MORPHOMETRICS, EVOLUTION, AND CYTOTAXONOMY OF MAINLAND BATS OF THE GENUS *MACROTUS* (CHIROPTERA: PHYLLOSTOMATIDAE)

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Abstract

Davis, Brent L., and Robert J. Baker (Dept. Biology, and The Museum, Texas Tech University, Lubbock, Texas) 1974. Morphometrics, Evolution, and Cytotaxonomy of Mainland Bats of the Genus Macrotus *(Chiroptera: Phyllostomatidae). Syst. Zool. 23:26–39.*—Chromosomal data reveal the existence of two mainland species of *Macrotus* that are parapatric in distribution. Individuals can be identified to species by both chromosomal features and cranial morphology. A stepwise multiple discriminant analysis and canonical variate analysis show that the two species are morphometrically divergent. The distribution of these two taxa is proof that parapatry occurs in species with high vagility. If chromosomal data had not been available or if chromosomal divergence had not accompanied speciation in *Macrotus*, this unique pattern of distribution probably would not have been detected. Allopatric, stasipatric, and centrifugal speciation are considered in light of the presently available data for *Macrotus*. [*Macrotus*; morphometrics; cytotaxonomy.]

Geographic variation in chromosome number is rather uncommon among bats of the family Phyllostomatidae. Of the 77 species of phyllostomatids thus far karyotyped (Baker, 1973), four species show geographic variation—*Uroderma bilobatum* (Baker *et al.*, 1972), *Macrotus waterhousii* (Nelson-Rees, *et al.*, 1968), and *Micronycteris hirsuta* and *Vampyressa pusilla* (Baker *et al.*, 1973).

Chromosomal variation in *Macrotus*, which previously was thought to have two chromosomal races, is of the Robertsonian type (the fundamental number remains constant but the diploid number varies). The cytotype with a diploid number ($2N$) of 46 and a fundamental number (FN) of 60 has been reported as having a geographic range from Alamos, Sonora, south through Morelos, and Guerrero (Nelson-Rees *et al.*, 1968). The $2N = 40$, $FN = 60$ cytotype had a known distribution from Carbo, Sonora, north to Arizona and California. Alamos (the most northern published record for the $2N = 46$ cytotype) is 335 kilometers south of Carbo (the most southern reported locality of the $2N = 40$ cytotype). Variation in diploid number, as understood at the outset of this study, was confined to the subspecies *M. w. californicus* (Anderson, 1968, 1969; Nelson-Rees, *et al.*, 1968).

The primary reason for studying the cytogenetics and morphometrics of *Macrotus* was to determine the mechanism of evolution and the degree of hybridization between the two cytotypes. Robertsonian variation is the most likely mechanism by which a chromosomal change of this nature could occur (Nelson-Rees *et al.*, 1972). If centric fusions or fissions are the mechanism by which the cytotypes evolved, loss or gain of genetic material probably would have occurred in the heterochromatic region (Jackson, 1971) and a cross between the two cytotypes should be successful, at least on a chromosomal basis of homology. Because of the relative ease with which specimens of *Macrotus* can be obtained and because the species is chromosomally variable, detailed studies were initiated.

Several possibilities are obvious from the outset. First, the two cytotypes could be freely interbreeding. If this were the case, the genetics of the system could be examined by using chromosome number, morphology, and meiotic studies. Second, the two could be sympatric and produce no hybrids. Third, the two could have allopatric distributions.

Our studies have revealed a parapatric distribution (as discussed for mammals by Vaughan, 1967) with no phenetic inter-

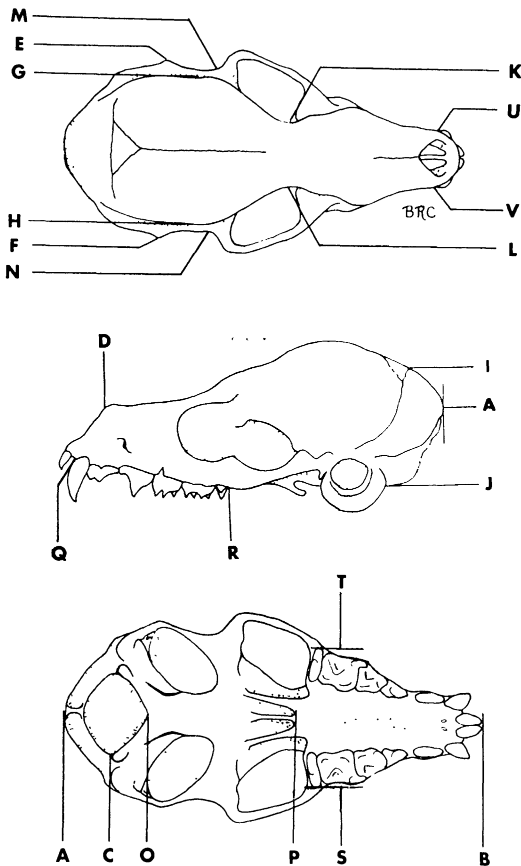

FIG. 1.—Skull of *Macrotus* showing cranial measurements used in statistical studies. Names of measurements are given in the text.

mediates. Baker (1970*a*) pointed out that ". . . studies of zones where two chromosomal races are contiguous could give considerable information on gene flow, dispersal potential, and isolating mechanisms in these populations." We have collected specimens for karyotypic analysis and, based on these and on specimens from museums, have evaluated the karyotypic and morphometric characters as potential population markers. In addition, this report evaluates the systematic status of mainland *Macrotus*.

MATERIALS AND METHODS

Bats were trapped in mistnets set over water or taken from caves or abandoned mines. Karotypes were prepared using a modification of the *in vivo* bone marrow techniques described by Patton (1967) and Baker (1970*b*). The modifications were: a 5-hour *in vivo* culture after injection of from .20 to .25 milliliters of 0.04% vinblastine sulfate (Velban of Eli Lilly); a 25-minute incubation of the bone marrow in 0.9% sodium citrate solution; and a total time in the first fixative of 25 minutes (a 10-minute fixation before disruption of the cell button, followed by 15 minutes of additional fixation). The remainder of the procedure was as described by Baker (1970*b*). At least five somatic spreads were examined for each specimen. Metacentric, submetacentric, subtelocentric, acrocentric, and fundamental number (*FN*) were used as defined by Patton (1967).

Cranial measurements were taken with dial calipers, calibrated in twentieths of millimeters, and rounded to the nearest tenth of a millimeter. The number before each measurement identifies that character at various places in the text and tables. Cranial measurements (Figure 1) were: (1) greatest length of skull, AB; (2) condylobasal length, CB; (3) occipitonasal length, AD; (4) mastoid breadth, EF; (5) braincase breadth, GH; (6) braincase depth, IJ; (7) interorbital breadth, KL; (8) posterior zygomatic breadth, MN; (9) postpalatal length, OP; (10) maxillary toothrow, QR; (11) width at M2, ST; and (12) canine breadth, UV. External measurements used were (13) length of forearm and (14) length of the third metacarpal.

Data were arranged into five procedures, labeled A thru E in text and tables. Procedure A (sexes pooled) consisted of all specimens and examined for intergroup divergence without regard for secondary sexual dimorphism. Procedures B (males only) and C (females only) examined for intergroup divergence and eliminated the effects of secondary sexual dimorphism.

Specimens in each of procedures A (sexes pooled), B (males), and C (females) were grouped for statistical analyses. Group 1 was all specimens with a karyotype of $2N =$

40 and all specimens north of Quiriego, Sonora, which were not karyotyped (localities 1–16 and the $2N = 40$ component of locality 17). See specimens examined and Figure 2 for population identification. Not all of the Quiriego specimens were karyotyped. Certain of these specimens were grouped with the $2N = 40$ cytotype based on morphometric analyses carried out prior to those reported here. These specimens were consistently classified with the $2N = 40$ cytotype and were treated as such. Group 2 consisted of the northern Sinaloan locality 18. Group 3 included all $2N = 46$ specimens and those from Quiriego that were (as in group 1) morphometrically similar to the $2N = 46$ cytotype (localities 19–21 and the $2N = 46$ portion of 17). Group 4 included the southern Sinaloan localities (22–24). Group 5 was the Jaliscan samples (localities 25–30). Group 6 included specimens from the Mexican states of Morelos, Puebla, Guerrero, and Oaxaca (localities 31–38).

The last two procedures, D and E, were designed to examine geographic variation within each cytotype. Procedure D analyzed the $2N = 40$ cytotype, sexes pooled. Specimens for Procedure D were grouped as follows (letters identify that group in the figures and text): (a) localities 1 and 2; (b) localities 3–5; (c) localities 6–8; (d) localities 9–14; (e) locality 15; (f) locality 16; (g) locality 17 ($2N = 40$ race); and (h) locality 18. Specimens of Procedure E, analyzing the $2N = 46$ cytotype, were divided into 7 groups as follows: (i) locality 17 ($2N = 46$); (j) localities 19–21; (k) localities 22–24; (l) localities 25–30; (m) localities 33–35; (n) localities 31–32; and (o) localities 36–38. See specimens examined and Figure 2 for population localities.

Statistical methodology has been described in detail by Baker *et al.* (1972). Univariate analysis of variance (ANOVA) was used to assess intergroup morphometric divergence. The effects of secondary sexual dimorphism was assessed by an ANOVA for a single population. It has been shown in mormoopid bats (Smith, 1972), in *Desmodus* personal communication Alberto

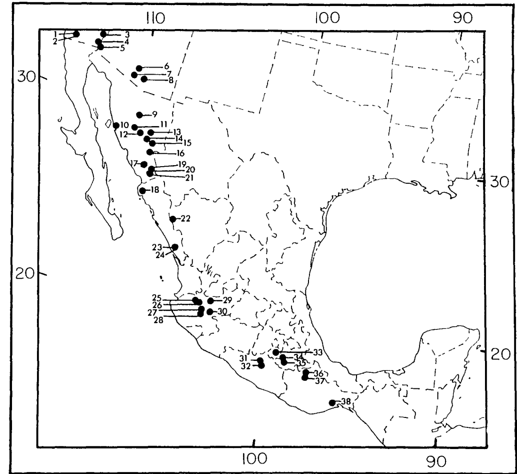

FIG. 2.—Localities from which specimens of *Macrotus* were studied. Numbers identify that locality in the list of specimens examined.

Cadena, and eluded to by Atchley (1971) for *Chironomus* Diptera: Chironomidae, that the amount of secondary sexual dimorphism may vary from population to population. Sample size prohibited multivariate analysis of sexual variation. For these reasons results of the ANOVA for secondary sexual dimorphism is not presented.

Canonical variate analysis aided in assessing the degree of multivariate divergence. A standardized canonical variate coefficient was computed by multiplying the canonical variate coefficients for a particular character by the pooled standard deviation. The standardized coefficient aids in indicating which characters account for the variation found within each canonical variate. The variables with a high coefficient are those that make the greatest contribution to the discrimination of the groups along that axis. The Mahalanobis generalized distance statistic ($\sqrt{D^2}$) was calculated for each pair of groups in each analysis and was also used to generate a probabilistic classification matrix to indicate the amount of phenetic overlap among groups. A stepwise discriminant analysis was performed, resulting in a listing of characters according to their discriminatory

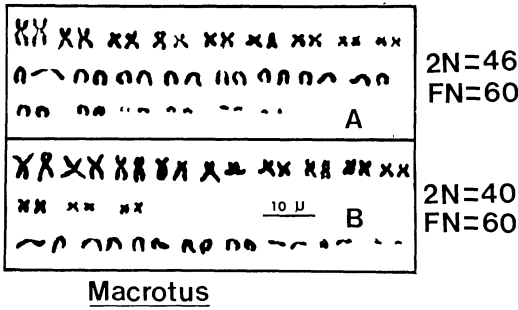

FIG. 3.—Karyotypes characteristic of *Macrotus*. A is the $2N = 46$ cytotype and B, the $2N = 40$ cytotype.

power (Baker *et al.*, 1972). The Wilks Lambda Statistic was employed for the overall test of equality of group means and a pair wise *F* test was performed to test the equality of mean vectors between each pair of groups. The BMDO7M program (Dixon, 1971) was used for these analyses.

In order to substantiate the results of the stepwise discriminant analysis a two-group discriminant analysis (BMDO4M program) was performed using the 14 variable data set, the optimal subset of variables (2, 4, 7, 12, and 13), and interorbital constriction (7). The two groups represented the two cytotypes.

RESULTS

The karyotypic analysis revealed that the $2N = 40$ form ranged from Quiriego, Sonora, north to Arizona. The $2N = 46$ form was found to be distributed from Quiriego, Sonora, southward. This study did not reveal the presence of hybrid cytotypes. The two races were sympatric in an abandoned mine located 11.9 mi. N Quiriego, Sonora (see specimens examined and Fig. 2). All 17 males of the $2N = 46$ race collected on 7 and 9 June 1972 had scrotal testes; however, none of the 17 males of the $2N = 40$ cytotype had scrotal testes. All female bats were in terminal stages of pregnancy.

Both cytotypes are as described by Nelson-Rees *et al.* (1968) and as shown in Figure 3. No chromosomal variation was

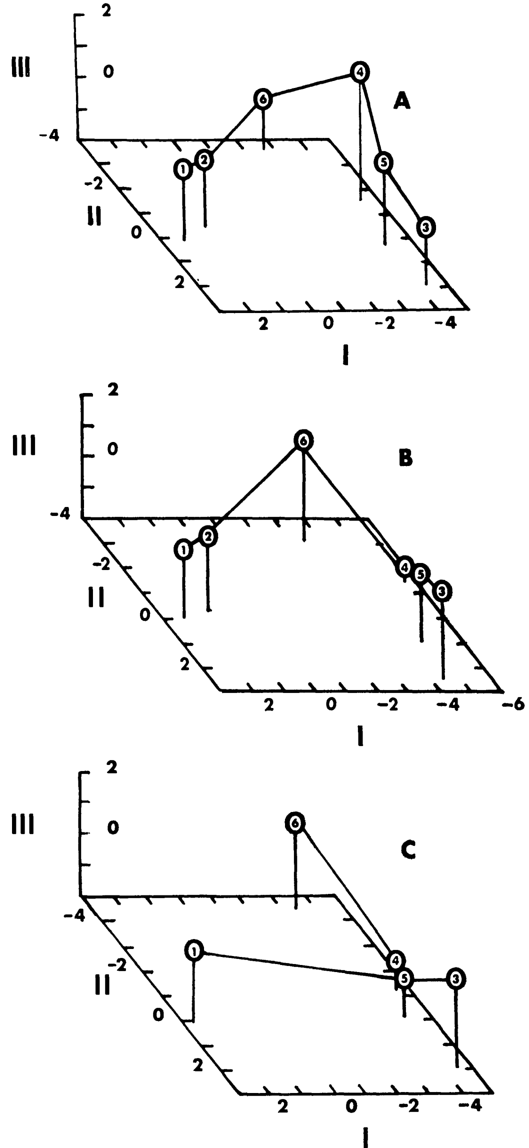

FIG. 4.—Projections of the canonical variables evaluated at group means onto the first three canonical variates for Procedure A (sexes pooled), Procedure B (males), and Procedure C (females). Lines connecting the groups represent the smallest paired D values.

observed within cytotypes. Figure 3B is the karyotype of the $2N = 40$ cytotype, which consists of ten pairs of size graded metacentric chromosomes, one pair of large submetacentric chromosomes, and eight

pairs of acrocentric chromosomes. The X is a medium sized metacentric and the Y is a small acrocentric chromosome. The $2N = 46$ karyotype is shown in Figure 3A and is composed of eight pairs of biarmed chromosomes, and 28 medium to small acrocentric chromosomes. The X is a medium sized metacentric chromosome, and the Y is a small acrocentric chromosome.

Results of the univariate analysis of variance for intergroup divergence in Procedures A (sexes pooled), B (males), and C (females) revealed all characters to be significant at the $P < .001$ level. For Procedure A (sexes pooled) canonical variate analysis indicated that the first canonical variate separated the $2N = 40$ cytotype (groups 1 and 2) from the $2N = 46$ cytotype (groups 3, 4, 5, and 6). The standardized canonical coefficients indicated that greatest length of skull, condylobasal length, and interorbital breadth contributed most to the separation. The second canonical variate separated group 6 (*M. waterhousii mexicanus*) from groups 3, 4, and 5 (*M. w. bulleri*); the farthest separation was between groups 3 and 6 (Table 1 and Figure 4A). The coefficients were more or less equal on the second canonical variate except for length of forearm. The third canonical variate was primarily a function of variation in greatest length of skull, condylobasal length, mastoid breadth, and posterior zygomatic breadth which separated out Group 4. Group 2 is separated from the other groups by the fourth canonical variate which reflected among-group variance in condylobasal length, posterior zygomatic breadth, maxillary tooth row, and length of the forearm. Canonical variate coefficients and canonical variables evaluated at group means are shown in Table 1 for Procedures A (sexes pooled), B (males), and C (females). Standardized canonical variables are presented in Table 2.

The classification matrix showed that no misclassification occurred between the $2N = 40$ cytotype and the $2N = 46$ cytotype. Misclassifications occurred, however, between the groups composing each cytotype. In

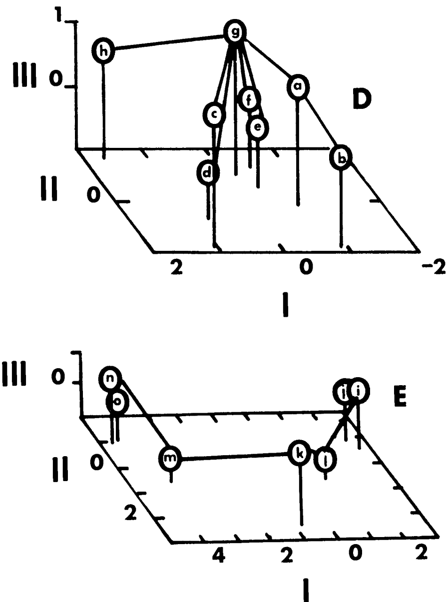

FIG. 5.—Three dimensional projections of the canonical variables evaluated at group means for Procedure D (geographic variation, $2N = 40$ cytotype) and Procedure E (geographic variation, $2N = 46$ cytotype). Lines between groups represent their smallest paired D value. Note in Procedure D that the groups diverge from the sympatric population g. See text and list of specimens examined for group identification.

the $2N = 46$ cytotype, misclassifications occurred among groups 3, 4, and 5 (*M. w. bulleri*).

The three canonical variables were projected into a three-dimensional plot and the samples joined by the smallest paired distance values ($\sqrt{D^2}$) shown in Table 3. These data in conjunction with the classification matrices indicated that the $2N = 40$ cytotype (groups 1 and 2) was phenetically distinct from the $2N = 46$ cytotype (groups 3, 4, 5, and 6). These distance values further indicated that females were slightly more divergent than males. In Procedures A (sexes pooled) and B (males) phenetic distance connections for the two cytotypes was lowest between groups 2 and 6, whereas in Procedure C (females) this distance value occurred between groups 1 and 5.

For each procedure the null hypothesis

TABLE 1. CANONICAL VARIATE ANALYSES FOR 14 VARIABLES, VARIANCE EXPLAINED, AND THE CANONICAL VARIATE COEFFICIENT EVALUATED AT GROUP MEANS.

Procedure A (Sexes Pooled)

Optimal subset of variables		I	II	III	IV
7	1	–1.88	–0.30	2.28	0.52
2	2	2.21	–0.42	–3.33	–1.89
4	3	0.86	–0.88	0.79	–0.93
13	4	–1.63	–0.06	4.86	–1.26
12	5	0.23	–0.94	–0.55	–0.25
10	6	–0.33	–0.35	–1.03	–0.10
1	7	–6.84	–0.87	–4.44	1.85
8	8	1.55	0.98	–2.89	–2.36
6	9	–0.37	–0.00	0.38	0.68
11	10	1.35	–1.18	–0.37	2.86
3	11	–0.66	–0.46	2.80	2.50
14	12	–2.06	–2.34	–2.18	0.42
5	13	0.05	–0.36	–0.02	0.50
9	14	–0.04	–0.02	–0.16	0.21
		Variance Explained			
		78.73%	17.39%	2.02%	1.16%
		Group Means			
	1	2.31	0.17	0.04	0.10
	2	1.29	–0.46	–0.14	–1.48
	3	4.26	1.94	–0.41	0.12
	4	–4.33	–1.49	1.79	0.22
	5	–4.00	0.32	0.38	–0.37
	6	–2.64	–3.64	–0.67	0.16

Procedure B (Males)

Optimal subset of variables		I	II	III	IV
7	1	–1.73	0.08	–0.84	–0.58
2	2	2.22	–0.53	2.96	1.71
4	3	0.34	–1.38	–1.48	0.70
13	4	–2.99	0.23	–4.50	1.15
12	5	0.36	–0.86	0.35	0.62
10	6	–0.73	–0.86	0.08	0.40
1	7	–5.32	2.22	4.72	–2.00
8	8	2.55	0.49	1.89	2.36
6	9	–0.56	–0.16	–0.51	–0.27
11	10	1.39	–0.83	0.18	–3.14
3	11	–2.01	–1.02	–3.60	–2.58
14	12	–2.93	–2.34	2.94	–0.86
5	13	0.11	–0.32	0.14	–0.40
9	14	–0.02	0.50	0.09	0.27
		Variance Explained			
		79.99%	14.88%	2.73%	1.60%
		Group Means			
	1	2.36	0.08	–0.08	–0.18
	2	1.48	–0.21	0.11	1.23
	3	–4.29	2.55	0.50	–0.26
	4	–5.65	–1.46	–1.92	–0.16
	5	–4.70	0.95	–0.13	0.56
	6	–3.41	–3.06	0.98	–0.20

TABLE 1. (*Continued.*)

Procedure C (Females)

Optimal subset of variables		I	II	III
7	1	–2.47	–0.47	–2.95
1	2	2.18	–0.34	2.93
14	3	1.16	–0.81	0.58
2	4	–1.46	–0.42	–5.10
12	5	–0.21	–1.19	0.25
13	6	–0.06	0.34	2.89
10	7	–7.76	0.63	2.36
4	8	1.34	1.29	3.06
8	9	–0.57	0.21	0.22
6	10	0.93	–2.07	–0.48
3	11	–0.53	–0.49	0.36
5	12	–2.55	–2.25	–0.04
11	13	0.14	–0.24	–0.10
9	14	–0.06	–0.31	0.43
		Variance Explained		
		79.02%	18.55%	1.70%
		Group Means		
	1	2.68	0.07	–0.01
	2			
	3	–4.26	2.00	0.50
	4	–4.49	–1.17	–1.24
	5	–4.04	0.11	–1.10
	6	–3.48	–4.53	0.58

of no morphometric divergence between groups was rejected at the $P < .001$ level using the Wilks Lambda Statistic. In the pair-wise F test, all pairs were significant at the $P < .001$ level.

A list of characters in order of their discriminatory ability is given in Table 1. For Procedures A (sexes pooled) and B (males) the order for the first three characters, interorbital breadth, condylobasal length, and mastoid breadth was the same. Procedure C (females) had the first character, interorbital breadth, common with Procedures A (sexes pooled) and B (males) but the next two characters were greatest length of skull and length of the third metacarpal.

An optimal subset of characters was determined according to their discriminating ability (Table 4), to facilitate the classification of unknown material. Table 4 gives the canonical coefficients, percent variation explained associated with each canonical variate, canonical variables evaluated at

TABLE 2. CANONICAL VARIATES, STANDARDIZED BY THE POOLED STANDARD DEVIATION. This coefficient shows which characters contribute to the variation found within each canonical variate. Absolute values are presented as the direction of the coefficient does not alter the contribution to variation on a particular axes.

	Procedure A (Sexes Pooled)				Procedure B (Males)				Procedure C (Females)		
	I	II	III	IV	I	II	III	IV	I	II	III
1	0.65	0.10	0.79	0.18	0.60	0.03	0.29	0.20	0.73	0.14	0.88
2	0.70	0.14	1.06	0.60	0.70	0.17	0.93	0.54	0.65	0.11	0.87
3	0.28	0.29	0.26	0.31	0.11	0.45	0.49	0.23	0.27	0.19	0.14
4	0.33	0.01	0.98	0.25	0.51	0.04	0.77	0.20	0.28	0.08	0.99
5	0.05	0.20	0.12	0.05	0.07	0.18	0.07	0.13	0.04	0.24	0.05
6	0.08	0.08	0.24	0.02	0.16	0.19	0.02	0.09	0.01	0.08	0.67
7	0.76	0.10	0.46	0.20	0.61	0.25	0.54	0.23	0.78	0.06	0.24
8	0.37	0.24	0.70	0.57	0.49	0.09	0.36	0.45	0.35	0.34	0.80
9	0.08	0.00	0.09	0.16	0.13	0.04	0.12	0.06	0.12	0.04	0.05
10	0.25	0.22	0.07	0.53	0.26	0.16	0.04	0.60	0.16	0.36	0.08
11	0.11	0.08	0.48	0.43	0.31	0.16	0.55	0.39	0.09	0.08	0.06
12	0.27	0.31	0.29	0.06	0.37	0.30	0.37	0.11	0.31	0.27	0.00
13	0.07	0.45	0.03	0.64	0.14	0.40	0.17	0.49	0.18	0.30	0.12
14	0.06	0.04	0.26	0.34	0.04	0.11	0.20	0.48	0.06	0.29	0.41

group means, and standardized canonical coefficients. For Procedure A (sexes pooled) the first canonical variate separated the $2N = 40$ cytotype from the $2N = 46$ cytotype with the highest canonical coefficient for interorbital breadth. Condylobasal length had the highest coefficient of variation along the second canonical variate which separated out Group 6 (*M. w. mexicanus*) and Group 3 (*M. w. bulleri*). Mastoid breadth had the highest coefficient on the third canonical variate which separated out Group 4 (*bulleri*). Similar results were obtained for Procedures B (males) and C (females).

The classification matrices based on the optimal subset of four variables was somewhat different from the matrices from the 14 variables. No misclassifications occurred between the two cytotypes. The difference between the optimal subset of variables and the 14 variable set was between the subspecies of the $2N = 46$ cytotype. In Procedure A (sexes pooled) three specimens (9.1%) of *mexicanus* were classified as group 4 (*bulleri*). In Procedure B (males) two specimens (1.2%) of *mexicanus* were classified as group 5 (*bulleri*). Procedure C (females) showed no misclassifications of *mexicanus*.

The null hypothesis of no morphometric divergence between groups can be rejected at the $P < .001$ level for both $2N = 40$ and $2N = 46$ cytotypes. A univariate analysis of variance within the $2N = 40$ cytotype showed most characters significant at $P < .001$, interorbital breadth was significant at $P < .01$, and the external measurements (13 and 14) were not significant. An ANOVA of the $2N = 46$ cytotype samples showed all

TABLE 3. MAHALANOBIS DISTANCE MATRIX. In each group the first value is for procedure A (sexes pooled), the second, in parentheses for procedure B (males), and the third for procedure C (females).

	1	2	3	4	5
2	4.81 (4.42)				
3	7.46 (7.63) 7.93	6.96 (7.12)			
4	7.72 (8.90) 7.99	6.98 (8.23)	5.11 (5.75) 4.73		
5	7.27 (7.99) 7.65	6.62 (7.33)	4.10 (4.46) 4.30	4.43 (4.89) 4.22	
6	7.31 (7.72) 8.54	6.53 (6.98)	6.91 (6.91) 7.53	5.27 (5.59) 5.61	5.78 (5.97) 6.19

TABLE 4. CANONICAL VARIATE ANALYSES FOR AN OPTIMAL SUBSET OF VARIABLES FOR PROCEDURE A (SEXES POOLED), B (MALES), AND C (FEMALES). Asterisks (*) indicate characters best explaining the variation seen along a particular canonical variable.

	Procedure A (Sexes Pooled)				Procedure B (Males)				Procedure C (Females)	
	I	II	III		I	II	III		I	II
2	1.54	−1.90	0.74	2	−1.53	−2.72	−1.42	1	−1.10	−1.59
4	−1.17	−0.48	−5.66	4	2.57	−0.78	6.58	2	1.67	1.15
7	−7.34	0.70	4.67	7	5.91	2.20	−5.18	7	−9.60	0.21
12	−2.16	−2.73	1.50	12	2.94	−3.02	−2.53	14	0.05	0.51
13	0.05	−0.39	0.22							
	Variance Explained				*Variance Explained*				*Variance Explained*	
	80.23%	17.90%	1.49%		82.68%	14.92%	2.37%		80.61%	18.59%
	Group Means				*Group Means*				*Group Means*	
1	2.12	0.16	0.03		−2.04	0.13	−0.01		2.32	−0.07
2	1.18	−0.55	−0.62		−1.28	−0.52	0.26			
3	−3.92	−1.76	0.35		3.81	2.12	−0.52		−3.77	−1.61
4	−3.71	−1.11	−1.14		4.38	−0.85	1.40		−3.75	−0.87
5	−3.63	0.29	−0.47		4.05	−0.64	0.39		−3.35	−0.28
6	−2.54	−3.40	0.51		3.89	−2.68	−0.72		−2.99	3.98
					Standardized Canonical Coefficient					
2	0.49	0.60*	0.24	2	0.48	0.86*	0.00	1	0.33	0.47*
4	0.24	0.10	1.14*	4	0.44	0.13	1.13*	2	0.48	0.34
7	0.82*	0.08	0.52	7	0.67*	0.25	0.59	7	0.96*	0.02
12	0.29	0.36	0.20	12	0.37	0.38	0.32	14	0.05	0.48*
13	0.06	0.49	0.28							

characters to be significant at the $P < .001$ level, except interorbital breadth which was not significantly different.

In the $2N = 40$ cytotype, 53 percent of all specimens were misclassified but no pattern of misclassification was apparent. Within the $2N = 46$ cytotype, misclassifications were less frequent and samples formed a definite pattern. Specimens of *M. w. bulleri* (groups i, j, k, and l) showed 22.8 percent misclassification with *M. w. mexicanus* (groups m, n, and o). Specimens of *mexicanus* had a 15.2 percent intergroup misclassification frequency with no specimens of *mexicanus* classified as *bulleri*.

Phenetic information for Procedure D (geographic variation; $2N = 40$ cytotype) and E (geographic variation; $2N = 46$ cytotype) is summarized in Figures 5D and 5E respectively. In the $2N = 40$ cytotype most groups varied from the phenetically central group g (the Quiriego $2N = 40$ population). In the $2N = 46$ cytotype the groups were connected by their smallest paired D value in the order j, i, l, k, m, o, n. A north to south order would be alphabetical i, j, k, l, m, n, o.

The results of the two group discriminant analysis for the 14 variable data set, the optimal subset of variables, and interorbital constriction showed the groups to be significant at the $P < .001$ level (F 197.55 with 13, 345 DF: F 508.75 with 4, 345 DF: and F 1904.47 with 1, 358 DF respectively). The Mahalanobis D^2 values are 34.72, 30.58, and 22.64 for the three data sets. When interorbital constriction was used as the discriminating variable the nine individuals with a 3.8 mm interorbital constriction width (3 from the $2N = 40$ cytotype and 6 from the $2N = 46$ cytotype) had discriminant function scores of −0.44981.

DISCUSSION

Chromosomal Data

The chromosomal data leads one to question the validity of the present systematics

of *Macrotus*. Of the 38 specimens karyotyped from Quiriego, 13 are $2N = 40$ and 25 are $2N = 46$. One would expect to find at least a narrow "hybrid" zone if the two cytotypes were freely interbreeding. The chromosomal and morphometric data suggest that specific status should be given the two cytotypes. In the following discussion, two species are recognized, *Macrotus californicus* Baird, and *M. waterhousii* Gray, the $2N = 40$ and $2N = 46$ cytotypes, respectively.

As shown by the chromosomal data the zone of sympatry between *M. waterhousii* and *M. californicus* is at most a narrow zone not exceeding 50 kilometers. In light of the mobility attributed to bats (as compared, for example, to rodents), this zone of contact is regarded as parapatric, a most important point. Perusal of the distribution maps in Hall and Kelson (1959) revealed that no more than two other genera of bats, *Pipistrellus* (*P. hesperus* and *P. subflavus*) and *Rhogeëssa*, are recognized as possibly having parapatric distribution. Unlike *Macrotus*, the species of *Pipistrellus* are easily recognized using external characters. With regard to *Rhogeëssa*, the systematic status of various taxa currently are under investigation (R. K. LaVal, personal communication). Several chromosomal races are involved in *Rhogeëssa* (Baker, unpublished data) and bats of that genus may prove most interesting in light of their distributional patterns.

Bats are generally considered by vertebrate biologists to be rather mobile creatures occupying fairly broad ranges. In the past, when the systematic status of a taxon was examined, grouping of specimens used for statistical analysis often covered fairly broad geographic areas. If the distribution of two similar species was parapatric, such lumping could have resulted in a "blending effect," therefore obscuring the recognition of distinct populational entities. The demonstration of a parapatric distribution in *Macrotus* poses a new dimension to the biology of this taxon and may have implications to the biology of bats in general. The karyotype has played a noteworthy role in the recognition of this phase of the biology of *Macrotus* because it has provided a means for recognition of individuals for grouping and proof that no hybrids were in the samples. The lack of such an index in other bat species may be one reason why this aspect of the biology of bats has not been recognized. It is hoped that future systematists will explore the possibility of parapatry in greater detail.

Morphometric Analysis of Intergroup Divergence

The degree of morphological divergence demonstrated by multivariate analysis revealed *M. californicus* (groups 1 and 2) to be phenetically separate from *M. waterhousii* (groups 3, 4, 5, and 6). Group 2 tended to separate as a distinct entity in most analyses. This group is composed entirely of male specimens.

The classification matrices for the 14 variable data set (Table 3) show distinct patterns of misclassifications. No specimens of *M. californicus* were classified as *M. waterhousii*, and conversely no *waterhousii* were classified as *californicus*. The groups of the subspecies *M. w. bulleri* (groups 3, 4, and 5) were misclassified with each other but remained distinct from other groups. In addition, no specimens from the other taxa were classified as *bulleri*. The same applies to group 6, *M. w. mexicanus*. When all of the phenetic data were summarized into a three dimensional projection a closer affinity between the two subspecies was realized. These taxa are therefore considered to be subspecies as defined by Lidicker (1962).

Specimens of the two species were easily separated by both the two group discriminant analysis using characters 2, 4, 7, 12, and 13. Separation along the first canonical variate, when figured on an optimal subset of variables, was primarily a result of interorbital breadth. An interorbital breadth of 3.8 mm or less is indicative of *M. californicus*, 3.8 mm or more being *M. waterhousii*. When a specimen from the zone of

contact has a 3.8 mm interorbital breadth and if it is a male, it should be a specimen of *M. californicus*; and if it is a female, it should be a specimen of *M. waterhousii*. Three males and no females of the 226 specimens of *M. californicus* had an interorbital breadth of 3.8 mm. Four females and two males of the 134 specimens of *M. waterhousii* had an interorbital breadth of 3.8 mm. The four females would be correctly classified by the above criteria. None of the males from the northern part of the range had a 3.8 mm interorbital constriction. The two males mentioned above came from the southern portion of the range (Guerrero and Oaxaca). If one exercises caution when assigning specimens with a 3.8 mm interorbital breadth and considers the locality and sex of the specimen in question, the two species are readily identifiable by interorbital breadth. If taxonomic placement of a specimen is doubtful, the optimal subset of variables would be sufficient in discriminating between the species.

The separation of *M. w. bulleri* from *M. w. mexicanus* is along the second canonical variate and is primarily a result of condylobasal length. This character is, on the average, larger in *mexicanus* but the difference between these two populations is more overlapping than the differences between the species.

Geographic Variation

Geographic variation does exist within each species as is evidenced by the amount of morphometric divergence among various populations for each species. This variation, however, is quite different for the two species. In *M. californicus* the mean canonical variable score and the Mahalanobis distance relationship show all populations, except b, to diverge from a phenetically central population, g. This population is the $2N = 40$ cytotype portion of the Quiriego sample. *M. californicus*, then, diverges phenetically from the known sympatric population (Fig. 5D).

Macrotus waterhousii shows a different pattern of geographic variation (Fig. 5E). The data show a general trend pointed out by Anderson (1965), of increase in size from north to south in all measurements as is evidenced by the means of the various groups. The data, however, do not indicate a perfect north to south relationship as Anderson (1965) implied. The multivariate analyses show that the groups are allied one to another nonclinally with respect to geography. Two distinct groups, however, are seen in the classification matrices and the three dimensional projections (Fig. 5E). These groups conform to the subspecies *M. w. bulleri* and *M. w. mexicanus*.

Role of Multivariate Statistics as a Population Indexer

The karyotype was defined earlier as a population marker or indicator as it relates to the biology of *Macrotus*. This index or other indices such as electrophoretic bands, though beneficial, are not prerequisites or necessities in recognizing the existence of a system such as described for *Macrotus*. Multivariate statistics provides a means by which the same ends are realized.

When specimens of *Macrotus* were grouped according to subspecies boundaries as defined by Anderson (1965), with misclassified animals regrouped along the columns of the classification matrix, six computations resulted in all but two animals being placed into the systematic relationship defined in this paper. The two misclassified animals were specimens of *M. californicus* with one classified as *M. w. bulleri*, the other as *M. w. mexicanus*. These classifications occurred early in the six computation series and were not reclassified to their respective groups. The localities of these two animals would lead one to question their classification. One is from locality 11, the other, 15, both considerably north of the zone of parapatry. When the first two canonical variables are plotted these animals appear as intermediates between the two groups. Nevertheless, the Quiriego sample was shown by this analysis to be sympatric (divided correctly according to

the karyotypes of the specimens). The northern Sinaloan locality 25 (Group 2) from Los Mochis was always classified in the matrix as *californicus*. This locality was attributed to *californicus* by its morphometric characters from the outset. No chromosomal data are available for this population. The Alamos $2N = 46$ specimens and the Quiriego $2N = 46$ specimens were classified as *bulleri*. The same distributional pattern is realized as was concluded from a chromosomal basis.

Evolutionary and Systematic Implications

If other closely related bat species have parapatric distributions, their current systematic status would be subspecific recognition. Methods are now available for testing to see if other species show such a distributional pattern. Genoways and Choate (1972) used cluster analysis of a distance matrix, principal components analysis, and discriminant function analyses in a study of the short-tailed shrew, *Blarina brevicauda*. Their results show that two subspecies of this shrew were found to occupy a parapatric distribution in Nebraska. Parapatric distributions in mammals are usually associated with species that have a low vagility especially as compared to bats. (For example: pocket gophers, Kennerly, 1959; Vaughn, 1967; Patton and Dingman, 1968; Reichman and Baker, 1972; *Blarina*, Genoways and Choate, 1972; *Neotoma*, Birney, 1970; *Perognathus*, Patton and Soule, 1967). Because a parapatric distribution seems improbable for a highly mobile species such as bats, several questions arise: (1) what is the biological significance of such a distribution, (2) what are the factors involved in maintaining this distribution, (3) how does such a distribution arise? Even if *Macrotus* is much less mobile in practice than one might conclude to be characteristic of a volant species, its vagility is certainly higher than that of the mammalian species mentioned above that show a parapatric distribution. Vagility is a most important factor in the discovery of parapatry in *Macrotus*. That is, parapatry is not a result of low vagility as implied by White (1969:89) but, as shown in *Macrotus*, is selected for and maintained in a mobile mammalian species.

The mechanisms involved in the maintenance of parapatry in *Macrotus* are unclear. Intrinsic factors such as philopatry or habitat selection could conceivably enter into the maintenance of parapatry in *Macrotus*. Three mechanisms seem probable in explaining the origin of this distributional pattern and speciation in *Macrotus*. First, the two cytotypes arose by classical allopatric speciation. A chromosomal change occurred and became established in a geographically isolated population. Baker (1973) hypothesized that chromosomal evolution in the phyllostomatid bats with a $2N$ greater than 32 occurred via fissions. If this is true one would expect that the $2N = 46$ cytotype to be the most derived species. Thus, the isolation and chromosomal changes would have most logically occurred in Baja, south central Mexico, or the Yucatan Peninsula.

The present distribution of *Macrotus* may be a recent development due to the activities of man. Mining has allowed the effective range of *Macrotus* to extend from the Sierra Madre Occidental toward the west coast. Mining activity being rather extensive in this region has expanded the available roosting sites and perhaps more important, maternity sites, thus playing a significant role in the present distribution of *Macrotus*.

A second interpretation is that *Macrotus* is a stasipatric species (White *et al.*, 1967; White, 1968, 1969; Key, 1968). This interpretation is best explained by assuming the $2N = 40$ cytotype to be derived. When one considers the climatic history of the Sonoran region and the habitat preferences of *Macrotus* this seems a tenable hypotheses. Conditions during late Pleistocene in northern Sonora and southern Arizona (Mehringer, 1967) would have been favorable to the $2N = 46$ cytotype, it having the more mesic to tropic habitat preference. Xeric conditions began to occur toward the end

of the Pleistocene so that by 7,500 to 7,000 B.P. modern conditions existed, as evidenced in southern Arizona (Mehringer, 1967; Martin, 1961). With these changing climatic conditions an alteration in diploid number could have occurred via centric fusions eventually resulting in a $2N = 40$ cytotype with a selective advantage over the $2N = 46$ cytotype. The changing populations could have come in contact with the $2N = 46$ cytotype with the eventual formation of a tension zone that could have shifted geographically with the changing climatic conditions (Key, 1968; White, 1969). At first, hybridization could have occurred across this tension zone. However, sufficient divergence evolved in the two types resulting in termination of hybridization with the tension zone now becoming the zone of parapatry, probably along the western side of the Sierra Madre Occidental. Assuming the $2N = 40$ cytotype to have evolved via a series of centric fusions from 46 to 44 to 42 then 40, waves of tension zones can be visualized, conceivably reducing the genetic input into the center of differentiation of the population. If the divergence of the two forms occurred in post-Pleistocene time, stasipatric speciation as described by White *et al.* (1967) is a plausible interpretation because there is no reason to assume isolation. This interpretation implies selection for a mesic versus a xeric habitat preference occurred between the two races.

Third, centrifugal speciation (Brown, 1957) may be operating in *Macrotus*. If so, southern Mexico could well be the evolutionary center of the range with the $2N = 46$ cytotype being the most derived. Genetic divergence occurs in the center of the range with concomitant dispersal resulting in the $2N = 40$ peripheral species. Fossil evidence from Antillean caves of late Pleistocene or sub-recent deposits indicate that forms of *Macrotus* "were not substantially different from those now in the same or nearby areas" (Anderson, 1969). Therefore *Macrotus* was well established on the islands by late Pleistocene. Assuming the insular forms are immigrants from the mainland the karyotypic information of these insular forms will be valuable in understanding the evolution of the karyotype in *Macrotus*. It is therefore, necessary to obtain information on the biology of *Macrotus* from the Antillean portion of its distribution before a final interpretation can be made concerning the evolution of *Macrotus*.

SPECIMENS EXAMINED

Specimens from The Museum of Natural History, The University of Kansas are identified by the letters KU and those of The Museum Texas Tech University are identified by the letters TT.

Macrotus californicus—CALIFORNIA: Riverside Co., (1) 35 mi. N Blythe, Riverside mtns., 7 females, 9 males, KU 45153–57, 45159–60, 49661–69; (2) Cave near Torres, 2 females, KU 9775–76. Imperial Co., (3) 3 mi. N Potholes, 2 females, 2 males, KU 22204–07; (4) Ft. Yuma, 8 females, 8 males, KU 14623, 14625, 45494–95, 45498–508; (5) 6 mi. SW mt. Yuma, 4 females, 2 males, KU 45410, 45412–16; ARIZONA: Pima Co., (6) Colossal Cave, 1 male, KU 34857; (7) 25 mi. S Case Grande, Old Mammon mine, 1 female, 2 males, TT 11825–27; (8) 22 mi. S, 7 mi. W Tucson, 2 males, KU 58944–45; SONORA: (9) 15 mi. E Carbo, Cueva de la Durado, 1 female, 2 males, TT 10598–600; (10) 2 km N Kino Nuevo, 2 males, KU 95150–51; (11) 3 mi. E Willard Railroad Station, 7 females, 12 males, TT 10533–52; (12) 0.7 mi. SE La Colorada, 2 females, 10 males, TT 10576–81, 10584–88; (13) 12.7 mi. W Novilla, 5 females, 2 males, TT 10525–27, 10529–32; (14) 2.6 mi. E, 0.4 mi. S Tecoripa, 2 females, 1 male, TT 10557–59; (15) 7 km. W Tonichi, 61 females, 20 males, TT 10607–15, 14100–11, 14113–28, 14130–33, 14135–38, 14140–55, 14158–65, 14167–77; (16) 26 mi. S Tonichi, ca. 1 mi. N La Dura, 8 females, 7 males, TT 14178, 14181–82, 14184–89, 14192, 14194, 14197–200; (17) 11.9 mi. N Quiriego, Mina Escondida, 4 females, 17 males, TT 14201, 14205–07, 14210, 14218–19, 14221, 14230, 14233, 14235–36, 14238–40, 14249–50, 14252–

53; SINALOA: (18) 12 mi. N, 3 mi. W Los Mochis, 16 males, KU 60782–83, 60786, 60792–97, 60799–803, 60805–06.

Macrotus waterhousii bulleri—SONORA: (17) 11.9 mi. N Quiriego, Mina Escondida, 18 females, 17 males, TT 14202–04, 14208–09, 14211–14, 14216–17, 14220, 14222–26, 14228–29, 14231–32, 14234, 14237, 14241–48, 14251, 14254–56; (19) 4.5 mi. N Alamos, 1 female, 1 male, TT 10565–66; (20) La Aduana water mine, 1 male, TT 6267; (21) 0.25 mi. W Aduana, 14 females, 1 male, TT 14257–63, 14265–72; DURANGO: (22) Santa Ana, 12 mi. E Casala Sinaloa, 2 females, 1 male, KU 90600, 90602–03; SINALOA: (23) 1 km. NE Panuco, 2 females, 3 males, KU 94075–76, 94083–85; (24) Panuco, 2 females, 6 males, KU 95745–47, 85611–15; JALISCO: (25) 10 mi. W, 9 mi. N Magdalena, 4 females, 3 males, KU 33330–31, 33334, 33337–39, 33341; (27) 4 mi. NNE Teuchitlan, 6 females, 6 males, KU 30879–86, 31847, 31849–51; (28) 22 mi. W, 8 mi. S Guadalajara, 3 females, 1 male, KU 36536–39; (29) 12 mi. S, 4 mi. E Yahualica, NW side Rio Verde, 1 male, KU 38248; (30) 11.5 mi. S, 10.5 mi. E Guadalajara, El Salto, 1 male, KU 31852.

M. w. mexicanus—GUERRERO: (31) 8 km. SE Teloloapan, 3 females, 5 males, KU 28384–89, 28391, 28393; (32) 1 mi. SE Apetlanca, 2 females, 2 males, KU 66331–33, 66335; MORELOS: (33) San Gabriel, 1 male KU 28297; PUEBLA: (34) 2 mi. SE Izucar de Metamores, 2 females, KU 60812, 60815; (35) 1 mi. E Raboso, 1 female, 1 male, KU 60816, 62345; OAXACA: (36) 4.5 km. N Cuicatlan, 3 females, 3 males, KU 20412, 29414–17, 29419; (37) 3 km. WNW Dominguillo, 2 females, KU 29421–22; (38) 3 mi. NW Tehuantepec, 5 females, 3 males, KU 60818–25.

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