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Electrophoretic Comparisons between Allopatric Populations of Five Australian Pseudomyine Rodents (Muridae)

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Abstract

Allopatric populations of the pseudomyine rodents *Pseudomys albocinereus*, *P. delicatulus*, the *P. nanus–P. gracilicaudatus* complex, *Zyzomys argurus* and *Mesembriomys gouldi* were surveyed for electrophoretic variability of 14–17 red cell and plasma proteins. Few or no electrophoretic differences were found to parallel the chromosomal differences between populations of *P. delicatulus*, *Z. argurus*, and *M. gouldi*. Populations of the *P. nanus–P. gracilicaudatus* complex, however, fell into two groups defined both chromosomally and electrophoretically. The western form (*P. nanus*) extends from Western Australia into the Northern Territory whilst the eastern form (*P. gracilicaudatus*) occurs only along the east coast of Queensland and New South Wales. The biochemical differentiation between South Australian, Western Australian mainland and Bernier Island *P. albocinereus* parallels the chromosomal, morphological and breeding data, all of which indicate that the Western Australian mainland and Bernier Island forms belong to one species (*P. albocinereus*), whilst the South Australian form represents a distinct biological species (*P. apodemoides*).

The relationship between electrophoretic variation and the biological species concept was explored using data from *Drosophila* and rodents. It was concluded that if two allopatric populations possess 'fixed' electrophoretic differences at at least 15% of their loci, then it is highly probable that they belong to different biological species. However, populations that differ by less than 15% of their loci need not necessarily belong to the same species especially if chromosomally they differ by several Robertsonian rearrangements.

Introduction

The endemic Australian rodents belonging to the subfamily Pseudomyinae include several species which are only known to occur in a few isolated populations, often on extreme sides of the continent. Many of these populations show recognizable structural and karyotypic differences.

We report here the results of a survey of the electrophoretic differentiation between such populations utilizing 14–17 red cell and plasma proteins encoded by 17–20 loci. In several cases, the genetic bases of the differences found between populations were checked by cross-breeding. The aims of the study were threefold:

(i) To determine whether the pattern of electrophoretic variation paralleled the structural and karyotypic variation.

(ii) To determine whether electrophoretic data would help clarify the taxonomic difficulties posed by such structural and karyotypic variation.

(iii) To gain some idea of the level of interpopulational electrophoretic variability that we might encounter in a forthcoming electrophoretic comparison of the Australian rodent fauna.

The species and species-pairs chosen and the reasons for choosing them are as follows:

(1) *Pseudomys nanus–P. gracilicaudatus. Pseudomys nanus* (Gould) is found in the Northern Territory and the Kimberleys area of Western Australia, whilst the morphologically similar *P. gracilicaudatus* (Gould) occurs in coastal Queensland and New South Wales. Both Ride (1970) and Mahoney and Posamentier (1975) suggested that the morphological differences may not be sufficient to warrant their recognition as different species.

(2) *Pseudomys albocinereus.* At present included in this species (Ride 1970) are four described forms: *P. a. squalorum* Thomas from Bernier Island, W.A., *P. a. albocinereus* (Gould) from the south-west of Western Australia, *P. apodemoides* Finlayson from South Australia and *P. glaucus* Thomas from northern New South Wales. No specimens of the latter form have been collected for several years and it has not been included in this study. Chromosomal analysis (Baverstock *et al.* 1977*a*) of the two populations from Western Australia and one from South Australia showed that all three were chromosomally distinct.

(3) *Pseudomys delicatulus.* Baverstock *et al.* (1977*a*) found that populations from Western Australia and Queensland differed chromosomally from those from the Northern Territory.

(4) *Mesembriomys gouldi.* Three subspecies have been named: *M. g. gouldi* (Gray) from the Northern Territory, *M. g. melvillensis* Hayman from Melville Island, Qld, and *M. g. ratoides* Thomas from Cape York. The only individual of *M. g. ratoides* available to us differed chromosomally from the nominate subspecies (Baverstock *et al.* 1977*a*). No *M. g. melvillensis* were available for the current study.

(5) *Zyzomys argurus.* Again two subspecies have been named, the nominate one from the Northern Territory and *Z. a. inductus* Thomas from Western Australia. Moreover Baverstock *et al.* (1977*a*) found that individuals from Western Australia and western Northern Territory (Victoria River) differed chromosomally from those from central Northern Territory (Nourlangie Rock) and Queensland.

Materials and Methods

Source of Animals

The animals utilized in the present study are listed in Table 1 together with their Institute of Medical and Veterinary Sciences (I.M.V.S.) number. Localities mentioned in Table 1 are shown in Fig. 1. Precise localities and habitat data appear in Robinson *et al.* (1977). When these animals die, the skulls and skins will be submitted to a museum and given a museum number. Museum numbers corresponding to I.M.V.S. numbers will be available from the I.M.V.S. or the South Australian museum.

Electrophoresis

Blood was collected by cardiac puncture under ether anaesthesia in syringes containing a dried film of heparin and centrifuged immediately at 2000 g for 10 min at 4°C. Plasma was removed, and the packed red cells resuspended in storage solution (400 ml ethylene glycol, 60 g trisodium citrate to 1 litre with distilled water). Both plasma and red cells were then stored at −20°C. To prepare the stored red cells for use, the samples were spun at 2000 g for 10 min at 4°C, the storage solution removed, and the packed cells lysed with 2 volumes of a solution containing 0·1 ml β-mercapto-ethanol, 0·1 ml Triton X-100 and 10 mg NADP per 100 ml.

Horizontal starch gel electrophoresis was conducted by the method described previously (Baverstock *et al.* 1976). For cellulose acetate gel electrophoresis, sheets 30 by 30 cm were divided to give sheets 15 by 30 cm. Approximately 30 samples could be applied to each sheet, the direction of migration

being across the 15-cm dimension. Approximately 0·5 μl of haemolysate was applied at each slot with a draughtsman's lining pen (Richardson and Cox 1973), following which a voltage of 150 V was applied for a period of 1½–2 h, depending on the enzyme studied. On completion of electrophoresis, the sheet was soaked in the appropriate staining mixture for 1 min then blotted, placed between two sheets of glass, sealed with plastic and incubated until bands were visible.

Table 1. I.M.V.S. numbers, sex and localities of animals studied

For more details of collection localities see Robinson *et al.* (1977). M, male; F, female

Species	Locality	I.M.V.S. No.
P. gracilicaudatus	Queensland	
	8 km SW. of Townsville	73F
	26 km NE. of Rockhampton	74M
	9 km NE. of Rockhampton	75M
	98 km NW. of Bundaberg	76F
P. nanus	Northern Territory	
	10 km S. of Nourlangie Camp	478F
	7 km SE. of Nourlangie Camp	255F
	14 km S. of Nourlangie Camp	473F
	Victoria River 346 km S. of Darwin	445F, 519F
	Western Australia	
	246 km E. of Derby	440M, 263F
	165 km E. of Derby	216M, 435F, 247M
	18 km NE. of Kimberley Research Station	444F
P. albocinereus	South Australia	
	20 km W. of Comet Bore on Pinaroo–Bordertown Road	37M, 38M, 39M, 40M, 41M, 42M, 43F
	Fairview Conservation Park	36F
	Western Australia	
	22 km NE. of Jurien	25F, 26F, 27M
	Bernier Island	29F, 30F, 31F, 32F, 33M
P. delicatulus	Queensland	
	40 km NW. of Townsville	55M
	Fairbairn Dam, 22 km SW. of Emerald	13M, 14M
	Northern Territory	
	5 km W. of South Alligator River Crossing	57M
	10 km SE. of Mudginberry HS, 185 km E. of Darwin	484F
	Western Australia	
	189 km S. of Broome	59F
M. gouldi	Northern Territory	
	12 km S. of Nourlangie Camp	85M
	Jabiru	86F
	7 km SE. of Nourlangie Camp	84F
	Queensland	
	28 km N. of Atherton	87F
Z. argurus	Queensland	
	Mt Simon, 22 km S. of Cooktown	94M
	Northern Territory	
	Nourlangie Rock, 20 km SE. of Nourlangie Camp	97M, 98F
	Victoria River, 246 km S. of Darwin	99F
	Western Australia	
	Fortescue River	107M, 108F
	165 km E. of Derby	104F, 105F
	West Bastion above Wyndham	100M

Blood samples from individuals of one species were run as a block, but individual specimens were randomized within the block. Samples were given a coded number and, following staining, were scored blind. All purported differences in mobility were checked by re-running the samples in a different sequence and again scoring blind. Only differences that were repeatable in this way were considered real differences.

Fig. 1. Map showing the localities mentioned in the text.

The proteins stained for and their abbreviations, used throughout this paper, are listed below:

Albumin	Alb	Hexokinase (EC 2.7.1.1)	HK
Haemoglobin	Hb	Aspartate aminotransferase (EC 2.6.1.1)	AAT
Glucose-6-phosphate dehydrogenase (EC 1.1.1.49)	G6PD	Phosphoglycerate kinase (EC 2.7.2.3)	PGK
Phosphogluconate dehydrogenase (EC 1.1.1.43)	PGD	Phosphoglucomutase (EC 2.7.5.1)	PGM
Lactate dehydrogenase (EC 1.1.1.27)	LDH	Adenylate kinase (EC 2.7.4.3)	AK
Malate dehydrogenase (EC 1.1.1.37)	MDH	Purine-nucleoside phosphorylase (EC 2.4.2.1)	PNP
Glyceraldehyde-phosphate dehydrogenase (EC 1.2.1.12)	GAPD	Dipeptide hydrolase[A] (EC 3.4.11 or 13)	DPH
Xanthine dehydrogenase (EC 1.2.1.37)	XDH	Aminopeptidase (cytosol) (EC 3.4.11.1)	AP(C)
Glucosephosphate isomerase (EC 5.3.1.9)	GPI	Adenosine deaminase (EC 3.5.4.4)	ADA
		Superoxide dismutase (EC 1.15.1.1)	SOD

[A] Using Val-Leu as the substrate.

Table 2 shows for each species comparison the proteins scored and the gel matrix used.

Results

Whether or not a particular staining system revealed intraspecific electrophoretic variability is given in Table 2. We have found that electrophoretic variation in both transferrin and plasma esterases frequently fails to follow a Mendelian pattern of inheritance in some pseudomyine rodents (Baverstock *et al.* 1977*b*) and in the present study we have refrained from placing any taxonomic weight upon variation in these two classes of proteins. The patterns of variation of all other proteins studied are summarized in Fig. 2.

P. gracilicaudatus–P. nanus

Of the 14 systems scored, seven showed variation. Three systems (MDH, PGM, and PNP) revealed clear-cut differences between Queensland specimens (*P. gracilicaudatus*) and Northern Territory and Western Australian specimens (*P. nanus*). SOD, Hb, LDH and Alb, however, varied in the following manner:

Sod. Both *P. nanus* and *P. gracilidaucatus* gave two zones of SOD activity, neither of which was common to both species. Whether the two zones of activity result from one or two loci has not been determined but it is apparent that at least one gene difference is indicated.

Hb. Three distinct patterns were found in wild-caught animals (Fig. 2). *P. gracilicaudatus* from Queensland possessed two bands of Hb, both of which had electrophoretic mobilities differing from any Hb bands of *P. nanus*. *P. nanus* from Northern Territory and Kimberley Research Station, W.A., possessed a triple-banded pattern whilst *P. nanus* from other Western Australian localities possessed only two bands, both being electrophoretically identical to two of the bands of the Northern Territory *P. nanus*. Because there are several possible genetic interpretations of these data involving multiple alleles and/or multiple loci, crosses were set up between and within haemoglobin types of *P. nanus*. The results to date indicate a complex pattern of inheritance, the basis of which has not been elucidated.

LDH. All *P. nanus* gave two intense zones of activity and up to three weak zones of LDH activity. We interpret this pattern, which is frequently encountered in pseudomyine rodents, as a slow migrating A_4 band and faster migrating A_3B band, the minor bands being satellites (Manwell and Baker 1970). *P. gracilicaudatus* gave five bands of approximately equal intensity, the slowest band corresponding in electrophoretic mobility with that of *P. nanus*. Whether these five bands correspond to the standard five LDH isozymes of other vertebrates or to only A_4 and A_3B with strong satellites is uncertain. However, under either interpretation the product of the B locus of *P. gracilicaudatus* would appear to be electrophoretically different from that of *P. nanus*.

Alb. All four *P. gracilicaudatus* studied possessed a slow-migrating albumin. Several *P. nanus* possessed the same band, whilst others possessed a slightly faster migrating band. Although these two parental forms were always clearly distinguishable from each other on a gel, the difference in electrophoretic mobility was minor. Offspring between the two parental forms gave a wider band of albumin (presumably a double-banded heterozygous type), but in about 20% of cases these could not be differentiated with certainty from one or other of the parental types. Two of the wild-caught *P. nanus* gave an albumin pattern that suggested that they were hetero-

Table 2. Electrophoretic conditions for each protein studied for each species or species group

Abbreviations: *P.g.*, *P. gracilicaudatus*; *P.n.*, *P. nanus*; *P.a.*, *P. albocinereus*; *P.d.*, *P. delicatulus*; *M.g.*, *M. gouldi*; *Z.a.*, *Z. argurus*. S, starch; C, cellogel; V, variation noted; — failed to stain or bands not scorable

Protein	*P.g.–P.n.*	*P.a.*	*P.d.*	*M.g.*	*Z.a.*	Buffer	Stain
Alb	S (V)	S (V)	S (V)	S	S (V)	Gahne (1966)	Amido black
Hb	S+C (V)	S (V)	S	S	S+C (V)	Brewer (1970) (as G6PD)	Amido black
G6PD	S	S	S	S	S	Brewer (1970)	Brewer (1970)
PGD	S (—)	S (—)	S (V)	S	S	Brewer (1970)	Brewer (1970)
LDH-A	S	S (V)	S	S	S	Selander *et al.* (1971)	Brewer (1970)
LDH-B	S (V)	S	S	S	S	Selander *et al.* (1971)	Brewer (1970)
MDH	S (V)	S	S	S	S	Selander *et al.* (1971)	Brewer (1970)
GAPD	S (—)	S (—)	S (—)	S (—)	S (—)	Brewer (1970)	Brewer (1970)
XDH			C (—)	C (—)	C (—)	Richardson[A]	Brewer (1970)
GPI	C	C (V)	C	C	C	Richardson[A]	Richardson[A]
HK	C (—)	C (—)	S	S	S	Richardson[A]	Richardson[A]
AAT	C	C	C	C	C	Richardson[A]	Richardson[A]
PGK	C (—)	C (—)	C	C	C (V)	Richardson[A]	Richardson[A]
PGM	C (V)	C	S (V)	S (V)	S+C	Richardson[A]	Richardson[A]
AK	C	C (V)	C	C	C (—)	Richardson[A]	Richardson[A]
PNP	C (V)	C (V)	C	C	C (V)	Richardson[A]	Richardson[A]
DPH (Val-Leu)	C	C	C	C	C	Richardson[A]	Richardson[A]
AP(C)	S	S	S	S	S (V)	Selander *et al.* (1971)	Brewer (1970)
ADA			S (—)	S (—)	S (—)	Spencer *et al.* (1968)	Spencer *et al.* (1968)
SOD	S (V)	S	S	S	S (—)	Brewer (1970) (as GAPD)	From GAPD; gels overstained
Systems scorable	14	14	17	17	15		
Systems variable	7	6	3	1	4		

[A] Personal communication, Dr B. J. Richardson, Research School of Biological Sciences, Australian National University, Canberra, A.C.T.

Species group	Protein	Pattern (− ⟶ +)	Animal numbers
P. gracilicaudatus – P. nanus	MDH		73F, 74M, 75M, 76F, (Qld) 255F, 445F, 473F, 478F, 519F (N.T.); 216M, 247M, 263F, 435F, 440M, 444F (W.A.)
	PGM		73F, 74M, 75M, 76F, (Qld) 255F, 445F, 473F, 478F, 519F (N.T.); 216M, 247M, 263F, 435F, 440M, 444F (W.A.)
	PNP		73F, 74M, 75M, 76F, (Qld) 255F, 445F, 473F, 478F, 519F (N.T.); 216M, 247M, 263F, 435F, 440M, 444F (W.A.)
	SOD		73F, 74M, 75M, 76F, (Qld) 255F, 445F, 473F, 478F, 519F (N.T.); 216M, 247M, 263F, 435F, 440M, 444F (W.A.)
	Hb		73F, 74M, 75M, 76F, (Qld) 255F, 445F, 473F, 478F, 519F (N.T.); 444F (W.A.) 216M, 247M, 263F, 435F, 440M (W.A.)
	LDH		73F, 74M, 75M, 76F, (Qld) 255F, 445F, 473F, 478F, 519F (N.T.); 216M, 247M, 263F, 435F, 440M, 444F (W.A.)
	Alb		73F, 74M, 75M, 76F, (Qld); 255F, 473F, 478F, 519F (N.T.) 444F (W.A.); 445F (N.T.) 216M, 247M, 263F, 435F, 440M (W.A.)
P. albocinereus	GPI		36F, 37M, 38M, 39M, 40M, 41M, 43F, 42M (S.A.); 25F, 26F, 27M (W.A.M.) 29F, 30F, 31F, 32F, 33M (W.A.B.I.)
	Alb		36F, 37M, 38M, 39M, 40M, 41M, 42M, 43F (S.A.) 25F, 26F, 27M (W.A.M.); 29F, 30F, 31F, 32F, 33M (W.A.B.I.)
	AK		36F, 37M, 38M, 39M, 40M, 41M, 42M, 43F (S.A.) 25F, 26F, 27M (W.A.M.); 29F, 30F, 31F, 32F, 33M (W.A.B.I.)
	PNP		36F, 37M, 38M, 39M, 40M, 41M, 42M, 43F (S.A.) 25F, 26F, 27M (W.A.M.); 29F, 30F, 31F, 32F, 33M (W.A.B.I.)
	Hb		36F, 37M, 38M, 39M, 40M, 41M, 43F, 42M (S.A.); 25F, 26F, 27M (W.A.M.) 29F, 30F, 31F, 32F, 33M (W.A.B.I.)
	LDH		37M, 38M, 42M (S.A.) 39M, 41M, 43F (S.A.) 36F, 40M (S.A.) 29F, 30F, 31F, 32F, 33M (W.A.B.I.) 25F, 26F, 27M (W.A.M.)
P. delicatulus	Alb		13M, 14M, 55M (Qld); 57M, 484F (N.T.) 59F (W.A.)
	PGD		13M, 14M, 55M (Qld); 57M, 484F (N.T.); 59F (W.A.);
	PGM_2		14M (Qld) 13M (Qld); 57M, 484F (N.T.); 59F (W.A.) 55M (Qld)
M. gouldii	PGM_1		87F (Qld); 85M, 86F (N.T.) 84F (N.T.)
Z. argurus	Hb		94M (Qld); 100M (W.A.) 99F (N.T.); 104F (W.A.) 97M, 98F (N.T.); 105F, 108F, 107M (W.A.)
	PNP		94M (Qld); 97M, 98F, 99F (N.T.); 100M, 104F, 105F, 107M (W.A.) 108F (W.A.)
	PGK		94M (Qld); 97M, 99F (N.T.); 100M, 104F, 105F, 108F (W.A.) 98F (N.T.); 107M (W.A.)
	Alb		94M (Qld) 97M, 98F, 99F (N.T.); 100M, 105F, 107M, 104F (W.A.)

Fig. 2. Electrophoretic patterns of proteins showing intraspecific variation in mobility together with corresponding animal numbers. In all cases the origin is to the left and the direction of migration is to the right. Broken lines indicate weak satellite bands. W.A.M., Western Australian mainland. W.A.B.I., Western Australia, Bernier Island.

zygotes (Fig. 2). Thus *P. nanus* appears to be polymorphic for a fast and a slow allele at the albumin locus, whilst *P. gracilicaudatus* seems to be fixed for the slow allele, although the small sample sizes may be misleasing.

Thus, differences between *P. nanus* and *P. gracilicaudatus* occur at at least 6 out of 14 loci. In contrast *P. nanus* from Northern Territory and *P. nanus* from as far away as Derby, W.A., apparently possess no fixed differences.

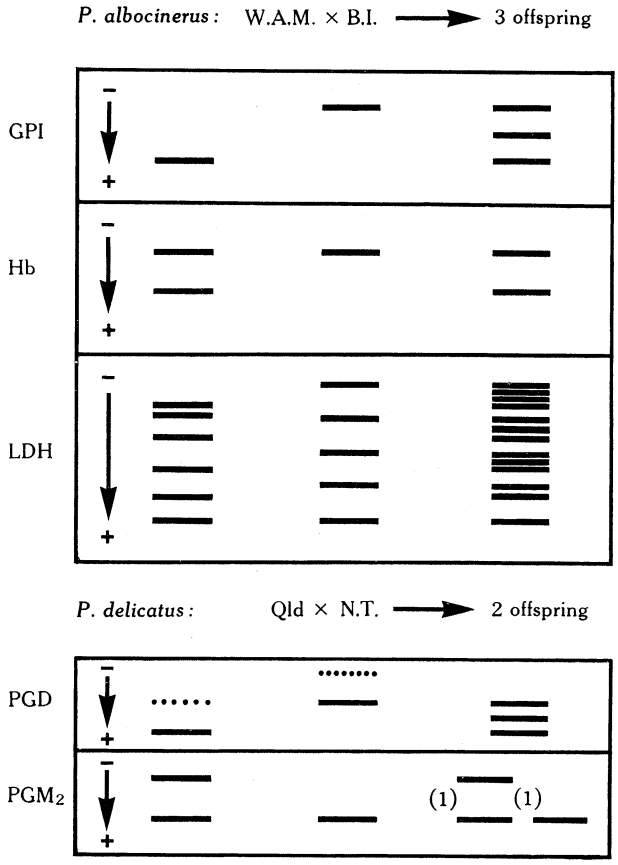

Fig. 3. Results of breeding studies for some variable proteins. Broken lines indicate weak satellite bands. W.A.M., Western Australian mainland. B.I., Bernier Island.

P. albocinereus

Six systems out of 14 showed electrophoretic variation (Table 2). Four of these (GPI, Alb, AK, and PNP) showed clear-cut differences between populations (Fig. 2). A cross between the Western Australian mainland form and the Bernier Island form yielded three offspring, all possessing both normal GPI bands and an additional band of intermediate electrophoretic mobility (Fig. 3). As GPI in rodents is a dimer (DeLorenzo and Ruddle 1969; Carter *et al.* 1972; Baverstock *et al.* 1977*b*), these data support the contention that the GPI difference between Western Australian mainland and Bernier Island animals is due to a single allelic difference.

In the case of Hb, South Australian and Western Australian mainland animals showed two bands whilst Bernier Island animals showed only one. It was postulated that the difference was due to the number of loci coding for Hb subunits. On this hypothesis, crosses between the double-banded and single-banded individuals should give offspring all of which were double-banded; this was the case (Fig. 3).

The subtle LDH variation within South Australian animals and between South Australian and Bernier Island animals seemed explicable in terms of satellites (Fig. 2). Western Australian mainland animals, however, apparently possessed the same allele coding for the B subunit but a different allele coding for the A subunit, since while the B_4 of Western Australian mainland animals had the same electrophoretic mobility as the B_4 of other animals, the remaining four zones (presumably A_4, A_3B, A_2B_2, and AB_3) failed to correspond. If this hypothesis is correct, a cross between Western Australian mainland and Bernier Island animals should yield offspring with a single band in the B_4 position but multiple bands due to the formation of multiple tetramers in the remaining four zones (Manwell and Baker 1970). The results (Fig. 3) completely fulfilled this expectation.

Assuming these interpretations to be correct, then out of 16 proteins examined, South Australian animals differ from Western Australian mainland animals by four proteins and from Bernier Island animals by five proteins. Western Australian mainland animals and Bernier Island animals differ by three proteins.

P. delicatulus

Of the 17 systems scored for *P. delicatulus*, only three showed variation. The Western Australian animal possessed an albumin differing from that of Queensland and Northern Territory animals whilst Queensland animals possessed a PGD differing from that of the Western Australian individual and the two Northern Territory animals. A cross between Queensland and Northern Territory animals gave offspring with three bands of PGD activity (Fig. 3), supporting the hypothesis that the PGD difference was due to a single gene difference. PGD is normally a dimer, so that heterozygotes possess a triple-banded phenotype (Manwell and Baker 1970). Although PGM_2 showed electrophoretic variation between Queensland animals, the pattern observed (Fig. 2) suggested that Queensland populations are polymorphic, possibly for three alleles. Results of a single cross between a supposed heterozygote and a supposed homozygote yielded segregation in the offspring (Fig. 3), supporting this hypothesis. PGM_2 thus showed no clear-cut differences between populations. In summary, of the 17 proteins studied, the single Western Australian animal differed from Northern Territory animals by one protein and from Queensland animals by two proteins. Northern Territory and Queensland animals differed by only one protein.

M. gouldi

Of the 17 systems scored for *M. gouldi*, only one (PGM_1) showed variation. The single Queensland animal was identical to two Northern Territory animals whilst a third Northern Territory animal possessed two instead of one band of PGM_1 activity (Fig. 2), suggesting that it may have been a heterozygote. Thus no clear-cut differences were found between Northern Territory and Queensland *M. gouldi*.

Z. argurus

Four of the 15 protein systems studied showed variation (Fig. 2). In the cases of Hb, PNP, and PGK, the variation was almost certainly due to polymorphism, the variants occurring within localities. In the case of albumin, the single Queensland animal possessed a form marginally but distinctly different from that of other specimens. However, a single animal from Western Australia (104F) possessed a broad albumin phenotype, suggesting that it was a heterozygote between the faster and slower types. Indeed offspring from a cross between the Queensland specimen and a Northern Territory animal yielded offspring with an identical broader zone of albumin (presumably indicating heterozygosity for the albumin locus), supporting the contention that 104F was heterozygous for the two albumin forms. Thus there were no 'apparently fixed' electrophoretic differences either between eastern and western subspecies (i.e. Queensland *v.* Northern Territory), or between eastern and western karyotypic forms.

Table 3. Electrophoretic diversity at five taxonomic levels of the *Drosophila willistoni* group

For the definition of subspecies and semispecies, and for terminology and calculations see Ayala *et al.* (1975). Unfortunatley those authors did not give ranges for these averages so that we have no idea of the degree of overlap in genetic distance between taxonomic levels

Taxonomic level	Average Nei distance	Average proportion of fixed differences
Local populations	0·031	0·02
Subspecies	0·230	0·18
Semispecies	0·226	0·08
Sibling species	0·581	0·40
Non-sibling species	1·056	0·60

Discussion

According to the biological species concept, two gene pools should be considered to constitute different species if they fail to exchange genes when given the opportunity to do so in the field. The mechanism preventing gene flow may be premating (behavioural) or postmating (developmental).

Where the populations are sympatric or parapatric, presence or absence of gene flow can be determined by morphological, behavioural, chromosomal, or electrophoretic studies. However, where the populations are completely allopatric, whether or not they should be treated as different species is largely a matter of interpretation, since it is usually difficult to determine whether any differences observed would be sufficient to prevent gene flow if the populations ever met. Nevertheless, recent results suggest that electrophoresis may be an exceptionally useful technique for arriving at a reasonably objective criterion for making a decision in such cases.

Ayala *et al.* (1975) compared levels of electrophotetic differentiation, expressed as the Nei distance, between various taxonomic levels of the *willistoni* group of *Drosophila*: namely local populations, subspecies, semispecies, sibling species and non-sibling species. The results are shown in Table 3. Sibling species show about twice as much electrophoretic diversity as subspecies and semispecies. However, a

similar relationship is obtained if we consider only 'apparently fixed' differences (i.e. which we may define as those with a genetic similarity of less than 0·2), as in Table 3. Thus simply knowing the number of 'apparently fixed' allelic differences is sufficient to determine whether two populations belong to one or two biological species. If the same situation applies to rodents, then electrophoresis becomes a powerful tool for determining the specific status of allopatric populations.

Table 4. Percentage of 'apparently fixed' electrophoretic differences between various taxonomic levels of rodents

Ranges (lowest to highest) are given in parentheses. *N* is the number of taxa compared in determining the mean percentage fixed differences

Group	Non-sibling species	*N*	Sibling species	*N*	Subspecies	*N*	Reference
Thomomys bottae v. T. umbrinus	—		0	2	0	4	Patton *et al.* (1972)
Thomomys talpoides karyotypic forms	—		2 (0–6)	6	—		Nevo *et al.* (1974)
Spalax karyotypic forms	—		0	4	—		Nevo and Shaw (1972)
Geomys	—		9 (6–15)	4	—		Selander *et al.* (1975)
Sigmodon	—		17	2	—		Johnston *et al.* (1972)
Dipodomys karyotypic forms	—		14	2	0	4	Patton *et al.* (1976)
Dipodomys	34 (0–50) or (14–50)[A]	3	—				Patton *et al.* (1976)
Dipodomys	37 (0–60) or (11–60)[A]	11	—		3 (0–6)	11	Johnson and Selander (1971)
Peromyscus	27 (20–33)	4	—		4 (0–13)	9	Kilpatrick and Zimmerman (1975)
Peromyscus	37 (18–50)	5	—		9 (5–14)	5	Avise *et al.* (1974), Selander *et al.* (1971), Smith *et al.* (1973)
Overall unweighted	34		7		2		

[A] The second range of values applies if the single comparison of *D. heermanni* and *D. paramintinus* (which were electrophoretically indistinguishable) is excluded.

In order to test this possibility, we have compared the levels of taxonomic differentiation, in terms of 'apparently fixed' differences, between taxonomic levels of various rodent groups from data available in the literature. An 'apparently fixed' difference was defined for the purposes of this analysis as one in which one allele at a locus occurred at a frequency of greater than 90% in one taxon and less than 10% in the other taxon (corresponding to a genetic similarity of less than 0·2). There are a number of biases in the technique of electrophoresis (Ayala 1975) which render it unwise to equate electrophoretic differentiation with genetic differentiation; however, in the present case we are attempting to determine whether electrophoretic differentiation *per se* is sufficient to differentiate biological species from lower taxonomic categories, so that these biases are of no concern to us here.

The studies used were chosen on the following bases: (1) at least 15 loci for each group were studied; (2) the taxonomic categories given in the study were those determined prior to the electrophoretic studies, so that there is no chance of circularity;

(3) the species groups studied have been well-worked taxonomically, so that categorization of species, sibling species and subspecies is probably correct.

Table 4 gives the proportion of 'apparently fixed' electrophoretic differences between different taxonomic levels of North American rodents. As in *Drosophila*, the distinction between non-sibling species and subspecies is most marked. Indeed when the ranges of differentiation are compared, there is only a small overlap around the 15% level. The only exception is the *Dipodomys heermanni–D. panamintinus* comparison, these species being electrophoretically indistinguishable. Moreover, the proteins albumin, transferrin, esterase and haemoglobin are important in subspecies differentiation whereas glycolytic enzymes are important in the full species differentiation. However, in contrast to *Drosophila* sibling species, the rodent sibling species show, on average, little more genetic differentiation than subspecies. Although the reasons for this are unclear, it may be significant that the sibling species of rodents listed in Table 4 are differentiated mainly by chromosomal differentiation arising from Robertsonian rearrangements whereas those differentiating sibling species of *Drosphila willistoni* involve paracentric inversions. Heterozygosity for the former is often sufficient by itself to cause reduced hybrid fertility (Ford and Evans 1973) whilst heterozygosity for the latter is not, at least in *Drosophila* (White 1973).

Thus electrophoretic data alone are not sufficient to distinguish biological species of rodents, especially where the populations being compared differ chromosomally by Robertsonian rearrangements. Although more data are needed, the results in Table 4 suggest that a one-way test could justifiably be proposed. Thus if two allopatric populations of rodents possess 'apparently fixed' electrophoretic differences at at least 15% of their loci, then it is highly probable that they belong to different biological species. The converse is not true, namely that if two populations differ by less than 15% 'apparently fixed' electrophoretic differences they are the same species, especially if chromosomally they differ by several Robertsonian rearrangements.

Because 'apparently fixed' electrophoretic differences are, for our purposes, as informative as coefficients of genetic difference, it is not essential to determine actual gene frequencies for polymorphic loci. Consequently only a small number of animals from each population need be characterized electrophoretically. Suppose, for example, there are two samples, each of three individuals, one from species 1 and one from species 2. The probability of choosing six A_1 genes from a population with a gene frequency for A_1 of 0·9 is $(0{\cdot}9)^6$. The corresponding probability in the second sample for A_2 is $(0{\cdot}9)^6$. The probability of both samples occurring simultaneously is $(0{\cdot}9)^6 \times (0{\cdot}9)^6 = (0{\cdot}9)^{12} = 28\%$. Hence if the gene frequencies of A_1 and A_2 are both less than 0·9 there is 72% probability of detecting this by the appearance of either A_2 in the first sample or A_1 in the second sample. With four animals per population the probability of detecting a heterogeneous sample becomes $1-(0{\cdot}9)^{16} = 81\%$.

What is far more important is the number of loci surveyed. For example, if two allopatric populations differ by 'apparently fixed' differences at 3 out of 10 loci, the *estimate* of electrophoretic difference is 30% with a 90% certainty that the difference is at least 11% (one-tailed test—Table $VIII_1$ of Fisher and Yates 1963). If, however, 6 out of 20 loci are different, the estimate is again 30% but the 90% limit is raised to 17%. It is, then, more informative to characterize electrophoretically a few individuals of each taxon for many loci than many individuals for a few loci.

The application and value of an electrophoretic approach to species problems is well illustrated by the *P. nanus–P. gracilicaudatus* situation. A discussion of this particular problem may be found in Ride (1970, pp. 31, 155) and Mahoney and Posamentier (1975). Briefly, *P. nanus* is a western form and *P. gracilicaudatus* is an eastern form. Specimens from Northern Territory, however, are morphologically intermediate. Martinez and Lidicker (1971), Taylor and Horner (1972), Parker (1973) and Covacevich and Easton (1974) referred Northern Territory specimens to *P. gracilicaudatus*, whilst Keith (1968) and Ride (1970) referred them to *P. nanus*.

In contrast to the taxonomic confusion resulting from the use of morphological criteria alone, the biochemical results appear to be clear cut. There are no fixed biochemical differences out of 14 proteins between populations of *P. nanus* taken up to 1000 km apart nor between populations of *P. gracilicaudatus* separated by 800 km. Yet between *P. nanus* and *P. gracilicaudatus* separated by 1600 km there are six 'apparently fixed' biochemical differences out of 14 proteins (proportion of fixed differences = 43%; one-tailed lower 90% limit = 24%). The karyotypic data (Baverstock *et al.* 1977*a*) show a similar pattern of uniformity within, but three chromosomal rearrangements between, *P. nanus* and *P. gracilicaudatus*. There can thus be little doubt that these two forms represent distinct species.

The *P. albocinereus* situation is less clear cut. The two Western Australian forms differ by three out of 14 proteins (21%; one-tailed lower 90% limit = 8%), the mainland Western Australian and South Australian forms by four proteins (29%; one-tailed lower 90% limit = 13%), and the Bernier Island and South Australian form by five proteins (36%; one-tailed lower 90% limit = 19%). These results are all the more significant when it is remembered that the 14 proteins studied specifically excluded transferrin and esterase. All three populations differ also in both their X chromosomes and Y chromosomes, although in a way which we believe has only minor taxonomic significance (Baverstock *et al.* 1977*a*). Finally, although specimens from South Australia have been bred in the laboratory, and Western Australian mainland animals have been crossed with Bernier Island animals, four South Australian × Western Australian crosses have failed to yield offspring after 15 months. We believe that these data, taken in conjunction with morphological criteria (Finlayson 1932), support a model in which the South Australian population is seen as having speciated in isolation from the Western Australian populations. The South Australian form should thus be referred to *P. apodemoides* (Finlayson 1932). The Western Australian mainland and Bernier Island populations also appear to have developed genetic differences in isolation, but to a lesser degree than the South Australian population, supporting their subspecific differentiation.

In contrast to the high levels of biochemical differentiation between *P. nanus* and *P. gracilicaudatus* and between populations of *P. albocinereus*, the remaining species—*Z. argurus*, *P. delicatulus* and *M. gouldi*—showed marked biochemical uniformity over their range, despite the chromosomal differences (Baverstock *et al.* 1977*a*). Indeed, from Queensland to Northern Territory, *M. gouldi* and *Z. argurus* showed no fixed differences, and *P. delicatulus* only one. From Northern Territory to Western Australia, *Z. argurus* showed no fixed differences and *P. delicatulus* a maximum of only one.

There are two possible interpretations of the apparent conflict between the chromosomal and electrophoretic data.

(1) Gene flow continues to occur between eastern and western populations, maintaining their genetic similarity. On this view populations in areas where the chromosomal forms meet should maintain a chromosomal polymorphism.
(2) The populations were contiguous until recently and the karyotypic differences have accumulated in the short period since their isolation.

On either view, the electrophoretic data give no indication that western and eastern forms of *Z. argurus*, *M. gouldi* and *P. delicatulus* represent different biological species.

One of the aims of our study was to gain some idea of the levels of electrophoretic variation we might encounter between allopatric populations of single species in a forthcoming electrophoretic comparison of Australian rodents. Because *M. gouldi*, *Z. argurus* and *P. delicatulus* show little interpopulation variation, only a few individuals of each species will be characteristic of the whole species. In the cases of *P. albocinereus* and *P. nanus–P. gracilicaudatus*, however, each population will have to be treated as a separate taxonomic unit.

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