THE DISTRIBUTION OF RED CELL LACTATE DEHYDROGENASE TYPES IN GREY KANGAROOS

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Abstract

Protein markers have been used to determine genetic differences in animal populations. Electrophoresis on disc acrylamide gels of red cell haemolysates from two reproductively isolated species of grey kangaroos, the eastern grey kangaroo, *Macropus giganteus* Shaw, 1790, and the western grey kangaroo, *M. fuliginosus* (Desmarest, 1817), revealed two patterns of lactate dehydrogenase isozymes, phenotypes D and E, and also E_m, a modified form of phenotype E.

Examination of phenotype frequencies in grey kangaroos revealed that the occurrence of lactate dehydrogenase type D was highest in *M. giganteus* from eastern Australia, significantly lower in the sympatric *M. f. melanops*, found infrequently in *M. f. ocydromus* from south-western Australia, and absent from *M. f. fuliginosus* from Kangaroo I. Disparity in lactate dehydrogenase phenotype frequencies between geographically distinct groups of grey kangaroos may result from the operation of the "founder" principle, or the maintenance of these differences in frequencies by natural selection, within reproductively isolated populations. This suggests that incipient speciation may be proceeding in western grey kangaroos.

I. INTRODUCTION

Grey kangaroos, *Macropus* spp., generally occur in the zone between the arid inland plains and the coast, including semi-arid mallee scrubs, shrub woodlands, and forests. Their distribution is continuous from northern Queensland, south and west to south-western Australia. Throughout that wide range, grey kangaroos show considerable variation in colour and morphology and it is not surprising that opinions have differed on the status of at least five taxa described since the early naturalists collected specimens from widely separated localities. Kirsch and Poole (1967, 1972) have differentiated two species of grey kangaroos by characteristic differences in length of cyclic reproductive processes; the presence or absence of one allele in a simple polymorphism for transferrin; and distinct serum antigens. These differences correlated with an apparent lack of hybridization in the wild, coat colour, and geographic distribution. The eastern grey kangaroo, *Macropus giganteus* Shaw, 1790, was found in all eastern states, plus south-eastern South Australia, while the western grey kangaroo, *M. fuliginosus* (Desmarest, 1817), occurred in south-western and southern Australia, including Kangaroo I., extending into western Victoria and south-western New South Wales, where the two species were sympatric.

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The distribution of the two species and the type localities of the described taxa are shown in Figure 1.

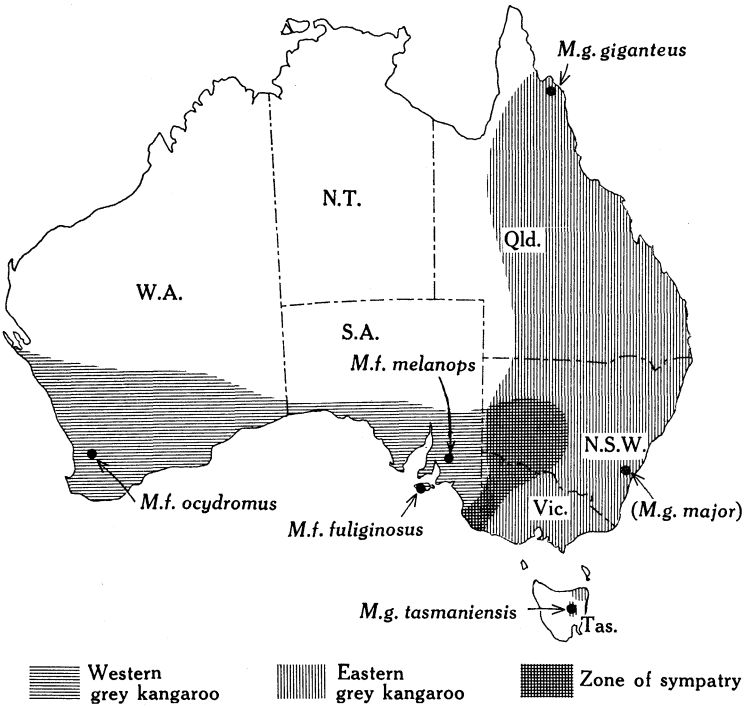

Fig. 1.—Distribution of eastern and western grey kangaroos adapted from Kirsch and Poole (1972). ● Type localities of described taxa.

In recent years electrophoretically detected polymorphisms of iron binding and other proteins of serum or red cell haemolysates have been used to characterize populations of a variety of animals. These protein markers have been successfully used in determining genetic differences in human populations and because these markers are products of one or two structural genes in each genome, genetic differences can be observed. The enzyme lactate dehydrogenase (E.C. 1.1.1.27) is the product of two genes, *a* and *b*, and each lactate dehydrogenase (LDH) isozyme is a tetrameric combination of the polypeptide gene products A and B. There are five possibilities for combination into tetramers: AAAA, AAAB, AABB, ABBB, and BBBB. Since the electrophoretic mobility of the A polypeptide is slow and that of B is fast, five isozymes can theoretically be produced and their compositions can be determined by their electrophoretic mobilities (Markert and Møller 1959; Markert 1962).

The tetrameric structure of LDH isozymes has been established for vertebrates from lampreys to man and since hydridization can take place between different species, Markert (1968) regards this as evidence of the evolutionary conservatism of the molecule. It is therefore considered that marsupial LDH is homologous with red cell LDH from other vertebrates.

An initial survey of LDH in red cells of several species of Macropodidae (Clark and Richardson 1968) established that two main groups of isozyme patterns occurred. Of these, group I had relatively little LDH 1 (fast tetramer), compared with the slower tetramers while group II patterns had a relatively large amount of LDH 1. Red kangaroos (*Megaleia rufa*) conformed to group I, while 14 grey kangaroos all had patterns conforming to group II. Of the 14 grey kangaroos, 13 were eastern grey kangaroos (*M. giganteus*) and all had LDH of type D, which consisted of four isozymes, LDH 1, 2, 3, and 4. Of these LDH 1 was the heaviest and LDH 4 the lightest band when stained. The fourteenth blood sample was from an insular form of the western grey kangaroo (*M. fuliginosus fuliginosus*) and its LDH pattern, called type E by Clark and Richardson (1968), differed from type D in having a prominent diffuse LDH 5 band and an increase in the amount of LDH 4 compared with LDH 2 and 3 (see Fig. 2). As these patterns were repeatedly reproducible when

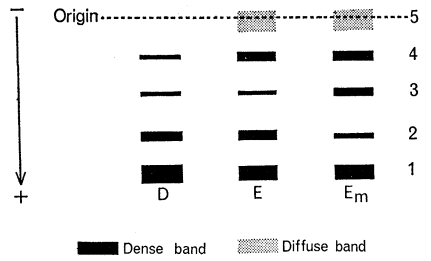

Fig. 2—Diagrammatic representation of LDH patterns in red cells from grey kangaroos. Four bands (isozymes) typify LDH type D and five bands LDH types E and E_m.

fresh blood samples were obtained from several individual kangaroos, it was considered that the difference between LDH types D and E was not an artefact. Certainly the presence of large amounts of LDH 5 in types E and E_m is unusual since it is more easily lost than other isozymes (Vesell 1965). Such a result could be expected if the ratio of A to B polypeptides was so large that excess A polypeptides remained despite the normal loss through breakdown during electrophoresis. Clark (1972) considered that the differences in patterns arise because of factors modifying the assembly of tetramers. Hence more A polypeptides are produced relative to B in type E than in type D. The regulating mechanism is not clear but may be due to some modifying factor such as a regulatory gene similar to that reported in mice by Shows and Ruddle (1968).

Consistent evidence for such a regulatory mechanism was found in the progeny from 14 matings of kangaroos of known LDH types (Clark 1970). The young tested resulted from 11 intraspecific matings and three from interspecific crosses. One type D × type D and five type E × type E matings produced expected results in the offspring. One interspecific cross (type E × type E) produced an atypical result (type D). All other matings (type D × type E) produced patterns intermediate between those of the parents but favoured type E due to the presence in large amounts of LDH 5.

Members of the three described taxa, comprising the western grey kangaroos, *M. f. fuliginosus*, *M. f. melanops*, and *M. f. ocydromus*, despite possessing certain serological and reproductive characters in common, are morphologically distinct (Kirsch and Poole 1972). As the one *M. f. fuliginosus* examined by Clark and

Richardson (1968) differed in LDH type from the other grey kangaroos tested it was decided to check more animals to ascertain whether this LDH type was common to all kangaroos from Kangaroo I., and whether LDH phenotype frequencies in these, and the mainland populations, would be useful as a taxonomic tool and provide supportive evidence for the findings of Kirsch and Poole (1972).

II. Methods

(*a*) *Collection of Blood*

Blood was collected from 138 kangaroos which included representatives of the two primary species and their possible subspecies. Many of the samples were drawn from animals shot in the field during the course of other investigations but the taking of protected species in some additional areas could not be justified in a preliminary survey of LDH phenotypes. Consequently blood was taken from several live kangaroos and some of their progeny both after capture on Kangaroo I. and in south-western Australia and held in captivity with several species of Macropodidae associated with studies of breeding biology at the CSIRO Division of Wildlife Research, Canberra. Animals originating from Kangaroo I. and held in menageries in New South Wales and South Australia were bled and whenever possible samples were taken from other grey kangaroos whose place of capture or parentage was known.

Blood (5 ml) was taken into an anticoagulant solution consisting of citric acid, potassium citrate, and dextrose to which inosine was added (World Health Organization 1967). Samples packed in ice were sent by air to the laboratory where red cells were separated from serum by centrifugation at 2500 r.p.m. for 15 min and washed three times with physiological saline (0·85% NaCl). The red cells were then haemolysed with 10 volumes of distilled water. Large particles were removed by centrifugation at 2500 r.p.m. for 10 min.

(*b*) *Electrophoresis*

LDH isozymes were separated by disc acrylamide electrophoresis according to the method of Ornstein (1964) and Davis (1964). The separating gel was 7·5% (pH 9·5) and the stacking gel 5% acrylamide. The buffer was Tris–glycine, pH 8·9. The duration of the electrophoretic run was 80 min at room temperature, the voltage applied being 240 V and the current 34 mA per bath of eight gels. The length of the run was 2·5 cm, determined by the use of a tracking dye (bromphenol blue 0·005 g per 100 ml water).

(*c*) *Staining*

The staining method was adapted from those of Goldberg and Cather (1963) and Allen (1961). Gels were removed from the glass tubes and incubated for 1 hr at 25°C in the dark in a mixture containing the following: to each 8 ml Tris–HCl buffer (0·05M, pH 7·4) was added 0·25 ml sodium DL-lactate (Sigma, grade DL-V); 0·5 mg NAD (Sigma, grade III); 0·2 ml phenazine methosulphate (2 mg/ml) (Sigma); 4 ml 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (2 mg/ml) (Sigma), and 2·5 ml KCN (0·06M). LDH isozymes appear as purple bands.

III. Results

During the course of the investigation it was observed that some of the LDH patterns did not conform to the type E described by Clark and Richardson (1968) and these were referred to as type E_m or E(modified). In this pattern there was a diffuse LDH 5 band as in type E but the relative amounts of isozymes LDH2 and LDH3 were reversed (see Fig. 2). This may be due to a restriction on the random association of A and B polypeptides as described in some fish (Markert 1968). No type E_m patterns were found in samples from any *M. f. melanops* or *M. g. tasmaniensis* examined.

Since these isozymes are products of more than one structural gene it is not possible in the case of LDH to determine gene frequencies, hence frequencies of phenotypes are compared. The phenotype frequencies of LDH in the species groups sampled are given in Table 1. Included in this list are 14 grey kangaroos previously typed by Clark and Richardson (1968) when an error during tabulation showed both *M. f. ocydromus* samples as LDH type D instead of one type D and one type E.

TABLE 1

LACTATE DEHYDROGENASE PHENOTYPE FREQUENCIES IN GREY KANGAROOS

Species	Distribution	No. of animals	Frequency of LDH phenotypes		
			D	E	E_m
Eastern grey kangaroos:					
M. g. giganteus	Eastern Australia	53	34	16	3
M. g. tasmaniensis	Tasmania	13	10	3	0
Western grey kangaroos:					
M. f. melanops	Southern Australia	30	11	19	0
M. f. ocydromus	South-western Australia	16	1	9	6
M. f. fuliginosus	Kangaroo I.	26	0	18	8

χ^2 tests for homogeneity of LDH phenotype frequencies are given in Table 2. Within eastern grey kangaroos there was no significant regional differences between kangaroos from the mainland and Tasmania, nor within the western greys was there a significant difference between the insular form *M. f. fuliginosus* and representatives of the mainland form *M. f. ocydromus* from the western end of their distribution. However, both these forms differed significantly ($P<0{\cdot}001$) from *M. f. melanops* at the eastern end of their range.

TABLE 2

TESTS FOR HOMOGENEITY OF LACTATE DEHYDROGENASE PHENOTYPE FREQUENCIES WITHIN AND BETWEEN SAMPLES OF EASTERN AND WESTERN GREY KANGAROOS

Species tested	χ^2	D.F.	Probability
Within eastern grey kangaroos:			
giganteus versus *tasmaniensis*	1·17	2	0·5558 (n.s.)
Within western grey kangaroos:			
ocydromus versus *fuliginosus*	2·02	2	0·3044 (n.s.)
melanops versus *ocydromus* + *fuliginosus*	22·35	2	0·0000 ($P<0{\cdot}001$)
Between eastern and western grey kangaroos:			
giganteus versus *melanops*	10·82	2	0·0045 ($P<0{\cdot}01$)
giganteus + *tasmaniensis* versus *ocydromus* + *fuliginosus*	46·56	2	0·0000 ($P<0{\cdot}001$)

Comparison of LDH frequencies between eastern and western grey kangaroos drawn from the sympatric species *M. g. giganteus* and *M. f. melanops* showed a

significant difference ($P<0{\cdot}01$) between the samples while there was a highly significant difference ($P<0{\cdot}001$) between all the eastern grey kangaroos (*M. g. giganteus* and *M. g. tasmaniensis*) and the combined sample of western greys from Kangaroo I. and south-western Australia (*M. f. fuliginosus* and *M. f. ocydromus*) (Table 2).

IV. Discussion

Statistically significant differences in LDH phenotype frequencies in grey kangaroos may reflect differences of geographical location with LDH type D highest in the eastern species (*M. giganteus*) and lowest in the most westerly group (*M. f. ocydromus*). This may indicate that a geographic cline exists but the samples from western grey kangaroos were drawn from a relatively small number of animals obtained from opposite ends of the known range of this species group. Confirmation of an east–west cline, or dissent from such a proposition, requires an intensive survey across Australia, throughout the known distribution of grey kangaroos.

No LDH type D was found in 26 individuals from Kangaroo I. where there is a discrete morphologically distinct population on an island estimated as separated from the mainland 9000–11,000 years ago (J. N. Jennings, personal communication). Despite the recording of one kangaroo with LDH type D in the sample of *M. f. ocydromus* their phenotype frequencies were not significantly different from that found in *M. f. fuliginosus*, but the recorded frequencies in both groups differed significantly from *M. f. melanops* at the eastern end of the range of western grey kangaroos. Kirsch and Poole (1972) consider *M. f. fuliginosus* a valid subspecies but, despite stating that kangaroos from south-western Australia can generally be distinguished on body shape, suggest that *M. f. melanops* and *M. f. ocydromus* may be found to integrate when sufficient morphological analysis has been done. Certainly all western grey kangaroos interbreed and share a common polymorphism for transferrin and serum antigens. Hybrids have not been found in the field but matings between captive western males and eastern females—the reverse mating never occurred—produce hybrids (Kirsch and Poole 1972). Such matings have involved western males of all three "subspecies".

In the absence of evidence for interbreeding between eastern and western grey kangaroos in the field, the migration of LDH type D-producing gene or genes must be discounted between those two populations. However, leakage of type D-producing genes may be occurring between *M. f. melanops* and *M. f. ocydromus* via the narrow coastal corridor to the south of the Nullarbor Plain.

The "founder" principle, by which phenotype frequencies in largely isolated colonies depend on the gene complements of individuals initiating the colony, may account for differences in LDH frequencies and in particular the absence of LDH type D on Kangaroo I. It is equally likely that the maintenance of different phenotypic frequencies is the result of the operation of some form of natural selection.

The presence of differences in LDH phenotype frequencies, considered in association with geographic barriers, suggests that the different groups may be reproductively isolated populations creating conditions suited to the occurrence of speciation. Incipient speciation may be occurring in the western grey kangaroos and while *M. f. fuliginosus* is distinct, the mainland forms *M. f. melanops* and *M. f. ocydromus* may also be approaching or have attained the status of subspecies, but this

may only be confirmed by the detection and investigation of other genetic markers in these grey kangaroos.

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