Heredity (1982), **48** (3), 435–438 0018-067X/82/04350435$02.00

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NOTES AND COMMENTS

LARGE GENETIC DIFFERENCES BETWEEN SIBLING SPECIES OF BATS, *EPTESICUS*, FROM AUSTRALIA

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Received 29.ix.81

1. Introduction

Populations isolated by geographic barriers accumulate genetic differences due to selection, mutation and drift. The genetic differences will be manifest to some extent in the phenotype, so that morphological divergence is expected to correlate generally with genetic divergence. Isozyme electrophoresis is a method of determining the extent of structural gene divergence between taxa, and one might therefore expect some sort of relationship between morphological divergence and electrophoretic divergence.

In keeping with this view, congeneric non-sibling species of mammals show genetic distances (Nei D's) of about 0·25–0·50 while sibling species typically show Nei D's of about 0·10 (Baverstock *et al.*, 1977). We herein demonstrate however that sibling species of the bat genus *Eptesicus* in Australia are characterised by genetic distances five to ten times higher than this.

2. Materials and methods

A total of 52 specimens of *Eptesicus* from the Canberra and southern N.S.W. area were available for electrophoretic study. Animals were killed in the laboratory and livers, testes and plasma removed and immediately frozen at −17°C. Erythrocytes were stored in ethylene glycol at −17°C (Vandeberg and Johnston, 1977). A total of 36 loci were scored electrophoretically by methods described previously (Baverstock *et al.*, 1980).

3. Results

Details of the allele frequencies at 36 loci in the three species of *Eptesicus* are available from the authors on request. In addition to the "fixed" genetic differences (*i.e.*, number of loci sharing no alleles) reported by Tidemann *et al.* (1981), many loci show extensive gene frequency differences between species. For example, *E. sagittula* and *E. regulus* share allele *b* at the Pep D locus, and therefore Pep D would not contribute to the fixed differences between these species. However, allele *b* occurs at a frequency of 78 per cent in *E. sagittula* and 16 per cent in *E. regulus*. Moreover, the alternative alleles are unique to each species. While such loci are not diagnostic of the species, they nevertheless clearly contribute

TABLE 1

Matrix of genetic similarities (I–upper right) and genetic distances (D–lower left) for three species of Australian Eptesicus

	E.s.	*E.v.*	*E.r.*
E. sagittula	—	0·44	0·37
E. vulturnus	0·82	—	0·64
E. regulus	0·99	0·44	—

to genetic divergence between the species. One way of incorporating such gene frequency differences into the total estimate of genetic divergence is to use the Nei distance (Nei 1972), which is in effect an estimate of the average number of codon substitutions per locus. When this is done for the present data (table 1), it is found that *E. sagittula* and *E. regulus* differ at an average of 0·99 substitution per locus and *E. vulturnus* and *E. regulus* at an average of 0·44 substitution per locus.

4. DISCUSSION

There seems little doubt that the three species of *Eptesicus* studied here constitute "sibling species" in the sense that they are morphologically very similar. Thus most recent reviews of Australian mammals have considered there to be one species of *Eptesicus* in Australia (Troughton, 1982; Ride, 1970) although various indications to the contrary exist in the literature (Wood-Jones, 1925; Hamilton-Smith 1966; McKean and Hamilton-Smith, 1967). Kitchener (1976) defined a new species, *E. douglasi* and McKean *et al.* (1978) separated Australian *Eptesicus* into five species based on an examination of bacula and other skeletal features. They recognised *E. pumilis*, *E. douglasi*, *E. regulus* and *E. vulturnus* as full species and designated a new species, *E. sagittula*. However, the diagnostic features used by these authors were not useful in separating females (Carpenter *et al.*, 1978). More recently, Tidemann *et al.* (1981) have used discriminant function analysis, based on 18 skeletal measurements, to separate into three species *E. vulturnus*, *E. regulus* and *E. sagittula*, animals of both sexes from near Canberra and the New South Wales south coast. However, it should be emphasized that discriminant function analysis is a means of enhancing differences between groups, and many of the variables included in the analysis were not useful in deriving the discriminant functions, because of a large degree of overlap between species. Moreover, no single morphological character will unequivocally classify all specimens. Finally, preliminary data on a larger series of *Eptesicus* throughout the range suggest that intraspecific geographic variation in *Eptesicus* is considerably larger than that found in the study area, so that the discriminant analysis that was successful there may fail when used over broader geographic areas. The evidence presented above clearly indicates that the three species studied here have undergone very little morphological divergence, and constitute sibling species as defined by Wilson (1975).

While extensive genetic divergence with little morphological divergence is known in invertebrates and lower vertebrates, its occurrence in mammals has not previously been recorded to our knowledge. Among rodents, for example, genetic divergence between sibling species is typically character-

ized by a D of 0·10 with a maximum of 0·22 (Baverstock *et al.*, 1977). Among mammals in general, genetic divergence between congeneric species is characterized by an average D of 0·36 with an upper limit of about 1·20 (Avise, 1974). Therefore the genetic divergences recorded here for *Eptesicus* are five to ten times higher than those found between sibling species of rodents, and in fact near the upper limit of those characterizing species of the same genus of mammal.

Sarich (1977) and Vawter *et al.* (1980) have shown empirically that Nei D is correlated with time of divergence (T). Sarich (1977) suggested that for "intra-cellular" loci T(MY) = 30 D. Our study included 30 loci that fall into this category (albumen, esterase and four peptidases were omitted) and these give a D of 0·81 for *E. sagittula* compared to *E. regulus* and *E. vulturnus*, and a D of 0·37 for *E. regulus* and *E. vulturnus*. The estimated times of divergence are therefore 24 MY for *E. sagittula* and 11 MY for *E. regulus* and *E. vulturnus*.

This is not to say that the divergence times are actually as ancient as 11 and 24 MY; rather that this is the time usually required for such large genetic differences to accumulate. Clearly therefore, either the rate of morphological evolution has been remarkably slow in Australian *Eptesicus*, or electrophoretic evolution has been remarkably rapid. It is difficult to see how these two hypotheses might be distinguished. There is no fossil record of *Eptesicus* in Australia. Even if there was, it is highly unlikely that the fossil record would be of help here, simply because, given that the extant forms are so similar, any fossil would be difficult to place on any of the branches leading to extant forms (Carlson *et al.*, 1978). What is clear is that even within mammals, morphological divergence and genetic divergence need not be correlated.

Whether such morphological conservation despite genetic divergence is characteristic of bats as a whole, remains to be determined. According to some authors, most notably Wilson (1975), morphological diversity goes hand in hand with chromosomal diversity. The present data are compatible with this point of view, since all three species are karyotypically indistinguishable, even when G-banded (unpublished data). Vespertillionid bats as a group tend to be karyotypically conservative (Bickham, 1979) and one might therefore expect to find other cases of sibling species of bats that are genetically divergent. We are currently investigating this possibility in other genera of Australian bats.

Acknowledgements.—We thank Dr C. H. S. Watts for valuable criticism of the manuscript, and M. Nagy for technical assistance.

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