Electrophoretic Resolution of Species Boundaries in Australian Microchiroptera. II.* The *Pipistrellus* Group (Chiroptera : Vespertilionidae)

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Abstract

Forty-four specimens of the *Pipistrellus* complex from Australia were analysed for 36 allozyme loci. The data show that four species were represented in the material, two of which are sympatric in the north, and two of which are allopatric in the south. The data are consistent with separate generic status for the two southern species.

Introduction

The genus *Pipistrellus* occurs primarily in Asia and Africa, with a few species ascribed to the genus occurring in North America, Europe and Australia (Honacki *et al.* 1982). Ride (1970) listed two species from Australia, *P. tasmaniensis* and *P. tenuis*. The distribution of *P. tenuis* extends from south-east Asia to the New Hebrides. It is relatively rare in Australia, where it is restricted to the north coast from the Kimberleys to Cape York (Tate 1952; McKean and Price 1978; Hall and Richards 1979; Koopman 1984). *P. tasmaniensis* is restricted to south-eastern and south-western Australia.

There has been considerable confusion over allocation of species to the genus *Pipistrellus* in Australia (e.g. Tate 1942; Ride 1970; Koopman 1984). There has also been some confusion over the generic status of *P. tasmaniensis* (e.g. Iredale and Troughton 1934; Tate 1942; Troughton 1943; Ride 1970), with Troughton (1943) placing it in the genus *Falsistrellus*.

The present study on *Pipistrellus* is the second in our series applying the technique of allozyme electrophoresis to species-boundary problems in Australian Microchiroptera. A full account of the rationale used can be found in the first paper in the series (Adams *et al.* 1987). Because our aim has been to clarify species boundaries within Australia, we have not attempted to compare Australian material with extralimital specimens. As discussed in the first paper in this series (Adams *et al.* 1987) it is far more important in electrophoretic analyses to sample as broad an area as possible for as many genetic loci as possible rather than to include many specimens from single localities. We have therefore concentrated our sampling efforts over as broad a geographic range as possible at the expense of sampling single localities in detail.

Based on morphological considerations in parallel with the electrophoretic results of the present study, Kitchener et al. (1986) recognized four species in

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Australia, two in northern Australia and two in southern Australia. The two northern species are *P. adamsi* (Cape York and coastal Northern Territory), and *P. westralis* (Kimberley, Northern Territory coast and gulf country of Queensland), with *P. tenuis* being restricted to the extralimital distribution. They have resurrected *Falsistrellus* Troughton, 1943 to accommodate the two southern species, *F. tasmaniensis* (Tasmania and coastal southeastern Australia) and *F. mckenziei* (south-western Western Australia). We follow the nomenclature of Kitchener *et al.* (1986) here.



Fig. 1. Sites of populations (indicated by a letter) and subpopulations (indicated by Arabic numeral) for the specimens studied. Sample sizes are given in the Accessory Publication: Key to populations: $\blacksquare P$. westralis; $\square P$. adamsi; $\blacksquare F$. tasmaniensis; $\bigcirc F$. mckenziei.

Materials and Methods

A total of 44 specimens was available for analysis. Full collecting details for the material used are given in an Accessory Publication^{*} and are summarized in Fig. 1. Methods of tissue handling and electrophoresis follow those given in Adams *et al.* (1987). Most of the specimens used in the present study were included in the morphometric analysis of Kitchener *et al.* (1986).

For the convenience of analysis, specimens from a single locality of a given genetic type are treated as a *subpopulation*. The subpopulations of the same genetic type were then grouped on the basis of geographic proximity to yield *populations*. In the present analysis, the 44 specimens were grouped into eight populations A to H (Fig. 1).

*Copies are available on request from the Editor-in-Chief, CSIRO, 314 Albert Street, East Melbourne, Victoria 3002, Australia.

Table 1. Allele frequencies, expressed as a percentage, for the eight populations at 36 loci
Alleles are designated alphabetically ('a' is the most cathodally migrating). Loci are designated
numerically according to increasing electrophoretic mobility. Where allele frequencies are not
given the frequency is 100. Pgk was invariant. $2N =$ the number of haploid genomes sampled
(G6pd is sex-linked and hence 2N has been adjusted accordingly)

Designation	F. mckenziei	F. tasmaniensis		P. westralis			P. adamsi		
Population	Α	В	C	D	Е	F	G	Н	
Locus 2	N 8	6	6	4	2	4	6	52	
Acon-1	c	c	c	a	b(50) a(50)	a	a	a	
Acon-2	e	d	d	b	b	c(50) b(50)	b	b(2) a(98)	
Ada	f	f(17) e(50) d(33)	e	c	a	a	b	b	
Adh	c	c	d(33) c(67)	а	а	а	c	b(10) a(90)	
Ak-I	а	а	a	с	с	с	b	bĺ	
Ak-2	b	b	b	_	а	a	а	а	
Alb	с	b	b	а	а	a	а	а	
Ca	b	с	с	а	b(50) a(50)	b(75) a(25)	а	b(69) a(31)	
Enol	b	b	b	а	b	b	b	b	
Fdpase	а	a	a	b(50) a(50)	b	b(75) a(25)	b(17) a(83)	а	
Fum	а	а	а	c	C	c	c(83) b(17)	c(88) b(12)	
Ga3pd	b	b	b	а	а	а	a	a`́	
Got-1	с	b	b	а	а	а	а	а	
Got-2	b	b	b	а	а	а	а	а	
G6pd	b	b	b	а	а	a	а	d(8) c(38) a(54)	
Gpd	с	b	b	а	a	а	а	a	
Gpi	c	c	c	c(25) b(75)	b	b	a	a	
Guk	b	b	b	a	а	а	а	а	
Idh-1	b	b	b	а	а	a	с	с	
Idh-2	d	d	d	c(75) a(25)	c(50) a(50)	c	b	b	
Ldh-1	а	a	а	b	b	b	b	b	
Ldh-2	b	b	b	а	а	а	а	а	
Mdh-1	b	b	b	а	а	a	а	b(6) a(94)	
Mdh-2	а	а	a	a	b(50) a(50)	а	а	а	
Mpi	а	b	b	а	a	а	с	с	
Np	а	а	а	b	b	b	с	с	
Pep-A	b	c	c	а	а	а	а	а	
Рер-В	e(87) d(13)	f (50) e(50)	e	c(25) a(75)	a	c(25) a(75)	b(17) a(83)	а	
Pep-C	a	c(17) b(83)	c(17) b(83)	a	a	a	b	b	
6Pgd	b	b	b(67) a(33)	d(75) c(25)	d	d(50) c(50)	f	f (44) e(56)	
Pgm-1	b(87) a(13)	b	b	c	c	c	c	e(35) d(25) c(40)	
Pgm-3	d	d	d	c	c(50) a(50)	c(50) a(50)	c	c(83) b(17)	
Sod	b	b	b	а	a	a	b	b`́	
Sordh	c	c	c	b	b	b	b(33) a(67)	b(15) a(85)	
Трі	а	а	a	b	b	b	b	b	

Results

A total of 36 loci yielded staining of sufficient intensity and resolution to be interpreted reliably. The proteins used were: aconitate hydratase (ACON, EC 4.2.1.3), adenosine deaminase (ADA, EC 3.5.4.4), alcohol dehydrogenase (ADH, EC 1.1.1.1), adenylate kinase (AK, EC 2.7.4.3), albumen (ALB), carbonate dehydratase (CA, EC 4.2.1.1), enolase (ENOL, EC 4.2.1.11), fructose diphosphatase (FDPASE, EC 3.1.3.11), fumarate hydratase (FUM, EC 4.2.1.2), glyceraldehydephosphate dehydrogenase (GA3PD, EC 1.2.1.2), aspartate aminotransferase (GOT, EC 2.6.1.1), glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49), glycerol-3-phosphate dehydrogenase (GPD, EC1.1.1.8), glucose-phosphate isomerase (GPI, EC 5.3.1.9), guanylate kinase (GUK, EC 2.7.4.8), isocitrate dehydrogenase (IDH, EC1.1.1.42), lactate dehydrogenase (LDH, EC1.1.1.27), malate dehydrogenase (MDH, EC 1.1.1.37), mannose-phosphate isomerase (MPI, EC 5.3.1.8), purine nucleoside phosphorylase (NP, EC 2.4.2.1), peptidases (PEP, EC 3.4.11 or 13.*), 6-phosphogluconate dehydrogenase (6PGD, EC1.1.1.44) phosphoglycerate kinase (PGK, EC 2.7.2.3), phosphoglucomutase (PGM, EC 2.7.5.1), superoxide dismutase (SOD, EC1.15.1.1), L-iditol dehydrogenase (SORDH, EC1.1.1.14), and triose-phosphate isomerase (TPI, EC 5.3.1.1).

Table 1 shows the allelic profiles of the eight populations for the 36 loci. These data were then converted into a matrix expressing the percentage of loci showing fixed differences between populations (Table 2). Table 2 also shows the genetic distance between populations expressed as Nei D corrected for small sample size (Nei 1978).

Species	Population	Α	В	С	D	Е	F	G	н
F. mckenziei	Α	_	22	25	83	83	81	83	81
F. tasmaniensis	В	28	_	0	89	92	89	81	81
	С	30	- 1	_	89	92	89	81	81
P. westralis	D	195	248	248	—	6	6	34	31
	Ε	189	257	257	6	_	0	31	31
	F	173	233	233	8	0		31	28
P. adamsi	G	179	164	170	44	44	45	_	3
	Н	178	174	174	51	48	45	10	-

Table 2. Genetic distances between populations of *Pipistrellus* and *Falsistrellus* Upper matrix-percentage fixed difference; lower matrix-Nei $D \times 10^3$

Fig. 2 shows a phenogram of the percentage fixed difference between populations, constructed by the average linkage cluster method of analysis (ALC). ALC analysis of Nei's D yielded an essentially identical phenogram which is not presented here.

The eight populations fall into four distinct genetic groups -A (corresponding to *F. mckenziei*); B-C (corresponding to *F. tasmaniensis*); D-E-F (corresponding to *P. westralis*); and G-H (corresponding to *P. adamsi*).

Group A and group B-C differ at 24% of loci examined, while group D-E-F and group G-H exhibit 31% fixed differences (Fig. 2). The corresponding values of D are 0.29 and 0.46 respectively (Table 2). The A, B-C cluster and the D-E-F, G-H cluster are genetically very distinct, with an average fixed difference of 84% and a corresponding D of 1.96.

By contrast, few genetic differences are evident between populations within the groups. Populations B (from Victoria) and C (from Tasmania) of *F. tasmaniensis* share alleles at all 36 loci. Populations E (from the Darwin area of Northern Territory) and F (from the gulf country of Queensland) of *P. westralis* also share alleles at all 36 loci. Population D of *P. westralis* (from the Kimberleys) showed fixed differences from populations E and F at two loci (*Ada* and *Enol*), although in view of the small sample sizes for these populations (2, 1 and 2 respectively),



Fig. 2. Phenogram constructed by average linkage cluster method using percentage fixed differences between populations.

shared polymorphisms at these loci may have been missed. Populations G (from the Darwin area of the Northern Territory) and H (from the top of Cape York) of *P. adamsi* differ at a single locus (Adh). The sample sizes here are relatively large (52 haploid genomes for H and six for G), so the *Adh* difference is probably not due to sampling error.

Discussion

The present study has confirmed the existence of at least four species of the *Pipistrellus-Falsistrellus* complex in Australia. *P. adamsi* and *P. westralis* clearly represent distinct biological species. Although the two forms were never found in strict sympatry, they were collected within 30 km of each other in the Darwin area. Here, the two forms show no less than 11 fixed genetic differences. The sample sizes for this area are small, but they are still sufficient to clearly show two species, even in the absence of other data. Thus on the null hypothesis that samples E (*P. westralis*) and G1 (*P. adamsi*), each consisting of one specimen, were drawn from the same population, the probability of not observing a heterozygote at the 11 loci showing fixed differences is $(0 \cdot 5)^{22}$ or $0 \cdot 0000002$ (Baverstock *et al.* 1983*a*). Such a difference is highly significant statistically. The biological significance of such a difference becomes even more apparent when the entire set of data for this pair of species is considered. For instance, *P. westralis* shows genetic uniformity across

the range sampled from Cape Bossut in Western Australia to the gulf country in Queensland (maximum of two fixed differences), while *P. adamsi* shows genetic uniformity from Darwin to the top of Cape York (one fixed difference). In this light, the presence of 11 fixed differences between the groups is highly significant.

There are at least two biogeographic explanations for the origins of P. adamsi and P. westralis—they may have speciated in situ in Australia (i.e. they are each other's sister species), or they may represent multiple invasions from the north. Additional data on the relationships of these species to those further north will be required to resolve this question fully.

F. mckenziei and F. tasmaniensis are not sympatric, so the same certainty of distinction cannot be applied in this case. However, the extent of genetic divergence between them is well beyond that which we would normally consider characteristic of populations of the same species. For mammals, and indeed most animals, the extent of divergence between populations of the same species seldom exceeds 10% fixed differences, and never more than 15% (Baverstock et al. 1977; Thorpe 1983; Richardson et al. 1986; although see Patton 1981 for a possible exception). In the present study, for example, even across large geographic distances, P. westralis showed a maximum of 6% fixed differences, P. adamsi 3% fixed differences, and F. tasmaniensis zero fixed differences (between Victoria and Tasmania). Such results are typical of the level of genetic divergence found between geographically isolated populations of the same species (Baverstock et al. 1977, 1980, 1983a, 1983b, 1984; Kitchener et al. 1984). The observation that F. mckenziei and F. tasmaniensis have 22–25% fixed differences at 36 loci strongly supports their recognition as distinct species.

At first sight it would appear that the primary isolating mechanism between F. mckenziei and F. tasmaniensis might be the Nullarbor Plain. Such a model has been proposed for east-west isolation of many groups (see Maxson and Roberts 1984). According to this model, glaciations during the Pleistocene led to a drop in sea level, exposing an east-west corridor across what is now the Great Australian Bight. The rise in sea level during interglacial periods then led to disruption of gene flow, thus allowing allopatric speciation.

Although the concept of a molecular clock for electrophoretic data should be viewed with some degree of caution (Richardson *et al.* 1986), electrophoretic data can be used to give an approximate estimate of divergence time (Sarich 1977; Vawter *et al.* 1980). The extent of genetic divergence between *F. mckenziei* and *F. tasmaniensis* (D about 0.29) is that which would normally be associated with a divergence time of about 6 million years (Sarich 1977). It therefore appears that the extent of genetic divergence between *F. mckenziei* and *F. tasmaniensis* is not compatible with Pleistocene glaciations. Other molecular studies of taxa supposedly isolated during the Pleistocene glacials (e.g. frogs of various genera) have similarly concluded that the events are far more ancient (Maxson and Roberts 1984; Barendse 1984).

The extent of genetic divergence between *Falsistrellus* and *Pipistrellus* ($D = 2 \cdot 0$) supports their recognition as separate genera (Kitchener *et al.* 1986). Congeneric species of mammals typically show Nei *D* values of less than $1 \cdot 00$, although the variance is very high (cf. Thorpe 1983). We would not recommend generic splitting on the electrophoretic data alone, but rather we point out that the data *support* a generic splitting.

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