

EVOLUTIONARY RELATIONSHIPS IN *MACROTUS* (MAMMALIA: CHIROPTERA): BIOCHEMICAL VARIATION AND KARYOLOGY

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Abstract

Greenbaum, I. F., and R. J. Baker (Department of Biological Sciences and The Museum, Texas Tech University, Lubbock, Texas 79409) 1976. *Evolutionary relationships in Macrotus (Mammalia: Chiroptera): biochemical variation and karyology*. *Syst. Zool.* 25:15-25.— Genetic variation at 21 loci as determined by isozyme electrophoresis was examined from 218 specimens of *Macrotus* representing two species and including both mainland and island populations. Karyotypic and electrophoretic data suggest that specimens from the Antillean population (*Macrotus waterhousii*) are conspecific with populations of the southern mainland taxa. This high degree of similarity between Antillean and the southern mainland taxa and the tendency for the average heterozygosity of populations to be higher in peripheral populations than at the zone of contact between the two mainland species are best explained by the classical allopatric model of speciation and the data do not support the centrifugal model of speciation in this genus. A reduced degree of average heterozygosity per individual as is thought to be characteristic of island populations was not found for specimens of *Macrotus* from Jamaica. A decrease in variation is indicated, however, by a reduced number of loci that are polymorphic with most of the heterozygosity of the Jamaican population being in the esterase loci. The high degree of exomorphological similarity in *Macrotus* is not reflected in genic similarities. *Macrotus* display the lowest range of interspecific similarities reported for mammalian sibling species and are comparable in protein identities to sibling species of *Drosophila*. [Chiroptera; karyology; speciation; biogeography.]

This study was designed to determine the genetic relationships of mainland and Antillean populations of the New World leaf-nosed bats of the genus *Macrotus* by electrophoretic and chromosomal analyses in order to support or refute possible mechanisms of speciation and elucidate the evolutionary relationships in the genus.

Analysis of genetic structure and a knowledge of changing patterns of genotype frequencies are essential to understanding evolution and speciation in natural populations. Estimates of genic heterozygosity and genic similarity within and between closely related species have been obtained for a wide variety of organisms. Analyses of genetic structure of natural populations of closely related mammalian species though have been limited almost exclusively to rodents.

Heterozygosity estimates have been obtained for four species of bats representing two families. Straney *et al.* (1975) reported low heterozygosity, 0.03, for one population of *Macrotus californicus* (a phyllostomatid

bat) and the highest level of heterozygosity for a vertebrate species, 0.144, for three populations of *Myotis velifer* (a vespertilionid bat). As would be expected, these two species representing two different families had a similarity value (S, Rodgers', 1972) of zero. In another study (Straney *et al.*, personal communication) two other vespertilionid bats, *Pipistrellus hesperus* and *Myotis californicus* were found to have heterozygosities of $\bar{H} = 0.026$ and 0.126 respectively. Bats then exhibit an extreme range of average heterozygosity. Selander and Johnson (1973) reported that genic similarity (S) between various species generally ranged from 0.08 to 0.32 for congeneric species and from .23 to .89 for sibling species. However, values outside of these ranges are not uncommon (Nevo and Shaw, 1972 and Nevo *et al.*, 1974). Heterozygosity estimates in a dozen various species (Le-wontin, 1974, Table 22) ranged from .56 to .184 with a mean of .106. Selander and Johnson (1973) summarized estimates of genic heterozygosity in vertebrate popula-

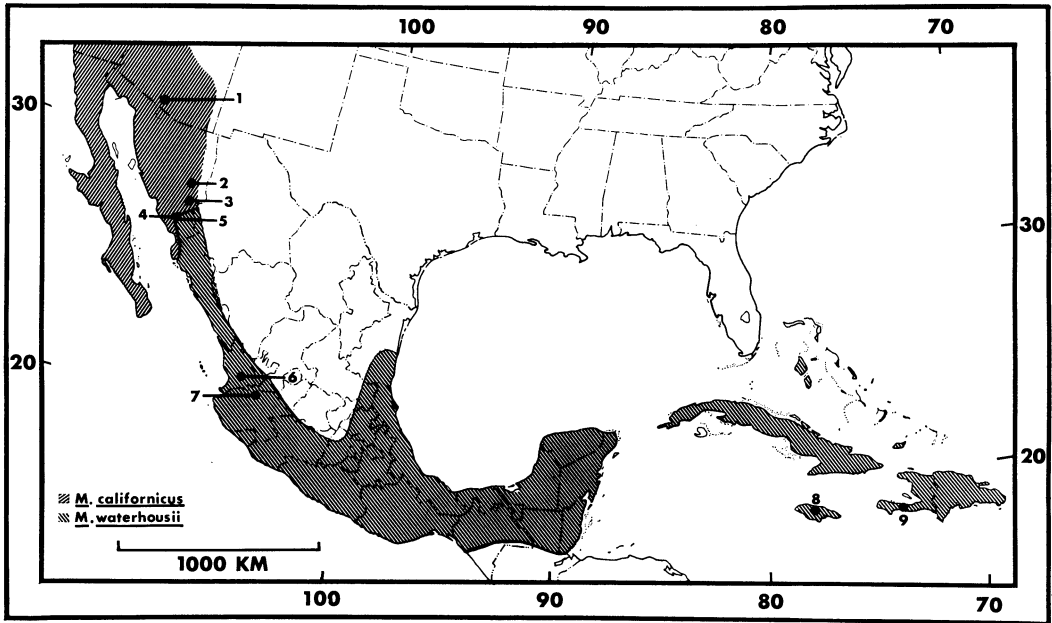


FIG. 1.—Distribution of *Macrotus*. Numbers refer to localities listed in text.

tions and reported a range from .03 to .112 with the highest value for a fish (*Astyanax*; Avise and Selander, 1971) and the lowest for the elephant seal (*Mirounga*; Bonnell and Selander, 1973) which experienced marked reduction in numbers over the last century. Patton *et al.* (1972), Nevo and Shaw (1972), Kim (1972), Nevo *et al.* (1974), and Selander *et al.* (1974) reported generally lower average heterozygosities for fossorial rodents (medians ranging from .000 to .07).

Distribution of bats of the genus *Macrotus* (Fig. 1) is such that an understanding of the genic relationships of the representative taxa can lead to a better understanding of the process of speciation and evolutionary divergence of bats. For a few years prior to karyotypic analyses *Macrotus* was considered a monotypic genus based on the morphological studies of Anderson and Nelson (1965). Currently two mainland species are recognized (Davis and Baker, 1974). *Macrotus californicus* has a diploid number ($2n$) of 40 and is distributed in Arizona, California, Baja California, the

northern three-fourths of Sonora, and the extreme northern coastal regions of Sinaloa. *Macrotus waterhousii* ($2n = 46$) is distributed over tropical Mexico northward to Tamaulipas on the eastern side of Mexico and into southern Sonora on the western side of Mexico. Although co-inhabitation was documented for a single locality the two species are believed to be parapatric with a narrow contact zone (Vaughan, 1967). Such a distributional pattern is rare if not unique among closely related species of bats (Davis and Baker, 1974). When Davis and Baker (1974) divided mainland *Macrotus* into two species, karyotypes of Antillean specimens were not available. The specific relationships of Antillean populations to the mainland species therefore remained in question.

Parapatric distribution of mainland species of this genus raises the question as to the presence or absence of introgressive hybridization at the zone of contact. Although no chromosomal hybrids were detected by either Nelson-Rees *et al.* (1968) or Davis and Baker (1974), electrophoretic

TABLE I. GEL TYPES AND BIOCHEMICAL STAINING PROCEDURES USED IN THIS STUDY.

Gel type	Tissue	Voltage or milliamperage (ma)	Time (hrs.)	Stains
1. Phosphate	liver	130V	5	Phosphoglucose isomerase (Pgi)
2. Continuous Tris-Citrate I	kidney	150V	3	Lactate dehydrogenase (Ldh) Malate dehydrogenase (Mdh) Isocitrate dehydrogenase (Idh)
3. Continuous Tris-Citrate II	liver	75ma*	5*	α -Glycerophosphate dehydrogenase (α -Gpd) Glutamic oxalate transaminase (Got) Alcohol dehydrogenase (Adh) Indophenol oxidase (Ipo)
4. Tris-Hydrochloric Acid	liver	75ma*	3*	Albumin (Alb) General protein (Gp)
5. Discontinuous Tris-Citrate (Poulik)	kidney	250V	5*	Glucose-6-phosphate dehydrogenase (G6p)
	liver	250V	5*	Esterases (Es)
6. Tris-Maleate	kidney	100V	5	6-Phosphogluconate dehydrogenase (6Pgd) Phosphoglucomutase (Pgm)

* Indicates variation from the techniques of Selander *et al.* 1971.

and further chromosomal analysis of the contact zone population and populations on either side of the contact zone might reveal or essentially discount introgressive hybridization between the two species of *Macrotus*.

Davis and Baker (1974) suggested that the mainland distribution of *Macrotus* could be explained by several models of speciation. These authors stated that a determination of the affinities of Antillean populations to mainland populations might be the key to understanding speciation in the genus. If the classical geographic isolation model best explains the evolutionary history of *Macrotus* then the island populations should be most closely related to adjacent mainland populations and more distantly related to the species occupying the northwestern part of the range of the genus. If speciation followed Brown's (1957) model of centrifugal speciation then the central populations should be more rapidly evolving and more distantly related to peripheral populations.

Morphological similarity, geographic chromosomal variation, and parapatric distribution of mainland forms provide a unique basis for studying speciation. In addition the availability of extending the chromosomal data to include specimens from Jamaica and Haiti, and the possibility

of correlating the above with isozyme data, present a unique opportunity for the extension of electrophoretic techniques to include intra- and interspecific comparisons of bats.

METHODS AND MATERIALS

Allozymic variation was analyzed for 100 *Macrotus californicus* (74 ♂ and 26 ♀) and 118 *M. waterhousii* (40 ♂ and 78 ♀). Specimens were collected at the following localities (the numbers in parentheses refer to Figure 1). *M. californicus*—(1) Arizona: 25 mi. S Casa Grande, N = 12; (2) Sonora: 5.5 mi. W Tonichi, N = 30; (3) 1 mi. N La Dura, N = 24; (4) 11.9 mi. N Quiriego, N = 34; *M. waterhousii*—(5) Sonora: 11.9 mi. N Quiriego, N = 7; (6) Nayarit: 0.5 mi. N, 0.7 mi. E Santa Maria del Oro, N = 23; (7) Jalisco: 1 mi. NW Tequila, N = 39; (8) Jamaica: 2 mi. E Discovery Bay, Green

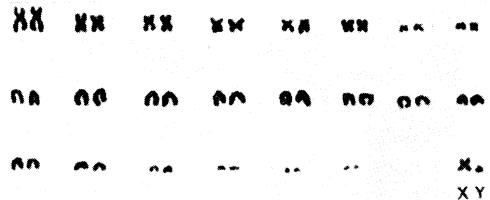


FIG. 2.—Karyotype of a male *Macrotus* from Jamaica.

TABLE 2. ALLELES AND FREQUENCIES (IN PARENTHESES) AT EACH LOCUS AND DIPLOID NUMBER IN THE EIGHT POPULATIONS ANALYZED ELECTROPHORETICALLY.¹

Pop.	1	2	3	4	\bar{h}
2N	40	40	40	40	
α -Gpd	100 (0.7220) 118 (0.2780)	100 (0.896) 118 (0.104)	100 (1.000)	100 (0.8970) 118 (0.1030)	0.12
Es-2	100 (0.7920) 110 (0.2080)	100 (0.845) 110 (0.155)	100 (0.833) 110 (0.167)	100 (0.8240) 110 (0.1760)	0.31
Es-3	100 (1.0000)	100 (1.000)	100 (1.000)	100 (1.0000)	0.00
Idh-1	100 (1.0000)	100 (1.000)	100 (0.979) 117 (0.021)	100 (0.4000) 117 (0.6000)	0.01
Ldh-1	100 (1.0000)	100 (1.000)	100 (0.979) 103 (0.021)	100 (1.0000)	0.01
Mdh-2	100 (0.9580) 114 (0.0420)	100 (0.917) 114 (0.083)	100 (0.896) 114 (0.104)	100 (0.9840) 114 (0.0160)	0.13
Pgi	-100 (1.0000)	-100 (0.950) -63 (0.050)	-100 (1.000)	-100 (1.0000)	0.03
Pgm	100 (1.0000)	100 (1.000)	100 (0.979) 75 (0.021)	100 (1.0000)	0.01
Got-2	-100 (1.0000)	-100 (1.000)	-100 (1.000)	-100 (1.0000)	0.00
Got-1	100 (1.0000)	100 (1.000)	100 (1.000)	100 (1.0000)	0.00
Ipo-1	100 (1.0000)	100 (1.000)	100 (1.000)	100 (1.0000)	0.00
Ipo-2	100 (1.0000)	100 (1.000)	100 (1.000)	100 (1.0000)	0.00
G6p	100 (1.0000)	100 (1.000)	100 (1.000)	100 (1.0000)	0.00
Adh	-100 (1.0000)	-100 (1.000)	-100 (1.000)	-100 (0.1000)	0.00
Alb	100 (1.0000)	100 (1.000)	100 (1.000)	100 (1.0000)	0.00
Gp-1	100 (1.0000)	100 (1.000)	100 (1.000)	100 (1.0000)	0.00
Idh-2	100 (1.0000)	100 (1.000)	100 (1.000)	100 (1.0000)	0.00
Ipo-3	100 (1.0000)	100 (1.000)	100 (1.000)	100 (1.0000)	0.00
Ldh-2	-100 (1.0000)	-100 (1.000)	-100 (1.000)	-100 (1.0000)	0.00
Mdh-1	100 (1.0000)	100 (1.000)	100 (1.000)	100 (1.0000)	0.00
6Pgd	100 (1.0000)	100 (1.000)	100 (1.000)	100 (1.0000)	0.00

¹ \bar{h} is the proportion of individuals heterozygous at a given locus and is averaged over the populations of *M. californicus*, mainland and Jamaican *M. waterhousii*.

Grotto, N = 49; (9) Haiti: Dept. du Sud; 1 km N Lebrun and 2 km N, 2 km E Lebrun, N = 6. Electrophoretic and chromosomal data were obtained from all of the above localities except locality 9 for which only chromosomal data were available. Specimens are on deposit in The Museum, Texas Tech University.

Karyotypic methods were described by Baker (1970). Heart, liver and kidney of each specimen were frozen and maintained in liquid nitrogen until processed. Heart and kidney extracts were processed together. Techniques of tissue preparation, electrophoresis, and biochemical staining were similar to those described in Selander *et al.* (1971) and Straney *et al.* (1975) except as

indicated in Table 1. Variations in techniques included regulation of the voltage to maintain constant amperage across the gel (in gel types that built up resistance with time) or increases in the time of runs to facilitate separation of allozymes. The six gel types (Electrostarch Lot 371, Otto Hiller, Madison, Wisconsin) used and the staining procedures are listed in Table 1.

For each locus the allele occurring in the highest frequency in *M. californicus* was designated as 100 if migration was anodal or -100 if cathodal. Other allozymic bands and their corresponding alleles were designated numerically as percentages of the 100 (or -100) allele. The most anodal locus in a system was number "1"; more cathodal

TABLE 2. (Continued)

Pop.	5	6	7	\bar{h}	8	\bar{h}
2N	46	46	46		46	
α -Gpd	100 (1.000)	100 (0.761) 118 (0.239)	100 (0.6180) 118 (0.3820)	0.31	100 (1.000)	0.00
Es-2	135 (1.000)	135 (1.000)	135 (1.0000)	0.00	135 (0.960) 123 (0.040)	0.08
Es-3	137 (1.000)	137 (0.978) 130 (0.022)	137 (0.8950) 130 (0.1050)	0.06	137 (0.490) 130 (0.510)	0.59
Idh-1	100 (0.800) 117 (0.200)	100 (1.000)	100 (1.0000)	0.00	100 (1.000)	0.00
Ldh-1	100 (1.000)	100 (0.978) 103 (0.022)	100 (0.9870) 97 (0.0130)	0.02	100 (1.000)	0.00
Mdh-2	100 (1.000)	100 (0.935) 114 (0.065)	100 (0.9620) 114 (0.0380)	0.18	100 (0.989) 114 (0.011)	0.02
Pgi	-125 (1.000)	-125 (1.000)	-125 (1.0000)	0.00	-115 (1.000)	0.00
Pgm	100 (1.000)	100 (1.000)	100 (0.9740) 150 (0.0260)	0.02	50 (1.000)	0.00
Got-2	-100 (1.000)	-100 (1.000)	-100 (1.0000)	0.00	-100 (0.979) -88 (0.021)	0.04
Got-1	150 (1.000)	150 (1.000)	150 (1.0000)	0.00	150 (1.000)	0.00
Ipo-1	95 (1.000)	95 (1.000)	95 (1.0000)	0.00	95 (1.000)	0.00
Ipo-2	55 (1.000)	55 (1.000)	55 (1.0000)	0.00	55 (1.000)	0.00
G6p	114 (1.000)	114 (1.000)	114 (1.0000)	0.00	114 (1.000)	0.00
Adh	-100 (1.000)	-100 (1.000)	-100 (1.0000)	0.00	-100 (1.000)	0.00
Alb	100 (1.000)	100 (1.000)	100 (1.0000)	0.00	100 (1.000)	0.00
Gp-1	100 (1.000)	100 (1.000)	100 (1.0000)	0.00	100 (1.000)	0.00
Idh-2	100 (1.000)	100 (1.000)	100 (1.0000)	0.00	100 (1.000)	0.00
Ipo-3	100 (1.000)	100 (1.000)	100 (1.0000)	0.00	100 (1.000)	0.00
Ldh-2	-100 (1.000)	-100 (1.000)	-100 (1.0000)	0.00	-100 (1.000)	0.00
Mdh-1	100 (1.000)	100 (1.000)	100 (1.0000)	0.00	100 (1.000)	0.00
6Pgd	100 (1.000)	100 (1.000)	100 (1.0000)	0.00	100 (1.000)	0.00

loci received progressively higher designations. Direct side-by-side comparison of mobilities of allozymes on the same gel was used for the comparison of alleles (see Smith *et al.*, 1973).

RESULTS

Karyology

All individuals from populations 1-3 had a diploid number of 40 and those from 6-8 had a diploid number of 46. Populations 4 and 5 represent a single locality at which both species (and therefore chromosomal types) were taken. No chromosomally intermediate individuals were found. The karyotype of specimens of *Macrotus* from the islands of Jamaica and Haiti is identical

to that of *M. waterhousii* from southern Mexico (Fig. 2). The diploid number is 46 and the fundamental number (FN) is 60. The karyotype is composed of eight pairs of biarmed chromosomes and 28 medium to small sized acrocentric chromosomes. The X is a medium sized metacentric chromosome and the Y is a small acrocentric chromosome.

Electrophoresis

Patterns of variability.—The 21 loci assayed, alleles found at each locus, and frequency of each allele are presented in Table 2. In *Macrotus californicus* seven loci (33 percent) segregate for more than one allele. In Mexican populations of *M. waterhousii* (pops. 5-7) six loci (20 percent)

segregate for more than one allele, whereas in the Jamaican population (pop. 8) there are only four such loci (13 percent). Of the four polymorphic loci in the Jamaican population, two, *Mdh-2* and *Got-2*, have frequencies of less than 5 percent (.02 and .04 respectively), and a third, *Es-2* has a frequency of only 8 percent (.08). The banding patterns of the allozymes agree with those found for *Macrotus* by Straney *et al.* (1975) and are similar to those found for *Peromyscus* by Selander *et al.* (1971). Alkaline phosphatase (*Akp*), studied by Straney *et al.* (1975) is not included in this study as this locus gave very light bands which could not be scored consistently. Leucine aminopeptidase activity was not detected. Sorbitol dehydrogenase (*Sdh*) was not included as its activity was affected by the multiple thawings necessary in the electrophoretic process. *Ipo-1*, *Ipo-2*, and *Ipo-3* which were not reported by Straney *et al.* (1975) are monomorphic in all samples. However, *Ipo-1* and *Ipo-2* are fixed for different alleles in the two species. *Es-3* and *Idh-1*, also not reported by Straney *et al.* (1975) were scorable and are included (Tables 1 and 2).

Alb and *Got-1* which were reported as polymorphic ($h = .044$ and $.022$ respectively) by Straney *et al.* (1975) for 45 specimens of *M. californicus* from locality 1 are scored as monomorphic in all populations in this study. *Pgi* and *Ldh-1* scored as monomorphic by Straney *et al.* (1975) for population 1 only, were found to be polymorphic at other localities (Table 2).

Two esterase loci were found in this study as opposed to only one reported for *Macrotus* by Straney *et al.* (1975). The most anodal system is designated *Es-2* to agree with Straney *et al.* (1975) and homology is inferred. The more cathodal system is designated *Es-3* and is not meant to be homologous with "Es-3" reported by Straney *et al.* (1975) for *Myotis velifer*.

Eight loci are monomorphic for the same allele in all populations of both *M. californicus* and *M. waterhousii* as follows:

Adh, *Alb*, *Gp-1*, *Idh-2*, *Ipo-3*, *Ldh-2*, *Mdh-1*, and *6Pgd*. Four loci: *Got-1*, *Ipo-1*, *Ipo-2*, *G6p* are monomorphic each for a different allele in the two species (Table 2). *Idh-1*, α -*Gpd*, *Ldh-1*, and *Mdh-2* are polymorphic for the same alleles in both species (Table 2). No heterozygotes between the different fixed alleles at these loci were detected. *Es-3* is fixed in all populations of *M. californicus* and at the contact zone (pops. 4 and 5) but is polymorphic in the southern Mexican and Jamaican populations of *M. waterhousii*. *Es-2* is polymorphic in *M. californicus*, monomorphic in Mexican populations of *M. waterhousii*, and polymorphic in Jamaican *M. waterhousii*, however, none of the alleles is shared by the two species (Table 2).

Pgm is essentially monomorphic in *M. californicus* except for a variant allele in low frequency in pop. 3. Two of the four mainland populations of *M. waterhousii* are polymorphic at the *Pgm* locus (Table 2), however, the Jamaican population is fixed for *Pgm*⁵⁰ which does not occur in any of the mainland populations.

Got-2 and *Pgi* also are essentially monomorphic except for a variant in low frequency in one population each, pop. 8 for *Got-2* and pop. 2 for *Pgi*.

Genic variability.—Calculations of genic variability are presented in Table 3. Average heterozygosity ranges from .030 to .041 in *M. californicus* and from .000 (for specimens from the contact zone) to .043 in *M. waterhousii*.

Genic similarity.—Coefficients of genetic similarity and distance were calculated for paired combinations of all populations (Tables 3 and 4). Two measurements of similarity were used: *S* (Rodgers, 1972) and *I*, the electrophoretic identity between two different taxa for a given number of average codon differences per locus (Nei and Chakraborty, 1973). *D*, a measure of genetic distance based on the gene differences detectable by electrophoresis (Nei and Chakraborty, 1973) is also given in Table 4. The two measures of genetic sim-

housii. Distance values for paired populations of mainland and Jamaican *M. waterhousii* (.0612 to .0659) do not fall within this range.

DISCUSSION

Speciation.—Chromosomal and electrophoretic data clearly indicate that the Antillean populations of *Macrotus* are more closely related to Mexican populations of *M. waterhousii* than to populations of *M. californicus*. These data are best explained by the classical allopatric model of speciation and not by Brown's (1957) model of centrifugal speciation. If it is assumed that the Mexican populations of *M. waterhousii* represent the central part of the *Macrotus* complex, then according to Brown's (1957) model these populations would show the most rapid rate of speciation and be the primary source of evolutionary change. *Macrotus californicus* and the Antillean populations of *M. waterhousii* should represent more slowly evolving and therefore less heterozygous peripheral populations. Brown's model predicts that peripheral populations will be more primitive than the central stock and may show greater similarity to each other than to the more rapidly evolving central stock populations. Bowers *et al.* (1973) presented data supporting these assumptions in *Peromyscus maniculatus* and *P. melanotis*. The genic variabilities and similarities, especially the tendency for average heterozygosity to be higher at the extremes of the range of the two species and the high degree of intra-specific similarity between mainland and Jamaican populations of *M. waterhousii* (see discussion below) do not support a centrifugal speciation model in *Macrotus*.

Decreased heterozygosity (Table 3), lack of heterozygotes at loci fixed for different alleles in the two species (Table 2), and absence of chromosomally intermediate individuals at the contact zone argue against the presence of introgressive hybridization between *M. californicus* and *M. waterhousii*.

Genic variability.—The average heterozygosity value for mammals is .056 (Selan-

der and Johnson, 1973) with a range from .000 for *Geomys tropicalis* (Selander *et al.*, 1974) to 0.144 for populations of *Myotis velifer* from Texas (Straney *et al.*, 1975). *Macrotus* has low heterozygosity values ranging from 0.00 (for seven individuals of *M. waterhousii* from the contact zone, pop. 5) to .043 (for the southernmost sample of *M. waterhousii*, pop. 7) (Table 3). Heterozygosity values are generally greater at the northern and southernmost extremes of the range of the two species and decreased at the zone of contact (Table 3). None of the loci examined for the seven specimens of *M. waterhousii* at the contact zone is polymorphic. Heterozygosity values reported here (Table 3) are in general agreement with those reported by Straney *et al.* (1975) of $\bar{H} = .03$ for *Macrotus* from population 1. The possible implications of low heterozygosity values in *Macrotus* are discussed at length by Straney *et al.* (1975).

The Jamaican population of *Macrotus* has an average heterozygosity value of .04. Compared with the average heterozygosity values of the other populations of *Macrotus* sampled this does not indicate a reduced degree of heterozygosity as is thought to be characteristic of island and isolated populations (Selander *et al.*, 1971; Selander and Johnson, 1973; Lewontin, 1974). However, as previously indicated, only 4 loci (13 percent) in this population are polymorphic (as compared to 33 percent in *M. californicus* and 20 percent in mainland *M. waterhousii*) and three of these are variable only in low frequency. One locus, Es-3, accounts for a large majority of the heterozygosity observed. If \bar{H} is calculated independent of this locus the value is .009. Therefore, although the average heterozygosity per individual is not reduced, the heterozygosity of 95 percent (20 of 21 loci) is severely reduced. Es-3 in the Jamaican population has a heterozygosity value of .59. Lewontin (1974, Table 23) reports two loci with heterozygosities above 50 percent for *Drosophila pseudoobscura*, Protein-8 with heterozygosity equal to .513 and Esterase-3 equal to .741. A number of mechanisms

can be invoked to explain heterozygosities above 50 percent. Differential migration between populations, natural selection, or gene duplication involving unequal translocation between short segments of homologous chromosomes could produce such high frequency of heterozygotes.

Some comments concerning the Jamaican populations of *Macrotus* are in order. Jamaica shows great differences in climate over short distances and *Macrotus* is widespread and one of the most common species of bats on the island. The Jamaican population is not small in a Sewell Wright sense. However, the number of polymorphic loci suggests some reduction in genetic variation.

The evidence of geographical variation in the Es-3 locus in this study may support selection for Es-3¹³⁰ allele in *M. waterhousii*. The northernmost population of *M. waterhousii*, population 5, is fixed for Es-3¹³⁷. Population 6 is polymorphic with Es-3¹³⁷ being essentially fixed with a frequency of .987 and Es-3¹³⁰ with a frequency of .022. Population 7 shows an increase in frequency of Es-3¹³⁰ = .105 with the frequency of the more common allele Es-3¹³⁷ equal to .895. These data show a north-south clinal increase in the frequency of the Es-3¹³⁰ allele in mainland *M. waterhousii*. Possibly, Jamaican *M. waterhousii* with Es-3¹³⁷ at a frequency of .49 and Es-3¹³⁰ at .51 are displaying selection for the Es-3¹³⁰ allele. If selection favors a new mutant the new allele should increase in frequency progressing toward fixation. At some point in time frequencies such as those seen for Es-3 in *M. waterhousii* in populations 6, 7, and 8 would be present. Several authors (Kimura and Ohta, 1971; Ohta and Kimura, 1973; Johnson, 1974; King and Jukes, 1969) questioned whether electrophoretically demonstrable variation is adaptively relevant or whether it is essentially neutral. Kimura and Ohta (1971) argued that most protein polymorphisms could be explained by random drift of effectively neutral alleles. Ayala *et al.* (1974) found that predictions from the hypothesis of selective neutrality

of protein polymorphisms were at complete variance with their empirical observations in the *Drosophila willistoni* species group. Es-3 polymorphism in *M. waterhousii* seems to argue against neutrality and explanation by random drift.

Genic similarity.—Comparative data from different variables are desirable in evaluating genetic distances between taxa. Data are available for *Macrotus* from morphometrics (Davis and Baker, 1974), karyology and electrophoresis. Based on a classical taxonomic revision Anderson and Nelson (1965) concluded that *Macrotus* were monotypic. Nelson-Rees *et al.* (1968) reported (what they considered to be) intraspecific chromosomal variation in *M. waterhousii*. Davis and Baker (1974) maintained there were two species involved: *M. californicus* and *M. waterhousii*. The recognition of specific status for the two taxa was based on karyotypic data, a zone of parapatry, and lack of evidence of hybridization. Morphological classification of specimens to species, as determined by multivariate analysis of measurements of cranial and wing characteristics (Davis and Baker, 1974) required a cautious consideration of interorbital breadth, capture locality, and sex of the specimen. We interpret the magnitude of exomorphological distinctness of the two species to be well within the range characteristic of sibling species of mammals.

Summaries of genetic similarities were published by Selander and Johnson (1973) and Nevo *et al.* (1974). Coefficients of genetic similarity (*S*) for conspecific populations are generally .90 and above. Increased distance between populations and physical barriers to gene flow tend to decrease similarity. The interspecific similarity values reported here for *Macrotus* (*S* = .5953 to .6661) fall within the reported range for sibling species, .23 to .89 (Selander and Johnson, 1973). However, *Dipodomys* is the only mammal which has been reported to have lower values for sibling species, *S* = .31 to .89 (Johnson and Selander, 1971).

Overall morphological similarity of mainland *Macrotus* is unreflected by interspecific genetic similarity, $S = .6116$ to $.6661$, indicative of considerable genetic change. Ayala *et al.* (1974) reported average genetic similarity (I) for comparisons between sibling species of the *Drosophila willistoni* group of $.587$ and average genetic distance of $.538$ with their most similar pair (*D. willistoni* and *D. tropicalis*) having $I = .673$ and the most different pair (*D. tropicalis* and *D. equinoxialis*) having $I = .510$. Mainland *Macrotus*, then with interspecific genetic similarities $I = .6313$ to $.6655$, are comparable in protein identities to sibling species of *Drosophila*. Examination of the genetic similarities between congeneric species (Selander and Johnson, 1973) reveals that *Drosophila* display the lowest similarity values for both sibling and nonsibling species.

The similarity values between Jamaican *M. waterhousii* and mainland *M. waterhousii*, $S = .9065$ ($I = .9362$ to $.9407$) are the lowest values of intraspecific similarity found in *Macrotus*. Likewise the coefficients of distance, $D = .0612$ to $.0659$ are considerably larger than those found for intraspecific comparisons of either *M. californicus* ($.0007$ to $.0197$) or mainland *M. waterhousii* ($.0050$ to $.0111$). These values fall within the expected range for conspecific taxa, which supports the conclusion of Anderson and Nelson (1965) that *Macrotus* from Mexico are conspecific with populations from Jamaica. However, we interpret this analysis of electrophoretic data as suggesting that the mainland *M. waterhousii* sampled represent a subspecies distinct from the Jamaican sample.

Nevo *et al.* (1974) found high similarity values between six karyotype groups of the *Thomomys talpoides* complex ($S = .71$ to $.97$). They suggested that speciation may occur with only a few genomic changes. If so, then the relatively large amounts of genomic changes suggested by samples of loci analyzed electrophoretically in *Macrotus* can be assumed to have accumulated

during periods of allopatric separation prior to assuming current parapatry or during independent evolution of the two species.

CONCLUSIONS

Analysis of electrophoretic and chromosomal data of populations of mainland and Antillean forms of *Macrotus* supports the conclusion of Davis and Baker (1974) that mainland *Macrotus* represent two distinct species, *M. californicus* and *M. waterhousii*, and clearly indicates that the Mexican populations of *M. waterhousii* are conspecific with Antillean populations although the Antillean populations sampled probably represent a subspecies distinct from those sampled on the mainland. No indication of introgressive hybridization was found.

High degrees of intraspecific similarity between mainland and Jamaican *M. waterhousii* as determined by electrophoretic identity and the tendency for average heterozygosity to be higher in peripheral than central populations are best explained by the classical allopatric model of speciation and not by Brown's (1957) model of centrifugal speciation.

The average heterozygosity of the Jamaican population does not indicate a reduced degree of heterozygosity as is thought to be characteristic of island and isolated populations. However the number of polymorphic systems and the heterozygosity of 95% (20 of 21) of the loci indicates a reduction of variation in island populations. One locus accounts for most of the heterozygosity found in the island population. Selection or gene duplication for a variant allele is suggested as a possible mechanism to explain such a high degree of variation.

Low interspecific genetic similarity in *Macrotus* is unreflected by morphological divergence and is the lowest range of similarity values reported for sibling species of mammals. Interspecific genetic similarities in *Macrotus* are comparable in protein identities to sibling species of *Drosophila*.

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