characters and the extent to which they separate animals with different chromosome numbers.

agilis ranges from the lower slopes of the San Jacinto Mountains in southern California southward through Baja California. This southern form occurs from sea level to the higher elevations of the Sierra de Juarez and Sierra San Pedro Martir (1,200 m). Thus, the northern and southern populations are karyotypically and, apparently, ecologically distinct.

If these chromosomal forms are parapatric, then an occasional hybrid (2N = 61) would be expected; yet, no hybrids have been found. More extensive sampling in the region south of Devore and north of Banning (Fig. 7) is desirable. Devore, San Bernardino, and Banning lie approximately within the 830 m topographic contour running along San Gorgonio Pass, which separates the San Bernardino and San Jacinto mountains (Fig. 7). This hiatus is currently occupied by highways, human habitation, the San Gorgonio River, and the Colorado Aqueduct. Thus, the two chromosomal forms may be geographically and reproductively isolated. The San Gorgonio Barrier also has been implicated as a major causal factor in Plio-Pleistocene patterns of differentiation in Baja California herpetofauna, and as a Holocene barrier to dispersal in several peninsula-restricted Baja California reptiles (Murphy, 1983).

Results of the electromorphic analysis corroborate the general pattern of geographic variation indicated by the karyotypic data (i.e., the greatest amount of genic differentiation occurs between the Wrightwood, Big Bear City, and Devore populations, and the nine populations of *D. agilis* south of San Gorgonio Pass; Figs. 2, 3). The nature of this geographic pattern is characterized not by the presence of uniquely fixed electromorphic differences between northern and southern populations, but by a nonclinal pattern of genic variation derived from the presence of unique alleles and abrupt shifts in the frequency of several alleles across the San Gorgonio discontinuity.

Populations of *D. agilis* south of San Gorgonio Pass in California (Banning, Hemet, Warner Springs) and those from Baja California (Laguna Hanson, Valle de Trinidad, Colonia Guerrero, Santa Catarina, San Borja, Rosarito) appear to constitute two homogeneous genic units that are more similar to each other than either is to the northern unit (Figs. 2, 3). These populations occur throughout the low elevation Mediterranean Climatic and Vegetation Zone and in the Vizcaino Region of northern Baja California (Cody et al., 1983). Geographic discontinuities separating coastal-lowland and foothill populations of these two genic units do not approach the magnitude of the San Gorgonio Barrier. Thus, the comparatively low degree of genic uniqueness that characterizes populations of *D. agilis* south of San Gorgonio Pass is likely attributable to the absence of major geographic barriers between populations in that region.

The distance-Wagner tree (Fig. 3) revealed two basic features regarding the inferred phylogenetic affinities among populations of *D. agilis*, and among *D. agilis*,

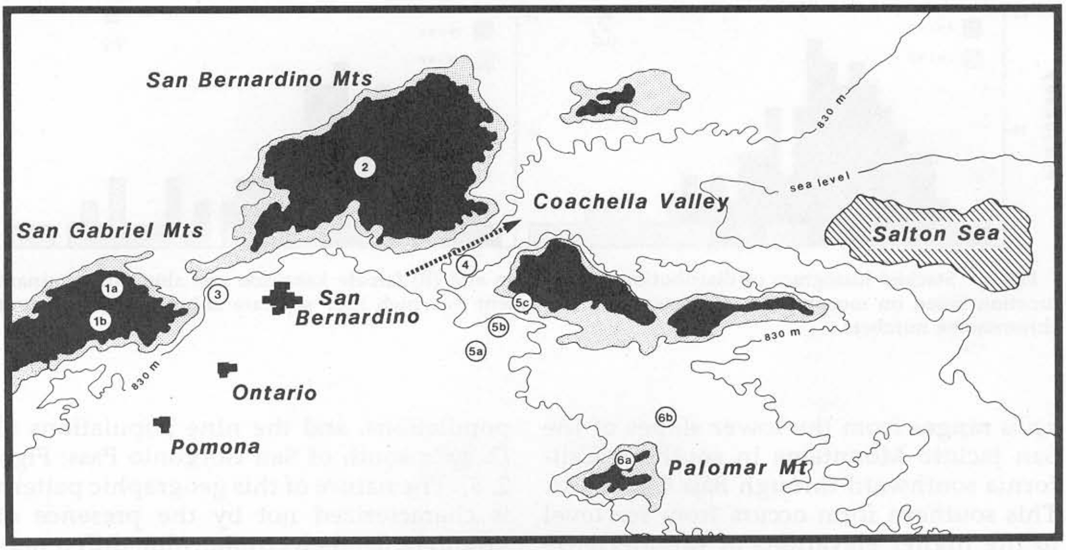

FIG. 7. Map of contact zone between two chromosomal forms of *D. agilis*. Extent of habitat above 1,525 m illustrated by shading. Region occupied by each city encompasses an area where existing human population is greater than 20,000. Numbered sampling sites correspond to those listed in Appendix. Arrow indicates location of San Gorgonio Pass.

D. gravipes, and *D. merriami*. First, two evolutionary lineages are suggested that correspond to a northern and southern form of *D. agilis*. Populations comprising these two lineages are separated geographically by the San Gorgonio Barrier, the largest geologic hiatus found within the range of *D. agilis*. This pattern of differentiation is congruent with the karyotypic data and the UPGMA phenogram. Second, *D. gravipes* appears to have shared a more recent common ancestor with *D. agilis* than *D. merriami*, as evidenced by the fact that *D. gravipes* differs from *D. agilis* by only one fixed allelomorph compared to three between the former two taxa and *D. merriami*. These relationships are consistent with those presented in previous studies (Grinnell, 1922; Setzer, 1949; Lidicker, 1960; Stock, 1974; Schnell et al., 1978).

Analyses of morphologic variation revealed a strong relationship between latitude and size for several characters. Best (1983b) found *D. agilis* in the northern and southern parts of its range were larger than those in the central portion. Thus, as latitude increased the size of several characters increased in the California and northern Baja California populations. Conversely, southern Baja California populations increased in size as latitude decreased. The populations examined herein were from the northern and central portions of the range of *D. agilis* and, as observed by Best (1983b), characters increased in size with increasing latitude. It seems that two patterns of differentiation are found within *D. agilis*. Based upon the present analyses and those of Best (1983b), we can speculate that the two forms of *D. agilis* are diverging morphologically in different ways at the extremes of their ranges.

Multivariate analyses of morphologic data indicated a clear separation of the two northern populations from the others. The San Gorgonio Barrier was the separating point for morphologic variation as well as chromosomal and genic variability. When *D. gravipes* and *D. merriami* were added to the data set, they were well separated from the populations of *D. agilis* by the analyses. The differences between *D. agilis* and these two species were greater than between the northern and southern populations of *D. agilis*. Thus, if species-level morphologic differences exist within *D. agilis*, they are not as marked as between *D. agilis* and *D. gravipes, D. agilis* and *D. merriami,* or *D. gravipes* and *D. merriami.* Our findings also support Best's (1978) conclusion that *D. antiquarius, D. paralius,* and *D. peninsularis* are conspecific with *D. agilis.*

No areas of sympatry were found that would have provided a test of biological species status. Although our data could be cited as providing support for recognition of these two chromosomal groups as evolutionary species (Wiley, 1981), we prefer to refrain at this time from inflating the taxonomy by affording species-level recognition to the northern and southern clusters, while calling attention to the pattern of divergence present in the species.

More intensive sampling may serve to elucidate morphologic differences between these chromosomal forms. Further, it is possible that examination of additional loci may help in more clearly delineating the northern from the southern chromosomal forms. Such an analysis would be useful, along with additional information on bacula, ecologic relationships, and an expanded analysis of other types of character sets in the potential zone of contact. Analyses of these data are necessary before a decision regarding the specific status of the two chromosomal forms of *D. agilis* can be made.

ACKNOWLEDGMENTS

We thank the numerous students who assisted us in collecting specimens, as well as Californian and Mexican authorities for granting collecting permits. This study was partially supported by grants from The National Science Foundation (GB-33062, GB-30814, DCB-8004685), National Institutes of Health (2-S06-RR-08139-10), and a Grant-in-Aid of Research from the Society of Sigma Xi.

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Received 17 May 1985; accepted 15 April 1986.

APPENDIX

Populations Sampled and Sample Sizes for Morphologic, Chromosomal, and Genic Analyses

Dipodomys agilis.—1, Wrightwood (sample size for morphologic analyses = 36 and 27, for males and females, respectively): (a) Angeles Nat. For., 6.4 mi W, 4.0 mi N Wrightwood (sample size for chromosomal analyses = 9, sample size for genic analysis = 8); (b) Angeles Nat. For., 11.2 mi W, 5.1 mi N Wrightwood (3, 9). *2, Big Bear City* (14, 18): 10 mi SE Big Bear City, Heart Bar Campground (6, 7). *3, Devore* (109, 80): 1 mi W Devore (3, 3). *4, Banning* (83, 82): 7 mi SE Banning (2, 4). *5, Hemet* (92, 87): (a) 5.3 mi N, 8.8 mi E Hemet (9, 14); (b) 7.3 mi N, 9.2 mi E Hemet (6, 16); (c) 6.3 mi N, 9.8 mi E Hemet (1, 4). *6, Warner Springs* (48, 32): (a) 6.7 mi NW (by road) Warner Springs (6, 4); (b) Cleveland Nat. For., Sourdough Springs (2, 3). *7, Laguna Hanson* (63, 54): Laguna Hanson (6, 7). *8, Valle de Trinidad* (145, 157): (a) 10.9 mi E, 20.0 mi S (by road) Valle de Trinidad (6, 8); (b) 19.9 mi E (by road) San Telmo (2, 4); (c) 16.0 mi S, 3 mi E Valle de Trinidad (2, 26). *9, Colonia Guerrero* (64, 51); 3 km SW Colonia Guerrero (4, 2). *10, Santa Catarina* (19, 25): (a) Rancho Santa Catarina (4, 26); (b) Santa Catarina Landing (2, 7). *11, San Borja* (22, 12): Mision de San Borja (3, 7). *12, Rosarito* (23, 12): 4.2 km NE Rosarito (8, 11).

Dipodomys gravipes.—Fifty-six males and 54 females from throughout range used in morphologic analysis.

Dipodomys merriami.—Four hundred forty males and 409 females from throughout range used in morphologic analyses.

Total number of specimens used in chromosomal analyses was 84, for genic analyses 170, and for morphologic analyses 2,314 (1,214 males and 1,100 females).