

## HYBRID ZONES IN *THOMOMYS BOTTAE* POCKET GOPHERS: GENETIC, PHENETIC, AND ECOLOGIC CONCORDANCE PATTERNS

JAMES L. PATTON, JOHN C. HAFNER, MARK S. HAFNER,  
AND MARGARET F. SMITH

*Museum of Vertebrate Zoology, University of California, Berkeley, California 94720*

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In a recent summary of geographic variability of both chromosomal and genic systems in *Thomomys bottae* pocket gophers, Patton and Yang (1977) presented data depicting extensive degrees of interpopulation genetic divergence. In several instances, adjacent or near adjacent pairs of populations were seen to differ by 10-15 fixed chromosomal rearrangements and to share less than 80% in overall allelic similarity in structural genes as measured by starch gel electrophoresis. Despite the enormity of observed interpopulation divergence (in many cases greater than that found between species of other mammals), available data suggested that these geographic segments retained the ability to interbreed. Evidence for this conclusion came from studies by other workers suggesting intergradation between several of the differentiated units based on the morphological intermediacy of some specimens. In no study, however, had the gross morphological indications of intergradation been fully supported by genetic analyses. The purpose of the present report is to provide a concomitant comparison of intergradation from a genetic, morphologic, and ecologic perspective between two of the most strongly differentiated, geographically adjacent units of *T. bottae* that were recognized by Patton and Yang (1977).

Pocket gophers of the conifer forest zones of the White and Sacramento mountains of south central New Mexico, described as *T. b. ruidosae* by Hall (1932), are characterized by moderate size, dark coloration, and a karyotype composed of nearly all biarmed autosomes ( $2n = 76$ ). This form meets *T. b. actuosus* Kelson

(1951) to the north and northeast. This race is larger in size and paler in coloration than *T. b. ruidosae* and has a diploid karyotype of 76 with 28-34 uniarmed autosomes. A survey of genic variation in *T. bottae*, which included several populations of each of these races, indicated that while the mean within race similarity values were high (Rogers'  $\bar{S}$ -value = .974 and .936 for *T. b. actuosus* and *T. b. ruidosae*, respectively), the mean between race similarity was relatively low ( $\bar{S} = .807$ , range .795-.824; Patton and Yang, 1977, and unpubl. data). Although Kelson (1951, p. 68) remarked on intergradation between the two races, a thorough analysis of this possibility has never been made. The extent of both chromosomal and genic differences between the two races might suggest extremely limited intergradation or none at all.

Since the level of reproductive compatibility between divergent populations is a key element both for understanding the relative evolutionary importance of chromosomal and genic change as well as for determining the systematic status of the varied *T. bottae*-group populations, we are making a concerted effort to examine contact zones from a genetic viewpoint. These data also permit an assessment of the relative resolving powers of both genetic and morphologic criteria in determining the consequences of hybridization. Until recently, hybrid zone analyses in many organisms have been examined largely from the viewpoint of external morphology. Hence, conclusions regarding the extent of reproductive compatibility between hybridizing populations have often been based on the criteria of zone

width and number of morphologically identified hybrids (e.g., Rudd, 1955; Thaler, 1968). These criteria need to be tested; it is possible that neither accurately reflects the actual extent of genic introgression between the populations in question.

#### MATERIALS AND METHODS

A total of 175 specimens of *Thomomys bottae actuosus* and *T. b. ruidosae* was used. Of these, 60 are from *T. b. ruidosae* populations outside a region of contact, 54 are *T. b. actuosus* from outside the zone, and 56 are individuals from within or adjacent to contact zones. All specimens are preserved as standard museum specimens (skin with skull) and are deposited in the collections of the Museum of Vertebrate Zoology, University of California, Berkeley (MVZ).

*Genetic analysis.*—Of the total sample, 99 individuals were karyotyped using standard methods (Patton, 1967). Tissue samples (blood, liver, and kidney) from all but seven individuals were available for separation of enzymes and other proteins by starch gel electrophoresis. Eighteen proteins encoded by 23 presumptive gene loci were analyzed according to procedures used in other studies (Patton et al., 1972; Patton and Yang, 1977).

*Morphological analysis.*—Assessment of overall morphological variation involved examination of both cranial and external morphometric characters and pelage color variables. For the former, 13 cranial measurements taken with dial calipers from cleaned skulls and four external measurements taken from specimen labels were utilized. Measurements are described in Cockrum (1962) unless otherwise defined here. These included total length (ToL), tail length (TaL), hind foot length (HF), ear length (EL), occipitonasal length (ON), basilar length of Hensel (BaL), zygomatic breadth (ZB), mastoid breadth (MB), least interorbital constriction (IOC), rostral length (RL), nasal length (NL), rostral width (RW), diastema length (DL), length of maxillary tooth row (MTRL), palatal width (PW, width across

palate at level of  $M^1$ ), bullar length (BuL, maximum length of tympanic portion of bulla), and bullar width (BuW, greatest width of exposed mastoid portion of bulla). Only specimens judged to be adult by closure of the supraoccipital-exoccipital and basisphenoid-basioccipital sutures were utilized in the analyses.

Variation in middorsal pelage color was examined by use of a Bausch and Lomb Spectronic 505 recording spectrophotometer equipped with a visible reflectance attachment. Values for dominant wavelength (=hue), relative brightness (=value), and excitation purity (=chroma or saturation) were computed for each specimen judged to have adult pelage.

Statistical methodology employed discriminant function analysis to assess the morphological relationship of known genetic hybrids to known parental reference populations. Males and females were treated separately for analysis of mensural characters due to secondary sexual variation. Computer analysis was performed with the SPSS (Statistical Package for the Social Sciences) programs on the University of California CDC 6400 computer.

*Field procedures and ecological analysis.*—To locate contact zones, transects were run between parental habitat types on the northern and northeastern flanks of the White Mountains. The distributional relationships of individuals of the two races were determined in the field by both pelage color and karyotype. All contact and near contact localities were sampled during a three week interval in August, 1976.

Ecological relationships across contact zones were examined by the following set of microhabitat data which was recorded from each trap site yielding a gopher: (1) nearest tree type(s); (2) distance to nearest tree; (3) density of shrub vegetation; (4) height of large annuals; (5) density of low grass cover; (6) distance from Nogal Creek; (7) depth of burrow; (8) percent of gravel in soil sample taken from burrow lining; and (9) brightness of soil. All ecological data were taken on the same day in August, 1976. Variables 3, 5, and 9

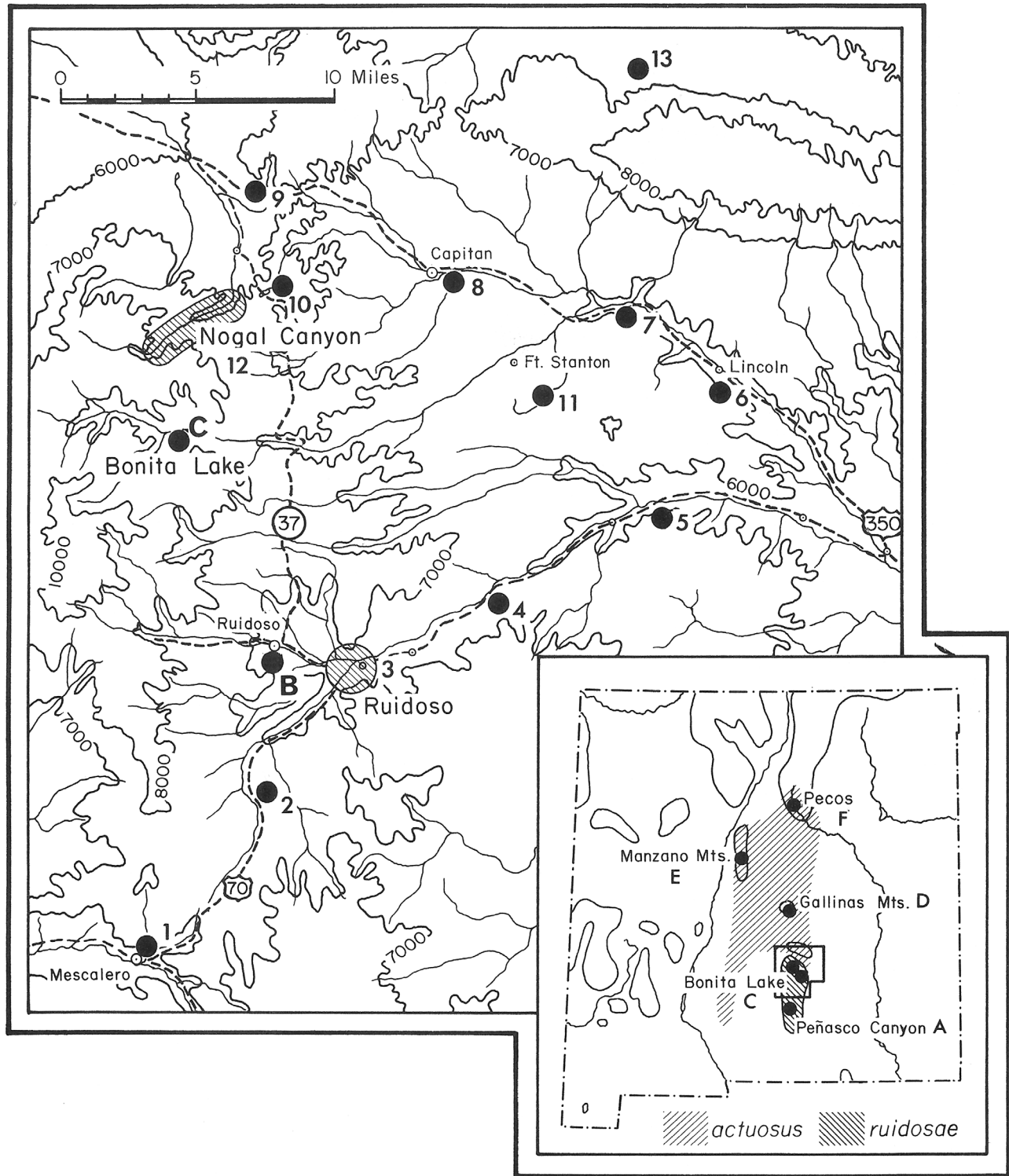


FIG. 1. Distribution of *T. b. ruidosae* and *T. b. actuosus* in New Mexico. Reference sample localities are indicated by letters; contact or near-contact zone localities are indicated by numbers. Names applied to mountain ranges are from Findley et al. (1975).

were qualitatively scored; the remainder were actual measurements. A habitat index was derived from the variables to quantify the rate of transition between the parental habitat types. Microhabitats surrounding burrow systems at the highest and lowest elevations along the transects were treated as parental type habitat.

Each trap site was then scored according to the degree of contribution by each of the two parental habitat types to that particular microhabitat structure. Individual habitat variables were scored as +1, 0, or -1 for similarity to *T. b. ruidosae* habitat, intermediate condition, or similarity to *T. b. actuosus* habitat, respectively. For a

few habitat features (e.g., number 1 or 2) a finer level of discrimination was possible and  $\pm 0.5$  intervals were scored. Scores were then summed across the nine habitat variables for each individual capture site to yield an overall index for that site. High positive scores thus indicate habitat composition similar to parental *T. b. ruidosae*, those with an extreme negative score closely resemble *T. b. actuosus* parental habitat.

*Specimens examined.*—Reference samples: *T. b. ruidosae* Hall. (A) Otero Co., jct. Willie White and Peñasco canyons, Sacramento Mts., 7900 ft (20 ♀♀, 4 ♂♂—MVZ 147037–147060). (B) Lincoln Co., 1 mi S Ruidoso (1 ♀—MVZ 50945); Ruidoso (5 ♀♀, 1 ♂—MVZ 50428–50430, 50432–50433, 50431 [holotype]). (C) Lincoln Co., Bonita Lake, 14 mi N Ruidoso, 7600 ft (23 ♀♀, 6 ♂♂—MVZ 147008–147036). *T. b. actuosus* Kelson. (D) Lincoln Co., Red Cloud Canyon, 2 mi S Rough Mt., Gallinas Mts., 7000 ft (11 ♀♀, 9 ♂♂—MVZ 146789–146808). (E) Torrance Co., 7 mi NW Tajique (by rd.), Manzano Mts., 7400 ft (9 ♀♀, 10 ♂♂—MVZ 144014–144032). (F) San Miguel Co., 3 mi N Pecos, 7100 ft (7 ♀♀, 8 ♂♂—MVZ 150277–150291). Contact and near contact samples: Otero Co.: (1) 0.5 mi NE Mescalero, 6600 ft (1 ♂—MVZ 150718). Lincoln Co.: (2) 6.6 mi S, 0.5 mi W Ruidoso, 7600 ft (1 ♀—MVZ 150719); (3) 1.3 mi S, 2.8 mi E Ruidoso, 6600 ft (1 ♀—MVZ 150721), 0.8 mi S, 2.7 mi E Ruidoso, 6500 ft (1 ♂—MVZ 150731), 0.3 mi S, 3.8 mi E Ruidoso, 6400 ft (7 ♀♀, 3 ♂♂—MVZ 150695–150699, 150732, 150744–150747); (4) 2.4 mi N, 9.0 mi E Ruidoso, 6200 ft (1 ♀—MVZ 150699); (5) 5.7 mi N, 15 mi E Ruidoso, 5800 ft (1 ♀—MVZ 150672); (6) 4.0 mi S, 11 mi E Capitan, 5800 ft (1 ♀—MVZ 150671); (7) 1.2 mi S, 7.0 mi E Capitan, 6000 ft (1 ♀—MVZ 150670); (8) 0.3 mi E Capitan, 6400 ft (1 ♀—MVZ 150673); (9) 2.7 mi N, 6.5 mi W Capitan, 6800 ft (1 ♀—MVZ 150720); (10) 1.4 mi S, 1.3 mi W Nogal, 6900 ft (4 ♀♀—MVZ 150676–150679); (11) 5.5 mi S, 4.5 mi E Capitan, 6700 ft (1 ♀—MVZ 150722); (12) 4.0 mi S, 3.2 mi W Nogal, 7800 ft (2 ♀♀—MVZ

150674–150675), 2.8 mi S, 2.0 mi W Nogal, 7300 ft (16 ♀♀, 11 ♂♂—MVZ 150680, 150685–150694, 150723–150726, 150733–150743), 2.4 mi S, 0.8 mi W Nogal, 6900 ft (2 ♀♀—MVZ 150727–150728), 2 mi S Nogal, 6800 ft (1 ♀—MVZ 150729); (13) 7.2 mi N, 6.8 mi E Capitan, 6800 ft (1 ♀, 2 ♂♂—MVZ 150681–150683). The above localities are plotted on the map, Figure 1.

## RESULTS

*Distribution and ecology.*—*T. bottae ruidosae* is known only from the higher elevations in the White and Sacramento mountains region of Lincoln and Otero counties, south central New Mexico. Populations of this race reach their densest numbers in the deep, humic soils of mountain meadows or along stream courses (canyon bottoms) within Transition or Canadian zone conifer forests. The lower elevational limit is marked approximately by the lower boundary of the yellow pine (*Pinus ponderosa*) belt. In contrast, *T. b. actuosus* ranges to the north, largely in rockier soils of the piñon-juniper woodland, in Lincoln, Socorro, Torrance, Bernalillo, Santa Fe, San Miguel, Doña Ana, and Mora counties of central New Mexico (see Kelson, 1951). It does occur in the highest elevations in the Gallinas, Manzano, and Sandia mountains in Transition and Canadian zone forests.

Kelson (1951, p. 68) recorded possible contact between *T. b. actuosus* and *T. b. ruidosae* in the Capitan Mountains to the northeast of the White Mountains, and from near Cloudcroft at the northern end of the Sacramento Mountains. We mapped two areas of contact between the two races during fieldwork in August, 1976: southeast of Ruidoso along the Rio Ruidoso between 6,400 and 6,700 ft, and Nogal Canyon, southwest of Nogal (see Fig. 1). The former area is heavily disturbed by human occupation in the form of building sites and cultivation. Hence, ecological analyses were restricted to the Nogal Canyon site which, except for grazing by cattle, presented no overt evidence of disturbance by man.

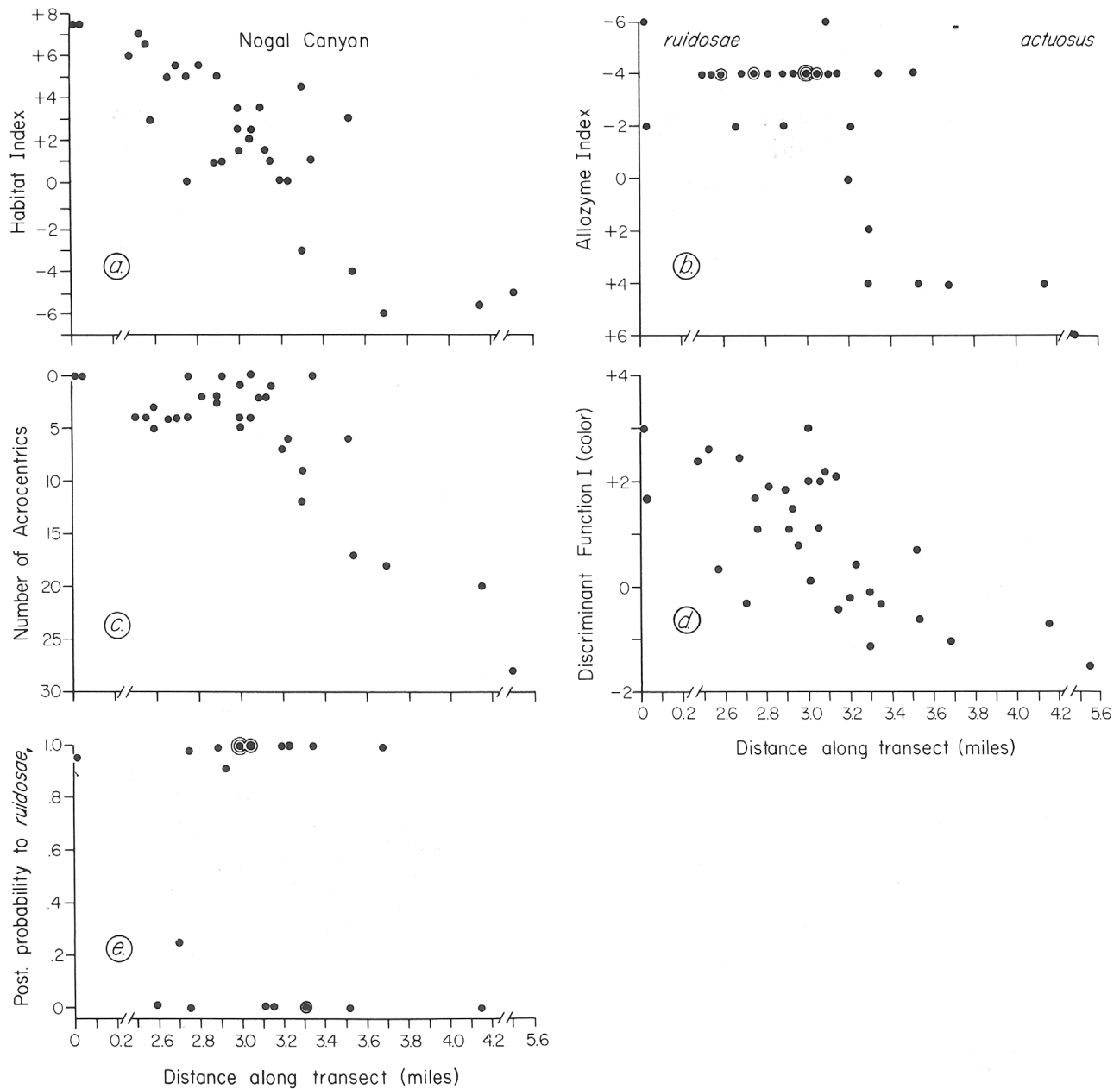


FIG. 2. Ecologic, genetic, and morphologic characteristics of individuals collected from the Nogal Canyon contact zone. (a) Gradient of individual ecological index scores across the transect. (b) Gradient of individual allozyme index scores across the transect. (c) Gradient of chromosomal characteristics (number of acrocentrics) of individuals across the transect. (d) Gradient of individual placement based on the first discriminant axis of color along the transect. (e) Gradient of individual placement based on external and cranial morphometric characters (as indicated by the posterior probability of assignment to *T. b. ruidosae* in the discriminant analysis) across the transect.

Nogal Canyon is a narrow canyon extending southwest to northeast, south of the town of Nogal (Fig. 1). The region is characterized by rolling hills clothed in piñon-juniper at the canyon mouth grading into successively steeper sides dominated by yellow pine in the higher reaches. Transition between the piñon-juniper woodland and pine forest occurs fairly abruptly, at about 7,000 feet. Nogal Creek courses the canyon, in places lined with

riparian trees such as walnut and oak. The soil throughout most of the canyon floor is sandy to gravelly with exposures of larger parent rock materials; the slopes are much rockier. Gopher activity was not great, and individuals were largely restricted to the canyon floor. Thirty-two individuals were collected at elevations ranging from 6,800 ft at the mouth to 7,800 ft in the upper portions of the canyon.

The rate of transition of the habitat index scores from characteristic *T. b. ruidosae* habitat to that typical of *T. b. actuosus* along Nogal Canyon is illustrated in Figure 2a. Due to the complex, mosaic nature of the vegetation in the canyon, no single trap locality received the maximal score of +9 or -9 indicative of "pure" parental habitat. Habitat index scores ranged from +7.5 to -6.0 across the 5.5 mi of the transect. A clearly demarcated and rather abrupt transition zone with an index change from +7.0 to -4.0 occurred over a one-mile distance (see Fig. 2a).

**Genetic analyses.**—Samples of *T. b. ruidosae* from four localities outside of the contact areas were available for karyotypic analysis. We karyotyped eight individuals from Bonita Lake (locality C) and seven from Peñasco Canyon (locality A), and Berry and Baker (1971) reported on five individuals taken from south of Cloudcroft and one from near Weed, both in the Sacramento Mountains. The number of acrocentric autosomes observed in these samples ranged from 0–2. Three populations of *actuosus* were sampled for karyotype, all of which are many miles distant from a potential contact zone with *T. b. ruidosae*. At the northern end of the range of *T. b. actuosus*, animals from near Pecos (locality F,  $N = 3$ ) have 26–28 unarmored autosomes; those from the Manzano Mountains (locality E,  $N = 16$ ) have 30–34 acrocentrics; and those from the Gallinas Mountains (locality D,  $N = 8$ ) have 30–32 acrocentrics. In *T. b. actuosus*, the interpopulation karyotypic variation recorded is mosaic in nature; no clinal patterns as observed in other sections of the species range (e.g., *T. b. grahamensis* [Patton, 1970] or *T. b. fulvus* [Patton and Yang, 1977]) are evident. Despite the variation in both races, it is clear that they are karyotypically quite divergent, differing in arm number by 13 to 16 pairs of chromosomes.

Since some karyotypic variability characterizes both races, absolute assignment of parental and hybrid individuals on chromosomal grounds alone is not possible. However, parental *T. b. ruidosae* are

TABLE 1. Allele frequencies for those loci which differ among the reference samples of *T. bottae ruidosae* and *T. bottae actuosus*.

Locus	Allele	<i>actuosus</i>			<i>ruidosae</i>	
		Pecos (F)	Manzano Mountains (E)	Gallinas Mountains (D)	Bonita Lake (B)	Peñasco Canyon (A)
<i>Pept-2</i>	121				.02	
	108				.02	
	100				.96	.98
	92	1.0	1.0	1.0		.02
<i>ADH</i>	-114	1.0	1.0	.93	.03*	
	-107				.97*	1.0
	-100			.05		
	-71			.02		
<i>δPGD</i>	100	1.0	.97	1.0		
	79				.98	.94
	67		.03		.02	
	60					.06
<i>Est-4</i>	105	.67	.50	.75	.57	.19
	101	.15	.05	.20	.43	.81
	100	.18	.40			
	94		.05	.05		
<i>preAlb</i>	101				.59	.42
	100	1.0	1.0	1.0	.41	.58

\* Given as .04 and .96, respectively, in Patton and Yang (1977). The presence of allele -114 is based on 2 heterozygotes in a sample of 29 individuals ( $P = .0345$ ).

expected to have 0, 1, or 2 acrocentrics; parental *T. b. actuosus* to have 26 or more acrocentrics. Hence, any given  $F_1$  hybrid is expected to have between 13 and 17 acrocentric autosomes, and first generation backcross hybrids ( $B_1$  generation) should have an acrocentric count between that of an  $F_1$  individual and one or the other parental class, depending upon the direction of the cross.

Based on genic data for five population samples of the two taxa from outside of contact areas, the taxa do not exhibit any fixed differences at any of the 23 loci examined. However, there are three loci (*Pept-2*, *ADH*, and *δPGD*) for which nearly fixed conditions do exist; that is, the degree of allele sharing is very minor (3% or less, Table 1; Patton and Yang, 1977). In two cases, the alleles concerned are only shared by populations well away from the contact zone (*Pept-2*<sup>92</sup> in Gallinas Mts. and Peñasco Canyon; *δPGD*<sup>67</sup> in Manzano Mts. and Bonita Lake). Only for *ADH*<sup>-114</sup> is there overlap in alleles be-

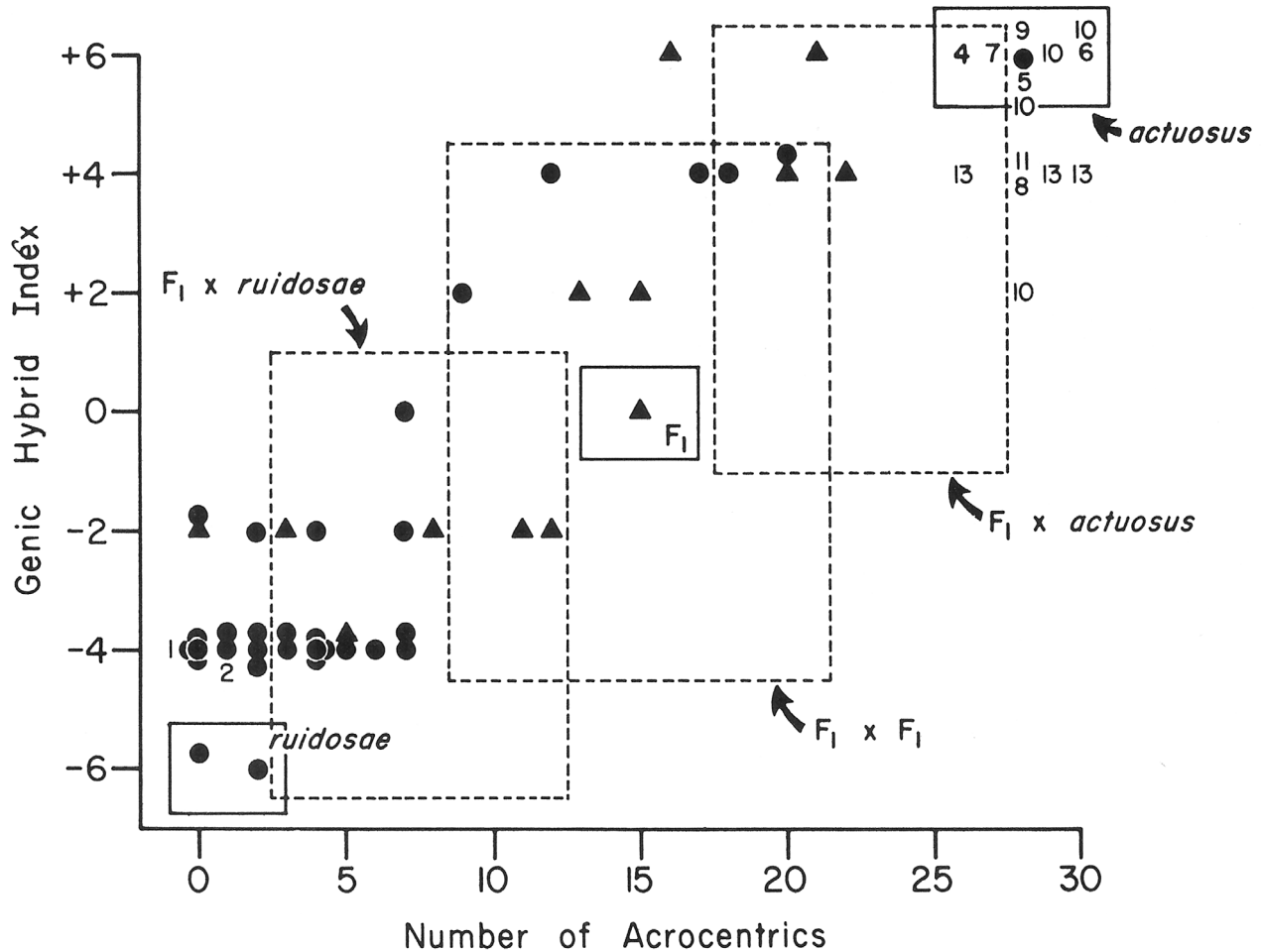


FIG. 3. Bivariate plot of genic index score and number of acrocentrics for all individuals from within or near contact zones of *T. b. ruidosae* and *T. b. actuosus*:  $\blacktriangle$  = Ruidoso contact individuals [locality 3];  $\bullet$  = Nogal Canyon contact individuals [locality 12]; numbers = individuals from near contact localities, identified by locality number. The expected range of variability for both genetic characters for parental *T. b. ruidosae*, parental *T. b. actuosus*, and  $F_1$  hybrids is indicated by solid boxes; expected range for about 65% of second filial and first backcross generation scores is indicated by the broken boxes. The latter was estimated from an assumption of random segregation and equal probability of mating between all classes of individuals.

tween the available parental samples geographically closest to a contact zone (Gallinas Mts. and Bonita Lake). The Bonita Lake sample of *T. b. ruidosae* was collected about 3 mi to the south of the Nogal Canyon contact; the Gallinas Mts. sample of *T. b. actuosus* comes from about 45 mi north of this contact (see Fig. 1).

Despite the low amount of allelic overlap between *T. b. ruidosae* and *T. b. actuosus*, the three loci can be used effectively in recognizing individuals of various hybrid classes. Thus, the extent of hybrid formation, and hence reproductive compatibility, can be estimated. If the three loci are considered to be fixed for distinct alleles in the two taxa, an index of hybrid status can be constructed for all individuals by assigning a +1 score for the pres-

ence of each allele characteristic of *T. b. actuosus* and a -1 score for each characterizing *T. b. ruidosae*. A parental *T. b. actuosus* individual will thus have a cumulative score of +6 (genotype *Pept-2*<sup>92/92</sup>; *ADH*<sup>-114/-114</sup>; *6PGD*<sup>100/100</sup>) while a parental *T. b. ruidosae* will have a -6 score (*Pept-2*<sup>100/100</sup>; *ADH*<sup>-107/-107</sup>; *6PGD*<sup>79/79</sup>).  $F_1$  hybrids, being heterozygous at all three loci, would have a combined score of 0; backcross hybrids or subsequent filial hybrids would have scores between +6 and -6.

Figure 3 illustrates the spread of genic index scores and number of acrocentric autosomes for each individual collected in or near the two contact zones. The range of expected parental and  $F_1$  scores for the two measures is indicated as are the ranges

for approximately 65% of all expected  $F_2$  ( $F_1 \times F_1$ ) and  $B_1$  ( $F_1 \times T. b. actuosus$  or  $F_1 \times T. b. ruidosae$ ) scores. Comparison of the observed genetic constitution of all individuals from contact areas with the expected thus gives a clear indication of the type and extent of hybridization occurring. For either contact zone there are few pure parental types of either race and only one identifiable  $F_1$  hybrid present. Most specimens have both chromosomal and genic scores widely intermediate between those of expected parental types, and thus most likely represent multiple backcross or filial generation hybrids. This conclusion is supported by the paucity of parental types and  $F_1$  individuals in the populations sampled.

The relationship between the hybrid status of an individual, based both on karyotype and genic index, and its placement along the transect through Nogal Canyon is illustrated in Figures 2b and 2c. The genetic measures are strongly concordant in relation to both individual hybrid status and individual placement along the transect. Most hybrids are closer genetically to parental *T. b. ruidosae* and occur in the middle to upper areas of the transect. Few hybrids are close to *T. b. actuosus* or occur near the mouth of Nogal Canyon. The distribution of individuals defined genetically differs somewhat from an individual's ecological placement (compare Fig. 2a with 2b and 2c). Individuals toward the middle of the transect (in the 2.4 to 3.0 mi region) are very similar to parental *T. b. ruidosae* in genetic characters yet occur in more intermediate microhabitats. Hence, an individual's habitat index score is not a good predictor of its genetic status, or vice versa.

*Morphological analyses.*—The morphological relationships between parental *T. b. actuosus*, parental *T. b. ruidosae*, and contact or near contact individuals were examined by discriminant function analysis of both the morphometric and color characteristics. These two sets of parameters were considered separately since they probably have dissimilar selective components and hence interact with both genetic and environmental backgrounds dif-

ferently. Replicate runs were made with localities A–C and D–F used separately or in combination as reference samples for *T. b. ruidosae* and *T. b. actuosus*, respectively. The results did not differ in detail, and only one set of data will be given below. Three categories of non-reference samples (contact or near contact localities 1–13) were treated as unknowns even though genotypes and karyotypes were known for each. Individuals were considered to be either (1) nearly genetically pure *T. b. ruidosae* (*T. b. ruidosae*-like) if they had a typical *T. b. ruidosae* karyotype (0–2 acrocentrics) and at least five of six *T. b. ruidosae* alleles (genic index of  $-6$  or  $-4$ ); (2) nearly genetically pure *T. b. actuosus* (*T. b. actuosus*-like) if they had 26–30 acrocentrics and a genic index of  $+6$  or  $+4$ ; or (3) intermediate hybrids if they had an acrocentric number and genic index score intermediate between those of individuals in categories (1) and (2) above. The distinction between these three categories is done only for illustrative purposes to emphasize that, while most if not all individuals from contact or near contact localities potentially contain some hybrid characteristics, some individuals are genetically much closer to one parental type than to the other. By such a distinction, a clearer relationship between an individual's genetic background and its morphology can be drawn without altering the multivariate procedures or results.

For the morphometric characters, three discriminant functions are needed to separate effectively the reference samples and to describe more than 94% of the explained variance for either sex (see Table 2). Females are strongly differentiated by overall size (ToL) alone while several variables weigh heavily in discrimination among males along each axis (Table 2). The a posteriori classification matrix summarizes the level of discrimination between the reference samples and the success of assigning genetically known contact or near contact individuals (Table 3). No reference sample individuals are misclassified as to race, but genetically known contact individuals are in several cases. Indeed, 27% of contact zone indi-



TABLE 2. Standardized coefficients for the first three discriminant functions in comparisons of females and males of reference samples of *T. b. ruidosae* and *T. b. actuosus*.

Variable	Females			Males		
	DFI	DFII	DFIII	DFI	DFII	DFIII
ToL	5.001	3.823	-2.326	.912	1.881	-1.830
TaL	-1.560	-.561	.893	-1.706	.601	1.751
HF	.067	-.640	.354	-.650	-.114	1.026
EL	-.358	-.374	-.053	-.047	-.937	.524
ON	-.555	-.708	2.540	9.155	-2.965	2.522
BaL	.046	.019	-.990	2.105	-1.907	.410
ZB	.329	-.366	-1.164	-6.574	1.511	-1.057
MB	-.135	.570	.068	2.154	.539	-.533
IOC	-.284	.122	-.245	-.956	-.451	.944
RL	-.111	.144	-.152	1.556	-1.114	-1.018
NL	.644	.016	-.075	-5.699	1.061	-1.450
RW	-.355	.100	.922	.741	1.597	-1.141
DL	-.276	-.147	-.465	-3.006	1.343	.963
MTRL	.049	-.287	-.813	1.934	-1.939	.577
PW	.737	.553	-.284	-2.676	.751	-.062
BuL	-.948	.203	.441	.015	-.608	.260
BuW	-.288	-.294	-.412	-2.199	-.309	-.704
Percent explained variance	52.2	26.4	14.9	69.7	15.4	11.5
Eigenvalue	6.529	3.294	1.858	24.312	5.363	3.997

TABLE 3. A posteriori classification matrix based on discriminant analysis of 17 external and cranial morphometric characters for adult individuals only. The number of individuals assigned to each reference group is given with the percentage classified in parentheses. Females are given above, males below in each couplet.

Sample	Reference sample					
	<i>ruidosae</i>			<i>actuosus</i>		
	A	B	C	D	E	F
Reference samples	A	15 (100) 4 (100)				
	B		3 (100) 1 (100)			
	C			20 (100) 5 (100)		
	D				9 (90) 8 (100)	1 (10)
	E				1 (17)	5 (83) 6 (100)
	F					
Contact zone individuals	Genetically <i>ruidosae</i> -like	2 (29) 1 (100)	1 (14)	1 (14)	1 (14)	2 (29)
	Genetically <i>actuosus</i> -like	1 (17) 1 (50)	1 (17)	1 (17)	2 (33) 1 (50)	1 (17)
	"Intermediate" hybrids	2 (14) 2 (25)	2 (14)	5 (36) 1 (13)	1 (07) 3 (37)	4 (29) 2 (25)

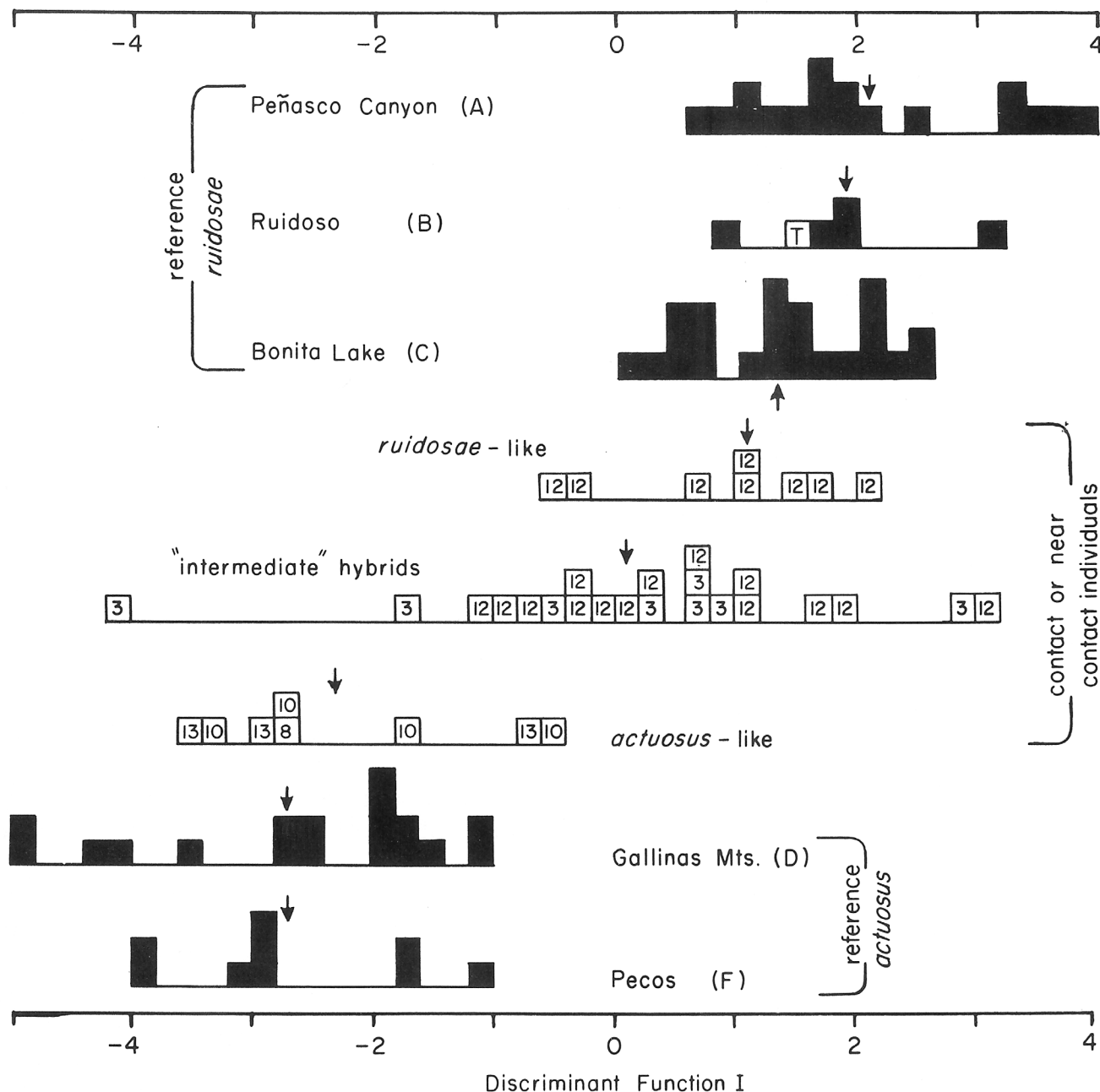


FIG. 4. Distribution of the first discriminant axis values for the color analysis of individual specimens of *T. b. ruidosae*, *T. b. actuosus*, and various genetically known hybrids. Reference samples of *T. b. ruidosae* and *T. b. actuosus* are indicated as are genetically known individuals from within or adjacent to contact zones (see text for explanation). For the latter, the numbers refer to the locality of each specimen. Arrows indicate group means. The specimen of *T. b. ruidosae* labeled "T" from Locality B is the holotype.

viduals that are genetically near *T. b. ruidosae* are classified morphologically as *T. b. actuosus*, and 50% of the genetic *T. b. actuosus*-like individuals are misclassified.

The reference samples of *T. b. ruidosae* and *T. b. actuosus* are readily separable on the basis of color. The latter is markedly paler in aspects of the dorsal color, and this is reflected in statistically significant ( $P < .05$ ) higher values for both pu-

rity and brightness. Only the Manzano Mts. sample of *T. b. actuosus* (locality E) approaches the rich, dark color characteristic of *T. b. ruidosae*. The color similarity of this sample to *T. b. ruidosae* is considered a convergent adaptation for crypticity in similar microhabitats rather than an indication of a common genetic background. In pocket gophers, pelage color variation is strongly related to soil color (see Ingles, 1950; Sands and Findley,

TABLE 4. A posteriori classification matrix based on discriminant analysis of 3 color variables for adult individuals only. The number of individuals assigned to each reference group is given with the percentage classified in parentheses.

Sample		Reference sample				
		<i>ruidosae</i>			<i>actuosus</i>	
		A	B	C	D	F
Reference samples	A	11 (65)	5 (29)	1 (06)		
	B	1 (17)		5 (83)		
	C	5 (22)	13 (56)	5 (22)		
	D				12 (67)	6 (33)
	F				3 (33)	6 (67)
Contact zone individuals	Genetically <i>ruidosae</i> -like	1 (13)	5 (63)	2 (25)		
	Genetically <i>actuosus</i> -like			1 (13)	3 (37)	4 (50)
	"Intermediate" hybrids	2 (10)	4 (20)	10 (50)	3 (15)	1 (05)

1959; Patton, 1973; for example). Thus to prevent convergence from obscuring color characteristics of contact individuals, the Manzano Mts. sample was excluded from the discriminant analysis.

Figure 4 presents a histogram of individual values of the first discriminant function based on color. This function effectively separates all reference samples and accounts for 92.3% of the total variation observed. Again, the nonoverlapping nature of the color characteristics of the reference samples of *T. b. actuosus* and *T. b. ruidosae* is emphasized. Unlike the morphometric analysis, contact or near contact individuals in the genetic class close to either parental type largely display parental color features; only four individuals of the 16 in this category tend to bridge the gap between reference samples, and only one of these is misclassified in the a posteriori classification matrix (see Fig. 4 and Table 4). However, several clearly intermediate genetic hybrids, rather than being intermediate in color, are indistinguishable from the parental types (Fig. 4). Despite these exceptions, an individual's color is a better indicator of its overall genetic background than is a multivariate assessment of its cranial and external morphometric characters.

The relationship between an individual's morphological state, as indicated by

either color or morphometric characters, and its geographic placement along the Nogal Canyon transect is indicated in Figures 2d and 2e, respectively. As is evident, the placement of individuals on the basis of morphometric features provides very little information relative to the genetic background of the individuals analyzed. Indeed, except for one somewhat intermediate individual, the contact appears to be composed solely of individuals of one or the other race with few or no hybrids (Fig. 2e). The color analysis, however, is more satisfying in that a clear gradient is seen across the transect from a *T. b. ruidosae*-like to *T. b. actuosus*-like pattern. Nevertheless, the color analysis is not strongly concordant with the gradient based on genetic background. In fact, an individual's color more closely matches its placement along the ecological gradient than along either of the two genetic gradients (compare Fig. 2d with 2a and 2b-c). As is true for the ecological analysis but not the genetic analysis, the range of individual color scores across the middle of the transect is intermediate between those of the parents rather than skewed toward *T. b. ruidosae*.

## DISCUSSION

*Racial contact or ecogeographic variation?*—The two sets of pocket gopher pop-

ulations examined here represent classical geographic races in the current literature. Each has a separate geographic range and distinct morphology (Kelson, 1951) and each is genically and chromosomally divergent (Patton and Yang, 1977). While there is interpopulation variation in both genetic parameters within each race, there is no overlap chromosomally between them and only minor allele sharing (3% or less) at the three diagnostic allozyme loci. Within each race populations occupy a variety of habitats ranging from Upper Sonoran piñon-juniper woodland to Canadian Zone conifer forests. As a result, morphological characters, particularly dorsal pelage color, show some geographic variability in both races reflective of local adaptive patterns. Nevertheless, non-contact individuals are clearly assignable to one race or the other by a combination of characters (see Kelson, 1951, and analysis above). For example, in the dark soils of the Manzano Mts., *T. b. actuosus* resembles *T. b. ruidosae* in having dark dorsal pelage. However, the pocket gophers of the Manzano Mts. otherwise have a *T. b. actuosus* cranial morphology, karyotype, and allozyme constitution and are readily identified as *T. b. actuosus*. Such is not the case in the contact zones. Here shifts in genetic characters and gross morphology accompany changes in pelage color along ecological transitions. Thus, it is clear to us that the observed gradients (genetic and morphologic) through Nogal Canyon and adjacent areas are indicative of a region of contact between two distinctive geographic races rather than simple ecogeographic variation.

*The genetics of contact zones.*—The genetic data presented above allow us to explore the nature and extent of hybridization between *T. b. actuosus* and *T. b. ruidosae* as well as to estimate the level of reproductive compatibility between the races. This capability provides a means for determining, rather specifically, the consequences of hybridization and hence provides a set of unbiased operational criteria to which other hybrid zone situations might be compared. These criteria are, of

course, for use when direct evidence regarding the viability and reproductive performance of hybrids is not available (e.g., evidence for sterility, embryonic mortality, and so forth). For example:

(1) Contacts involving individuals with only parental genic index scores ( $-6$  and  $+6$  in this case) and karyotypes would indicate no hybridization and hence complete reproductive isolation between the races. In this case the forms are obviously behaving as full biological species.

(2) Contacts involving both parental type individuals ( $-6$  or  $+6$ ) but only occasional numerical intermediates (0 score) indicate hybridization limited to the  $F_1$  generation only and hence the presence of an isolating barrier (either premating or postmating) of some force.  $F_1$  production by itself will not provide a means for genic introgression and hence, in these cases, genetic isolation is complete while reproductive isolation is not.

(3) Contacts involving parental,  $F_1$ , and other hybrid class individuals (scores of  $+4$ ,  $+2$ ,  $-2$ , and  $-4$ ) indicate that hybridization is successful at least through one backcross generation, if not more. Reproductive isolation is thus not complete and the possibility for genic introgression, selective or otherwise, is present. The frequency of the number of individuals in the sample that fits each class will reflect the potential freedom of interbreeding and extent of introgression. For example:

(a) Some degree of reproductive impairment would be indicated by individuals clustered around the expected  $F_1$  or  $B_1$  scores.

(b) The presence of few  $F_1$  but many other hybrid class individuals would suggest multiple filial and backcross generations and hence a nearly complete mixing of the two gene pools in the contact zone.

It should be emphasized that, except for (1) above, distinction between each type of contact is not absolute. These descriptions merely represent static points along a potential continuum of reproductive compatibility and consequent gene flow. In the absence of other data to the con-

trary, documentation of a level of interbreeding suggested by category (3) is sufficient, in our opinion, to serve as an operational definition of population conspecificity based on genetic criteria. Certainly, level (3b) represents near total, if not complete, reproductive compatibility.

The data presented here for hybridization between *T. b. ruidosae* and *T. b. actuosus* match the expectations given in (3b) above, and we conclude that the two races freely interbreed. The evidence for this conclusion comes from (1) an examination of the range of genotypes and karyotypes of hybrids from the contact zones, (2) the apparent introgression of alleles beyond the boundaries of the ecologically and morphologically defined contact zones, and (3) the discordance observed between the genotype and phenotype of several hybrid individuals.

Even though sample sizes are insufficient to determine if deficiencies of certain genotypic arrays result from poor survivorship and/or lowered fertility of hybrid individuals (as in *Sceloporus grammicus*, see Hall and Selander, 1973), the paucity of F<sub>1</sub> individuals and the number and wide range of other hybrid class individuals from both contacts (Fig. 3) argues strongly that hybridization is not only extensive but that all hybrids are viable and fertile. These data also suggest that the condition of hybridization has been rather stable through time. The genotypes of most hybrids intermediate between parental types and F<sub>1</sub>s in genic index are most likely the result of multiple generations of backcrossing. This requires a relatively stable hybrid situation and a general lack of reproductive impairment of hybrid class individuals. The low frequency of F<sub>1</sub>s in the hybrid zone is also a testament to the reproductive and competitive success of other hybrid class individuals. The demonstrated large number of hybrids within the zone means that the probability of matings between "pure" parents (and hence expected F<sub>1</sub> production) should be low, but only if backcross hybrids are fertile and ecologically successful.

Secondly, there is an indication that the

consequences of genetic intergradation extend beyond the confines of the zones as defined morphologically and ecologically. Indeed, while a zone so defined in Nogal Canyon extends for only about one mile (see Figs. 2a and 2d), genetic characteristics of each parent occur in individuals of the opposite parental type many miles distant from the contact. For example, two of 29 morphologically "pure" *T. b. ruidosae* individuals from Bonita Lake and others from 6 and 15 mi southwest of the Ruidoso contact (localities 1 and 2) contain some alleles characteristic of *T. b. actuosus* (e.g., *ADH*<sup>-114</sup> and *Pept*-2<sup>92</sup>; Table 1). Similarly, individuals classed on morphometric or colorimetric grounds as *T. b. actuosus* from north and northeast of the Nogal Canyon contact contain some alleles more typical of *T. b. ruidosae* (*ADH*<sup>-107</sup>, localities 8 and 11). Finally, the three individuals collected from the Capitan Mts. (locality 13) shared *ADH* alleles found in both *T. b. ruidosae* and *T. b. actuosus*, yet morphologically these individuals are clearly *T. b. actuosus*.

While it cannot be stated with certainty that the presence of alleles characterizing one race but found in minor frequencies in populations of the other has actually resulted from introgression, the data are consistent with this interpretation. Significantly, the Bonita Lake reference sample of *T. b. ruidosae*, which contains two individuals with *T. b. actuosus* alleles at the *ADH* locus, also shows slight tendencies toward *T. b. actuosus* in the color discriminant analysis (see Fig. 4). Thus, genic introgression appears to extend beyond the zone of contact defined on ecological or morphological grounds alone, and this is reflective to some degree in certain morphological traits.

The path of gene flow between *T. b. ruidosae* and *T. b. actuosus* is clearly bidirectional, but hybridization is strongly weighted in favor of *T. b. ruidosae* in the Nogal Canyon contact (Fig. 2b, 2c). This skewness probably results from the disproportionate representation of parental individuals of the two races in the general contact area rather than to a higher degree

of fertility and viability of individuals backcrossed in the direction of *T. b. ruidosae*. During our field work, a dense population of *T. b. ruidosae* occurred in the higher reaches of Nogal Canyon whereas *T. b. actuosus* individuals were scarce in the rockier, more shallow soils of the canyon mouth and beyond to the east and north. This is amply evident by the dispersion of individual trap sites along the transect (see Fig. 2). Hence, the unbalanced pattern of hybridization can be explained simply by random breeding within the zone.

Finally, there is a general lack of correspondence between the genetic and morphologic characters of several individuals from the contacts. In general,  $F_1$  individuals should be approximately intermediate between parental forms in morphological features while animals with largely one or the other parental genetic makeup should bear more morphological resemblance to that parental type. This, of course, assumes that our genetic measures (genic and chromosomal) of the hybrid status of given individuals are representative of the entire genome. Figure 5 plots the relationship between the genetic characters of individual hybrids and their morphology as indexed by color. While there is a general correlation between these measures of an individual's genetic background, it is not a particularly strong one. Importantly, individuals with the genetic characters of *T. b. actuosus* may strongly resemble *T. b. ruidosae* in color (i.e., with a posterior probability of 1.0), or vice versa.

This apparent lack of genetic-phenetic concordance at the individual level may seem paradoxical, but we find it predictable under the conditions of continual backcrossing suggested for the *T. b. ruidosae-actuosus* contact zones. There is no reason to suspect that the diagnostic allozyme loci and those controlling aspects of cranial morphology or pelage color are tightly linked. Hence, the repeated backcrossing suggested by the genetic data should result in a somewhat random reassignment of the genetic characters of both races in individuals of hybrid origin. Thus

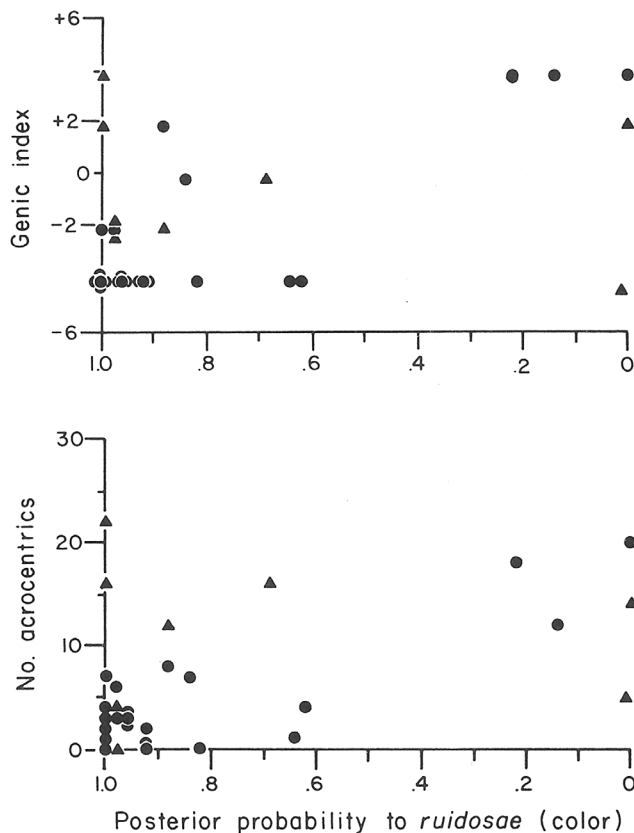


FIG. 5. Bivariate relationship between the genetic and morphologic characteristics of contact zone individuals. The former is based on both genic hybrid index (upper graph) and number of acrocentrics (lower graph); the latter is based on the posterior probability of assignment to *T. b. ruidosae* on color features. ▲ = individuals from the Ruidoso contact; ● = individuals from the Nogal Canyon contact.

the lack of correlation between an individual's genetic and morphologic characters is evidence for a history of free interbreeding at the contact points between *T. b. actuosus* and *T. b. ruidosae*.

If interbreeding is so free within the contact zones, why then has there not been more extensive introgression between the parental gene pools? The classical interpretation for the lack of extensive introgression beyond narrow hybrid zones involves the presumed reduction in fitness in backcross generations caused by disruption of coadapted parental gene complexes (Mayr, 1963). Another factor, or group of related factors, which must be considered, however, involves the history of contact, including the time and extent of such. We have no direct data pertinent to either of these possibilities, but for the

moment favor the second alternative as a major component.

Individuals of *T. b. actuosus* are relatively scarce at the base of the White and Sacramento mountains, and potential contacts between the two races are limited to a few narrow canyon systems which, because of relatively poor habitat, do not harbor many animals. Hence, there may simply be insufficient continual contact between *T. b. ruidosae* and *T. b. actuosus* to permit extensive introgression even if the contact between them is old and there is no other limitation to introgression. Moreover, there are no data to suggest lowered reproductive fitness of any hybrid individual. All adult males were scrotal with enlarged testes and accessory glands, indicative of complete competence; each adult female hybrid displayed evidence of present or previous pregnancies with the average litter size (based on embryo or placental scar counts) well within the range characteristic of both parental forms. It is possible, however, that the spread of alleles into parental populations is partially limited due to lowered fitness of hybrid phenotypes in parental habitat. This would likely be due to pelage-color mismatching and hence to disproportional predation pressure rather than to intrinsic problems associated with genic or chromosomal "fitness."

The rather complete genetic mixing which occurs where *T. b. actuosus* and *T. b. ruidosae* meet is somewhat surprising since the two races differ by nearly 20% of their genome as measured by starch gel electrophoresis. This degree of genic differentiation is not, however, unique to this pair of *T. bottae* regional units. Indeed, in central California the large, pale San Joaquin Valley gopher, *T. b. pascalis*, has been shown to intergrade by morphological criteria with the smaller, reddish Sierran foothills race, *T. b. mewa* (Thaeler, 1968). Nevertheless, these races share only 78% of their genomes based upon the same set of allozyme loci examined herein (Patton, unpubl. data). These findings reinforce the conclusions presented by Patton and Yang (1977) that, at least in

*T. bottae* pocket gophers, genic differentiation is largely a function of the historical sequences through which populations have passed. The extent of such is not a particularly useful index of the degree of reproductive isolation achieved between differentiated geographic segments of *T. bottae* and, by inference, other organisms as well.

*Genetic-phenetic relationships in contact zones.*—It is clear from Figure 2 that there is a general correspondence between gradients based on an individual's ecologic, morphologic, or genetic characters along the Nogal Canyon transect. The degree of correspondence, however, differs between the three measurements used; the color gradient appears more reflective of the ecological one than is either genetic gradient. The spread of individual points in the colorimetric and ecologic gradients are more nearly balanced along the transect while both genetic gradients are strongly skewed toward parental *T. b. ruidosae*. We interpret these differences to mean that there is a stronger interaction between an individual's morphology (particularly color) and the microhabitat in which it occurs than between either of these features and its genotype. The marked correspondence between color and microhabitat along the transect is not surprising. Pocket gophers are known to be colored cryptically to match the background soil color (see Patton, 1973, for example). Furthermore, the association between color and habitat but not genic index or karyotype is evident by the color convergence of the Manzano Mts. sample of *T. b. actuosus* to *T. b. ruidosae* even though that sample shows no tendency toward *T. b. ruidosae* in either genetic character (see Table 1 and Patton and Yang, 1977). Thus, while color is probably responding directly to environmental selective pressures, neither the allozymes examined nor karyotypes are. If both of the latter do, in fact, have a selective base, it appears to be different from that operating on color.

This situation has other, broader implications. In many groups, but particularly

in fossorial rodents whose distributions are most often parapatric, the evolutionary status of populations has been heavily weighted by knowledge of the dynamics of contact zones between them. In situations where hybrid formation has been identified or suspected, evidence for the level of reproductive isolation is often derived from two sources: (1) the proportion of hybrids and parental types found and (2) the actual geographic limits of the hybrid zone so identified (for example, Thae-ler, 1968, 1974; Patton, 1973; Nevo and Bar-El, 1976). While the relationships of these features of hybrid zones are indeed important, either or both might give misinformation with regard to the actual dynamics of a given zone, particularly if they are considered in the absence of genetic data. For example, gross morphology may inadequately describe actual levels of gene exchange. Moreover, zone widths, as classically defined by morphology, may be strongly influenced by ecological gradients and have little to do with the genetic conditions of the zone. These points are pertinent in the analysis of the contact between *T. b. actuosus* and *T. b. ruidosae*. Here, the evidence indicates that a morphologically based zone delineation closely parallels the ecological gradient within that zone while genetic data suggest introgression beyond a zone so identified. This situation is similar to that recently described for the Australian frog *Pseudophryne* (McDonnell et al., 1978), but here there is evidence for embryonic mortality in hybrids and hence for a postmating reproductive barrier. In the present case, the conclusions reached regarding the degree of gene exchange between these pocket gopher races might have been different without the genetic information. The discriminant function analyses of the morphometric characters, in particular, were insufficiently sensitive to identify the actual proportion of hybrid individuals in the sample. More importantly, the crucial hybrid class individuals upon which evidence for introgression must necessarily rest (e.g., backcross individuals) cannot be unambiguously so identified by morphol-

ogy, if such are recognized as hybrids at all.

#### SUMMARY

An analysis of hybridization between two races of *Thomomys bottae* in central New Mexico (*T. b. ruidosae* Hall and *T. b. actuosus* Kelson) is given in the context of the genetic, morphologic, and ecologic characteristics of contact zones between them. The data show extensive hybrid formation resulting from multiple generations of backcrossing and hence free interbreeding between races which differ at nearly 20% of their genomes, as measured by allozyme analysis, and up to 16 fixed chromosomal arm number differences. The contact zone is narrowly defined by both genetic (genic and chromosomal) and morphologic (morphometric and colorimetric) characters which change abruptly over a distance of less than two miles. These changes coincide with an equally sharp ecological gradient, although the concordance between an individual's placement along the transect by morphology and ecology is much closer than that of either of these and the individual's genetic characters. None of the data available suggest that the various hybrid class individuals experience lowered fitness, and the allozyme data show genic introgression extending into the parental populations well beyond the zones defined ecologically or morphologically.

A series of operational criteria are defined for the consistent interpretation of the evolutionary status of hybridizing populations of gophers which can be applied to other organisms as well.

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