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Ecological Genetics of the Wild Rabbit in Australia. II.* Protein Variation in British, French and Australian Rabbits and the Geographical Distribution of the Variation in Australia

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Abstract

A survey for genetic variation was carried out using 21 proteins controlled by 26 loci in rabbits from Britain, Mediterranean France and Australia. Five enzymes, adenosine deaminase, phosphogluconate dehydrogenase, carboxylesterase, carbonate dehydratase and dihydrolipoamide reductase (NAD^+) were found to be polymorphic. The average heterozygosity in wild rabbits was 6%. The genetic distances separating the various populations indicated that three different stocks were present in these populations. The rabbits from Britain and mainland Australia belonged to one group, those from France to a second group and the rabbits from southern Tasmania were a distinctive third group. Highly significant differences in gene frequency were found between the various local populations studied from mainland Australia. This variation showed no clear pattern and was attributed to genetic drift due to small effective population sizes. Bottlenecks in population size occur regularly in local rabbit populations in Australia through, for example, drought, myxomatosis outbreaks or rabbit control programs.

Introduction

The rabbit came originally from the Iberian peninsula and North Africa and was spread elsewhere in Europe as a semidomesticated animal by the Romans (Zeuner 1963). It reached Britain during Norman times. More recently the rabbit has been taken by European settlers to many other parts of the world, including Australia.

Mainland Australian rabbit populations are derived from a release of about a dozen rabbits near Geelong in southern Victoria in 1859. From there rabbits spread throughout temperate Australia, colonizing much of Victoria, New South Wales and southern Queensland by 1890 and reaching south-western Australia in about 1910 (Rolls 1969; Myers 1971). Rabbits were brought to Australia earlier, but established only local populations, apparently of little consequence, in South Australia, around Sydney, Melbourne and perhaps at other places on the mainland. It is, of course, possible that genes from these rabbits were eventually incorporated into the gene pool derived from the Geelong rabbits. In Tasmania, rabbits were introduced early in the 19th Century and, consequently, were derived from European sources independent of the Geelong release on the mainland (Rolls 1969).

A survey of various loci for genetically inherited variation was carried out using rabbits from Australia, Britain and Mediterranean France. In this paper the results

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of these studies are reported, and possible causes of the observed genetic divergence between the various populations considered. This divergence is also compared with that reported for morphological characters.

Methods

Blood and liver samples were taken from rabbits collected by spotlight shooting. Additionally, blood samples were taken from animals live-trapped in treadle-operated box traps at locations where more detailed ecological and genetic studies were in progress. These latter animals made up the entire sample from Grassy Creek and part of the samples from Urana, Snowy Plains, Chidlow, Cape Naturaliste and Quobba.

The samples were collected and preserved as described by Coggan *et al.* (1974*a*). Lysates were prepared by adding 2 volumes of 0·8% (w/v) β-mercaptoethanol to packed red cells or to a liver sample. Liver samples were broken up with a glass rod and the cell debris was removed by centrifugation (2000 *g*, 10 min). Normally electrophoresis was carried out, using cellulose acetate gel (Cellogel, Chemetron, Italy) as the support medium, following the technique employed by Richardson and Cox (1973). The enzymes studied, the specific stains employed and the optimum running conditions are given in Table 1. The adenosine deaminase phenotypes in liver were typed using the starch-gel electrophoresis system of Spencer *et al.* (1968). The carboxylesterase phenotypes were also determined following starch-gel electrophoresis (Schiff and Stormont 1970).

In the survey for new polymorphisms approximately 50 English, 50 French, 50 Tasmanian and 50 mainland Australian animals were studied. Other animals were then typed, but only for the polymorphic loci already found.

Table 1. Enzymes studied and running and staining conditions used

Compositions of the running buffers are given on the first occasion each buffer is used. The amperage to be supplied is per centimetre width of gel. PMS, phenazine methosulfate; MTT, 3-(4,5-dimethylthiozol-2-yl)-2,5-diphenyltetrazolium bromide

Enzyme	Running and staining conditions
Phosphogluconate dehydrogenase (decarboxylating) (EC 1.1.1.44)	*Gel*: TEB (15 mM Tris, 5 mM EDTA, pH adjusted with boric acid) pH 8·2 with 10 mg/100 ml NADP; 0·8 mA/cm for 1·5 h *Stain*: 1·0 ml 0·1 M Tris-HCl, pH 8·6; 0·1 ml 6-phosphogluconate (25 mg/ml); 0·1 ml NADP (10 mg/ml); 0·1 ml 0·1 M $MgCl_2$; 0·1 ml PMS (2 mg/ml); 0·1 ml MTT (4 mg/ml)
Adenosine deaminase (EC 3.5.4.4)	*Gel*: TMII (50 mM Tris, pH adjusted to pH 7·8 with maleic acid); 0·8 mA/cm for 1 h *Stain*: 1·0 ml 0·1 M phosphate buffer, pH 6·5; 0·1 ml adenosine (25 mg/ml); 0·1 ml PMS (5 mg/ml); 0·1 ml MTT (5 mg/ml); xanthine oxidase 1·2 i.u.; nucleoside phosphorylase 1·2 i.u.
Carbonate dehydratase (EC 4.2.1.1)	*Gel*: TEB (9·1) (6 mM Tris, 2·6 mM EDTA, 0·7 mM boric acid, pH 9·1); 1·2 mA/cm for 15 min *Stain*: Dip gel in 0·2% (w/v) bromothymol blue in 0·4% (w/v) NaOH, blot and spray with CO_2
Carboxylesterase (EC 3.1.1.1)	*Gel*: TEB pH 7·8; 0·8 mA/cm for 1·5 h *Stain*: 35 mg α-naphthyl acetate in acetone, 50 mg fast blue, 6 ml distilled water. Filter stain and lay gel face down on a sheet of stain-saturated filter paper
Dihydrolipoamide reductase (NAD^+) (EC 1.6.4.3)	*Gel*: TEB pH 7·8; 0·8 mA/cm for 1·5 h *Stain*: 1·0 ml 0·1 M Tris-HCl, pH 8·5, 0·2 ml NADH (10 mg/ml), 0·1 ml dichlorophenolindophenol (10 mg/ml), 0·1 ml MTT (2 mg/ml)

Table 1 (*Contd*)

Enzyme	Running and staining conditions
Glycerol-3-phosphate dehydrogenase (NAD^+) (EC 1.1.1.8)	*Gel*: TM (7·8) (100 mM Tris, pH adjusted to pH 7·8 with maleic acid); 1·1 mA/cm for 2·5 h *Stain*: 1·0 ml 0·1 M Tris-HCl, pH 7·4; 0·1 ml α-glycerophosphate (25 mg/ml); 0·1 ml NAD (10 mg/ml); 0·1 ml PMS (2 mg/ml); 0·1 ml MTT (4 mg/ml)
Alcohol dehydrogenase (EC 1.1.1.1)	*Gel*: TM (7·8); 1·1 mA/cm for 2·5 h *Stain*: 1·0 ml 0·1 M Tris-HCl, pH 7·4; 0·1 ml ethanol (25 mg/ml); 0·1 ml NAD (10 mg/ml); 0·1 ml PMS (2 mg/ml); 0·1 ml MTT (4 mg/ml)
Glutamate dehydrogenase (EC 1.4.1.2)	*Gel*: TM (7·8); 1·1 mA/cm for 2 h *Stain*: 1·0 ml 0·1 M Tris-HCl, pH 7·6; 0·1 ml glutamic acid (25 mg/ml); 0·1 ml NADP (10 mg/ml); 0·1 ml PMS (2 mg/ml); 0·1 ml MTT (4 mg/ml)
Isocitrate dehydrogenase ($NADP^+$) (EC 1.1.1.42)	*Gel*: TM (7·8); 1·1 mA/cm for 1 h *Stain*: 1·0 ml 0·1 M Tris-HCl, pH 8·0; 0·1 ml sodium isocitrate (25 mg/ml); 0·1 ml NADP (10 mg/ml); 0·1 ml PMS (2 mg/ml); 0·1 ml MTT (4 mg/ml); 0·1 ml 0·005 M $MnCl_2$
Lactate dehydrogenase (EC 1.1.1.27)	*Gel*: TMII; 0·8 mA/cm for 1 h *Stain*: 1·0 ml 0·1 M Tris-HCl, pH 8·0; 0·1 ml lactic acid (25 mg/ml); 0·1 ml NAD (10 mg/ml); 0·1 ml PMS (2 mg/ml); 0·1 ml MTT (4 mg/ml)
Malate dehydrogenase (EC 1.1.1.37)	*Gel*: TMII; 0·8 mA/cm for 1 h *Stain*: 1·0 ml 0·1 M Tris-HCl, pH 8·0; 0·1 ml malic acid (25 mg/ml); 0·1 ml NAD (10 mg/ml); 0·1 ml PMS (2 mg/ml); 0·1 ml MTT (4 mg/ml)
Fumarate hydratase (EC 4.2.1.2)	*Gel*: TMII; 0·8 mA/cm for 1·5 h *Stain*: 0·6 ml 0·1 M Tris-HCl, pH 7·5; 0·1 ml sodium fumarate (80 mg/ml); 0·2 ml NAD (10 mg/ml); malate dehydrogenase 60 i.u.; 0·1 ml PMS (2 mg/ml); 0·1 ml MTT (4 mg/ml)
Ornithine carbamoyltransferase (EC 2.1.3.3)	*Gel*: TNM (50 ml 1·0 M Tris-maleate+60 ml 1·0 M NaOH in 1000 ml); 0·9 mA/cm for 3 h *Stain*: 100 ml 0·5 M Tris-maleate, pH 7·2; 50 mg carbamoyl phosphate (lithium salt); 300 mg L-ornithine hydrochloride; 5 ml lead nitrate (2% w/v); filter, incubate gel in solution for 10 min, rinse, immerse in 1% (w/v) ammonium sulfide, rinse. Black zones of activity appear
Purine-nucleoside phosphorylase (EC 2.4.2.1)	*Gel*: TB (4·8 mM Tris, 1·3 mM barbital, 4·7 mM sodium barbital); 0·8 mA/cm for 2 h *Stain*: 1·0 ml 0·05 M phosphate buffer, pH 7·5; 0·1 ml inosine (25 mg/ml); 1·2 i.u. xanthine oxidase; 0·1 ml PMS (2 mg/ml); 0·1 ml MTT (4 mg/ml)
Adenylate kinase (EC 2.7.4.3)	*Gel*: TM (8·2) (50 mM Tris, pH adjusted to pH 8·2 with maleic acid); 0·8 mA/cm for 2 h *Stain*: 1·0 ml 0·1 M Tris-HCl, pH 8·0; 0·1 ml ADP (10 mg/ml); 0·1 ml 0·1 M $MgCl_2$; 0·1 ml glucose (20 mg/ml); 0·1 ml NADP (20 mg/ml); 2 i.u. glucose-6-phosphate dehydrogenase; 2 i.u. hexokinase; 0·1 ml PMS (2 mg/ml); 0·1 ml MTT (4 mg/ml)
Aspartate aminotransferase (EC 2.6.1.1)	*Gel*: TMII; 0·8 mA/cm for 1 h *Stain*: 0·6 ml 0·1 M Tris-HCl, pH 7·4; 0·2 ml L-aspartate (53 mg/ml); 0·1 ml NADH (10 mg/ml in buffer); 0·1 ml α-ketoglutarate (25 mg/ml) neutralized; malate dehydrogenase 8 i.u.

Table 1 (*Contd*)

Enzyme	Running and staining conditions
Glyceraldehyde-phosphate dehydrogenase (EC 1.2.1.12)	*Gel*: TM (8·2); 0·8 mA/cm for 2 h *Stain*: 0·8 ml 0·1 M Tris-HCl, pH 7·4; 20 μl glyceraldehyde 3-phosphate; 0·2 ml NAD (25 mg/ml); 0·1 ml sodium arsenate (25 mg/ml); 0·1 ml sodium pyruvate (75 mg/ml); 0·1 ml PMS (2 mg/ml); 0·1 ml MTT (4 mg/ml)
Glucosephosphate isomerase (EC 5.3.1.9)	*Gel*: TM (8·2); 0·8 mA/cm for 1·5 h *Stain*: 1·2 ml 0·1 M Tris-HCl, pH 8·6; 0·1 ml fructose 6-phosphate (25 mg/ml); 0·1 ml NADP (10 mg/ml); 0·1 ml PMS (2 mg/ml); 0·1 ml MTT (4 mg/ml); glucose-6-phosphate dehydrogenase 2 i.u.
Phosphoglucomutase (EC 2.7.5.1)	*Gel*: TEB; 0·6 mA/cm for 2 h *Stain*: 0·7 ml 0·1 M Tris-HCl, pH 8·0; 0·1 ml glucose 1-phosphate (25 mg/ml); glucose 1,6-diphosphate (0·25 mg added to glucose 1-phosphate stock); 0·1 ml NADP (40 mg/ml); 0·1 ml PMS (2 mg/ml); 0·1 ml MTT (4 mg/ml); 0·1 ml 0·1 M $MgCl_2$; glucose-6-phosphate dehydrogenase 2 i.u.
Superoxide dismutase (EC 1.15.1.1)	*Gel*: TM (8·2); 0·8 mA/cm for 2 h *Stain*: 0·8 ml 0·1 M Tris-HCl, pH 7·4; 0·1 ml PMS (2 mg/ml); 0·1 ml MTT (4 mg/ml); white bands on blue background
Fructose-bisphosphate aldolase (EC 4.1.2.13)	*Gel*: TEM (50 mM Tris, 5 mM EDTA, adjust pH to 7·2 with maleic acid); 0·8 mA/cm for 2 h *Stain*: 1·0 ml 0·1 M Tris-HCl, pH 7·5; 0·3 ml fructose 1,6-diphosphate (25 mg/ml); 0·2 ml NAD (25 mg/ml); triosephosphate isomerase 2 i.u.; 0·1 ml sodium arsenate (25 mg/ml); glyceraldehyde-3-phosphate dehydrogenase 2 i.u.; 0·1 ml 0·1 M $MgCl_2$; 0·1 ml PMS (2 mg/ml); 0·1 ml MTT (4 mg/ml;

Results

A total of 26 loci were studied. The number of loci, and where relevant, the number of alleles present for each protein surveyed are given in Table 2. The phenotypes and the genetic interpretation of each polymorphism are shown in Fig. 1 as they appear following Cellogel electrophoresis and staining.

A series of carboxylesterase polymorphisms are found in rabbit blood and liver (Schiff and Stormont 1970). The red cell esterase-1 locus with two alleles (*A* and *B*) in laboratory rabbits was studied in this program. The pattern of the esterase-1 polymorphism on Cellogel electrophoresis differed from that found following starch-gel electrophoresis (Schiff and Stormont 1970) in that a second, slower, set of bands is also found. These bands appear to be monomers of the usual esterase-1 pattern, i.e. bands may occur in a fast position, a slow position, or both, and this pattern correlates exactly with the *A*, *B* or heterozygote esterase-1 phenotype of the sample run (Daly 1979). A third, null, phenotype has been found in Australian rabbits (Daly 1979). This allele is expressed as a null phenotype in red cells but as a *B* phenotype in liver. The red cell esterase-1 polymorphism is linked to a second esterase polymorphism (esterase-2). Red cell esterase-2 has a complex mode of inheritance and is related to serum esterase (van Zutphen 1974). A third polymorphic red cell

esterase system (esterase-3) has also been described in rabbits. Variation in esterase systems 2 and 3 is present in wild rabbits but is not included here. The null allele, which can be detected only in red cells, was found at Urana and Grassy Creek at frequencies of 0·144 and 0·170, respectively; however, it was not found at Snowy Plains where a large number of blood samples was typed. Its distribution elsewhere is therefore unknown, as liver samples were examined at most locations.

Table 2. Number of loci and alleles found for each enzyme studied

Enzyme	No. of loci	No. of alleles	Designation
Phosphogluconate dehydrogenase	1	2	Pgd^1, Pgd^2
Adenosine deaminase	1	3	Ada^1, Ada^2, Ada^3
Carbonate dehydratase (CAII)	1	2	CA^F, CA^S
Carboxylesterase	1	3	$Est\text{-}1^A$, $Est\text{-}1^B$, $Est\text{-}1^N$
Dihydrolipoamide reductase (diaphorase)	1	2	Dia^F, Dia^S
Glycerol-3-phosphate dehydrogenase	1	1	
Alcohol dehydrogenase	1	1	
Glutamate dehydrogenase	1	1	
Isocitrate dehydrogenase	1	1	
Lactate dehydrogenase	1	1	
Malate dehydrogenase	2	1 each	
Aspartate aminotransferase	2	1 each	
Glyceraldehydephosphate dehydrogenase	1	1	
Glucosephosphate isomerase	1	1	
Phosphoglucomutase	2	1 each	
Superoxide dismutase	1	1	
Fumarate hydratase	1	1	
Ornithine carbamoyltransferase	1	1	
Purine-nucleoside phosphorylase	1	1	
Adenylate kinase	2	1 each	
Aldolase	2	1 each	

The esterase gene frequencies (Table 3) differed significantly from location to location (Table 4) but there was no clear pattern in this variation. Smith's H is a statistic that measures divergence from Hardy–Weinberg equilibrium. It is an unbiased estimate of half the difference between the expected and observed proportion of

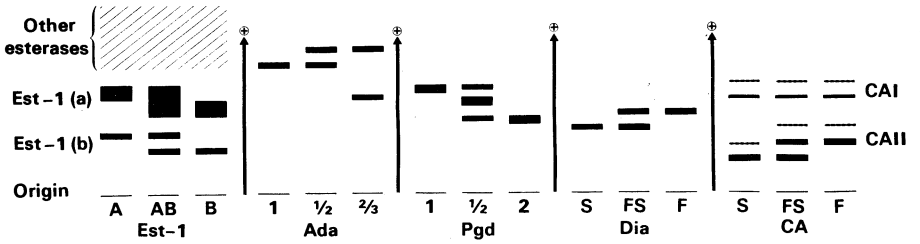

Fig. 1. Diagrammatic representation showing the electrophoretic patterns observed and the nomenclature used for the five polymorphic loci studied. Only three of the six adenosine deaminase patterns observed are shown.

heterozygotes. Consequently it is zero at equilibrium, positive when there are too many homozygotes and negative when there are too many heterozygotes. H values (Table 3) showed that the populations were out of equilibrium at three localities in

Table 3. Gene frequencies (*P*), Smith's *H* values and sample sizes (*n*) for adenosine deaminase (*Ada*), phosphogluconate dehydrogenase (*Pgd*) and esterase (*Est-1*) loci from each of the geographical locations

Numbers before locations identify populations in Fig. 2

Geographical location	*Ada* locus					*Pgd* locus			*Est-1* locus		
	P_1	P_3	H_1	H_3	2*n*	*P*	*H*	2*n*	*P*	*H*	2*n*
South-eastern Australia											
1 Werribee	0·56	0·02	0·065	—	48	0	—	180	0·77	0·002	44
2 Bemboka	0·63	0·02	−0·001	—	46	0	—	46	—	—	—
3 Mogo	0·55	0·02	0·002	—	60	0	—	58	—	—	—
4 Canberra	0·62	0·05	−0·010	0·006	240	0·04	0·004	394	0·50	−0·005	158
5 Cooma	0·69	0·05	−0·013	−0·002	74	0·19	0·054	134	—	—	—
6 Snowy Plains	0·61	0·02	0·011	0	756	0·03	0·002	740	0·53	0·037[A]	822
7 Grassy Creek	0·67	0·04	−0·012	0·002	382	0·02	0	396	0·69[B]	0·080[A]	366
8 Batlow	0·49	0·09	−0·01	−0·007	160	0·09	0·004	162	0·56	−0·018	162
9 Adelong	0·55	0·14	−0·020	0·002	294	0·08	0	296	0·57	0·029	296
10 Urana	0·54	0·03	0·001	0·008[A]	730	0·07	0·006	788	0·55[B]	0·006	788
Western Australia											
11 Cape Naturaliste	0·52	0·09	0·023[A]	0·007[A]	1066	0·03	0·007	214	0·64	0·007	422
12 Chidlow	0·66	0·11	0·023[A]	0·010[A]	870	0·04	0·001	148	0·49	−0·023	422
13 Belton	0·79	0·07	−0·007	0·004	56	0·14	0·014	64	0·66	0·042	64
14 Quobba	0·65	0	0·003	—	190	0	—	190	0·65	0·059	122
North-eastern Australia											
15 Charleville	0·83	0·03	0·042	0·033[A]	60	0	—	74	—	—	—
16 Mitchell	0·55	0·06	0·007	0·021[A]	86	0·08	0·041	86	0·27	0·048	86
17 Injune	0·74	0·02	−0·017	—	42	0·04	−0·001	54	—	—	—
18 Tambo	0·53	0·06	−0·007	−0·002	36	0·03	0	36	0·47	0·064	36
Tasmania											
19 Deloraine	0·67	0·20	0·022	0·020	438	0·01	—	438	0·56	0·044[A]	434
20 Bogan Gap	0·59	0·11	0·024	0·027	54	0	—	54	0·41	0·024	54
21 Oatlands	0·94	0	−0·003	—	78	0·14	0·033	78	0·40	−0·001	78
22 Sorell	0·87	0	−0·015	—	46	0·17	0·028	46	0·30	−0·099	46
Britain											
23 Norwich (East Anglia)	0·40	0·21	0·030	0·028	84	0	—	96	0·60	−0·030	96
24 Fishguard (Wales)	0·45	0·19	−0·060	−0·034	48	0	—	58	0·69	0·011	58
France											
25 Camargue	0·75	0·01	0·007	—	338	0·01	—	338	0·69	0·108[A]	168

[A] Denotes sample out of Hardy–Weinberg equilibrium. [B] Denotes the presence of a third null allele in these areas.

Australia having, in each case, too many homozygotes. At Grassy Creek this was due, at least in part, to the presence of the null allele which causes null heterozygotes to be typed as apparent *A* or *B* homozygotes, thus artificially inflating these classes. At Snowy Plains and at Deloraine the reduction in the number of heterozygotes was probably due to a Wahlund effect because of structuring within the populations sampled. At Snowy Plains, for example, the gene frequency ranged from 0·3 to 0·8 in different subpopulations.

The adenosine deaminase polymorphism is most conveniently explained as a system of three alleles at one locus (Fig. 1; Coggan *et al.* 1974*b*). Alleles *1* and *2* are also found in laboratory rabbits. The gene frequencies for adenosine deaminase also vary between locations (Tables 3 and 4; Fig. 2). The British rabbits had the lowest frequency of allele *1* but otherwise no pattern was apparent. Two populations

Table 4. Chi-squared values and degrees of freedom (in brackets) for homogeneity tests of the set of data specified

Mainland includes all the available locations on mainland Australia while eastern mainland includes the north-eastern and south-eastern Australian locations. *** $P < 0{\cdot}001$. ** $P < 0{\cdot}01$

Comparison	Adenosine deaminase	Phosphogluconate dehydrogenase	Carboxyl-esterase	Carbonate dehydratase	Dia-phorase
All locations	557(48)***	250(24)***	149(19)***	1032(16)***	36(6)***
Within Tasmania	48(6)***	63(3)***	19(3)**	7(3)	—
England, Wales and France	91(4)***	—	2(2)	107(2)***	—
Mainland, England and Wales	307(38)***	169(19)***	111(14)***	124(11)***	—
Mainland	263(34)***	157(17)***	107(12)***	104(9)***	6(5)
Eastern mainland	137(26)***	127(12)***	82(7)***	40(6)***	—

were out of Hardy–Weinberg equilibrium (Table 3) and again a Wahlund effect is the likeliest explanation as subpopulations at each location differ in gene frequency.

The phosphogluconate dehydrogenase polymorphism (Coggan *et al.* 1974*a*), is the product of two alleles (Fig. 1). Allele *2* was not found in any of the British samples (Table 3). It is present in France and Australia, however, and so presumably it is also present in Britain. It was missing from the eastern Australian coastal samples (Table 3).

Bernoco (1969) described a two-allele polymorphism for carbonate dehydratase in laboratory rabbits analogous to the polymorphism found in wild rabbits in this study. The polymorphism is in the slower migrating form of the two carbonate dehydratase loci (Fig. 1) and this form (see Hopkinson *et al.* 1974) has esterase characteristics similar to that of CAII. The carbonate dehydratase gene frequency of the French rabblts (Table 5) was quite different to that found elsewhere. British and Tasmanian populations had similar gene frequencies but differed from the other Australian mainland populations (Table 5). However, the various mainland populations differed significantly in gene frequency from each other. One population (Sorell, Tas.) was out of Hardy–Weinberg equilibrium in the direction of excess

heterozygotes. The significance of this is uncertain, though, given the number of tests made, it can probably be ascribed to chance.

The dihydrolipoamide reductase (NAD^+) polymorphism, postulated as a two-allele system (Fig. 1), is not found in liver cells and consequently data from fewer locations are only available (Table 5). The French population was fixed for the slow allele, though the presence of what is presumably the same polymorphism in 'petite russe' rabbits (Vergnes *et al.* 1974) implies that the polymorphism is present in European rabbits. The gene frequencies of the Australian populations did not differ significantly (Table 5). However, fewer animals were typed for this locus. The average heterozygosity in Australian rabbits over the 26 loci studied was 5·9%.

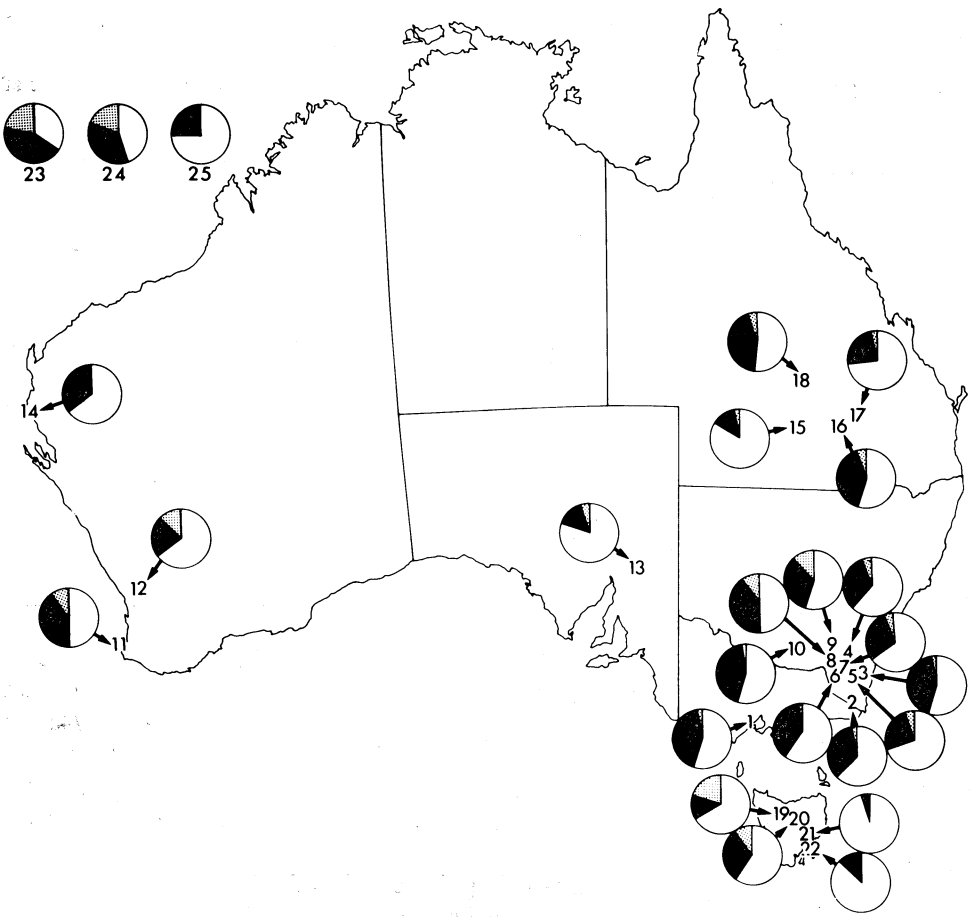

Fig. 2. Geographical distribution of adenosine deaminase gene frequencies. The numbers refer to the locations sampled and are given in Table 3. o Ada-1. • Ada-2. Stipple, Ada-3.

Discussion

The level of interpopulation variation in gene frequency found in mainland populations is similar, and high, in four of the five polymorphic proteins studied (Table 4). Yet all populations are presumed to derive from a single relatively recent source. The genetic data can be used to examine this presumption. The variability

in gene frequency between populations is reflected in the genetic distances (Rogers 1972) found between local populations which range (using four loci) from 0·03 to

Table 5. Gene frequencies (*P*), Smith's *H* values and sample sizes (*n*) for each geographical location for carbonate dehydratase and diaphorase loci

Numbers before locations identify populations in Fig. 2

Geographical location	Carbonate dehydratase locus			Diaphorase locus		
	P	*H*	2*n*	*P*	*H*	2*n*
South-eastern Australia						
6 Snowy Plains	0·10	0·012	1158			
7 Grassy Creek	0·05	−0·002	386	0·75	0·033	130
8 Batlow	0·06	0·010	162			
9 Adelong	0·04	−0·002	296			
10 Urana	0·11	0·008	1200	0·76	−0·038	108
Western Australia						
11 Cape Naturaliste	0	—	422	0·71	2·240	112
12 Chidlow	0	—	156			
14 Quobba	0·03	−0·001	100	0·81	−0·033	86
North-eastern Australia						
16 Mitchell	0·04	−0·001	46	0·72	0·067	58
17 Tambo	0·03	—	36	0·86	−0·016	36
Tasmania						
19 Deloraine	0·26	−0·009	436			
20 Bogan Gap	0·15	0·018	54			
21 Oatlands	0·30	−0·033	78			
22 Sorell	0·39	−0·107[A]	46			
Britain						
23 Norwich	0·13	−0·015	96			
24 Fishguard	0·22	−0·013	58			
France						
25 Camargue	0·66	−0·030	338	1·00	—	102

[A] Denotes sample out of Hardy–Weinberg equilibrium.

Table 6. Genetic distance between 15 populations calculated from four variable loci

Rogers' genetic distance is given (Rogers 1972): 1, Snowy Plains; 2, Grassy Creek; 3, Batlow; 4, Adelong; 5, Urana; 6, Cape Naturaliste; 7, Chidlow; 8, Quobba; 9, Mitchell; 10, Tambo; 11, north Tasmania; 12, south Tasmania; 13, England; 14, Wales; 15, France

Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	—	0·07	0·06	0·06	0·04	0·07	0·07	0·06	0·11	0·05	0·10	0·20	0·08	0·12	0·22
2		—	0·09	0·07	0·10	0·06	0·09	0·04	0·15	0·10	0·13	0·24	0·10	0·10	0·18
3			—	0·03	0·04	0·06	0·09	0·08	0·09	0·05	0·13	0·23	0·08	0·12	0·26
4				—	0·05	0·06	0·07	0·07	0·10	0·07	0·11	0·22	0·08	0·12	0·25
5					—	0·07	0·09	0·09	0·10	0·06	0·11	0·21	0·08	0·12	0·24
6						—	0·08	0·05	0·12	0·06	0·14	0·27	0·08	0·10	0·24
7							—	0·09	0·11	0·06	0·11	0·20	0·13	0·16	0·25
8								—	0·14	0·08	0·13	0·25	0·09	0·11	0·20
9									—	0·07	0·19	0·20	0·16	0·20	0·32
10										—	0·14	0·22	0·10	0·14	0·27
11											—	0·16	0·11	0·10	0·18
12												—	0·26	0·25	0·24
13													—	0·06	0·23
14														—	0·18
15															—

0·09 (Table 6; Fig. 3). The distances between the two British populations and between the various Australian mainland populations are of this order.

The genetic relationships of the populations summarized in Fig. 3 show that the French population differs markedly from the stocks present in Britain and therefore, from the Australian stocks derived from them. The origin of Camargue rabbits is unknown. Presumably they were introduced to the area in Roman times though they might be indigenous (Rogers 1979).

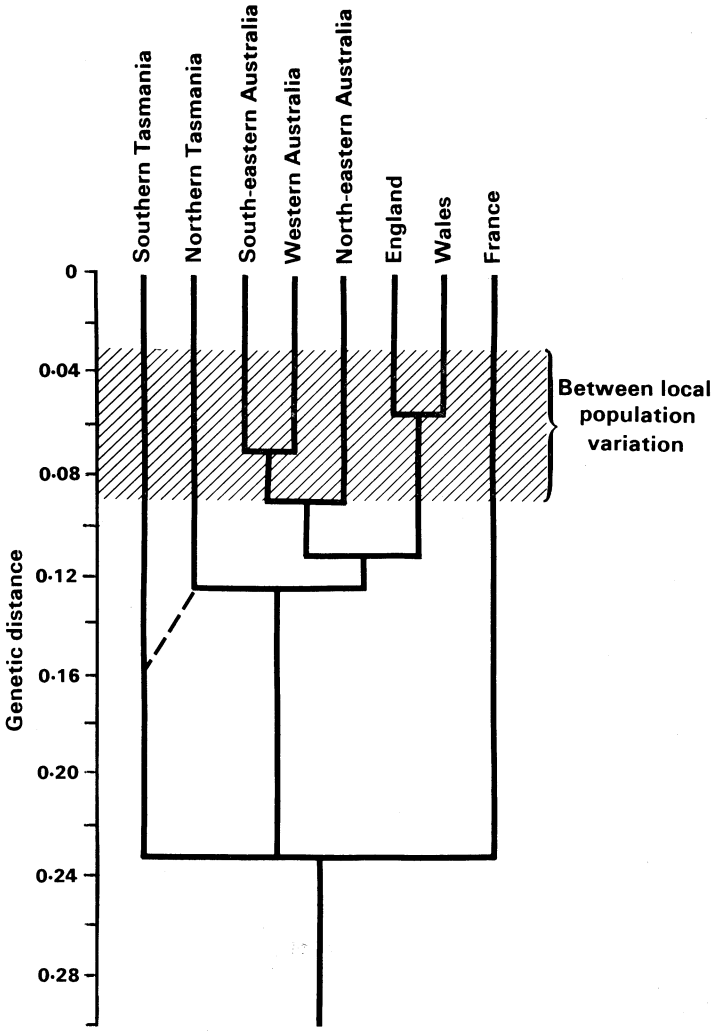

Fig. 3. Genetic relationships of the populations as deduced from the Roger's genetic distances between populations in Table 6. The dashed line shows the genetic distance separating southern and northern Tasmania.

The English and Welsh rabbits are similar to the Australian mainland rabbits, emphasizing the close relationship between all these groups. Clearly the bottleneck in the size of the gene pool which occurred when the rabbit was introduced into Australia has not lead to any great lasting change in the gene frequencies at the loci studied. The Tasmanian data shows that the two southern populations from Oatlands and Sorell differ significantly from the northern Tasmanian populations. In fact

the southern Tasmanian populations differ markedly from all other populations studied, being equidistant from both the French and British stocks. The northern Tasmanian populations show marked similarities with the Australian mainland and British populations.

Our results thus indicate that there were at least two releases of rabbits in Tasmania, one in the south and one in the north, and that they have not reached a common equilibrium. The rabbits introduced into Tasmania were apparently derived from semidomesticated hutch rabbits (Rolls 1969). Both releases may have been in the early days of white settlement but it is also possible that the populations in northern Tasmania include a component derived later from mainland sources. Though the northern Tasmanian rabbits are similar to the mainland rabbits they do differ significantly from them, and are more closely related to the southern Tasmanian population (genetic distance 0·16) than are the mainland populations (genetic distance 0·23). The fact that these populations are significantly different implies that effective migration is very limited in rabbit populations. The lack of any evidence of systematic area differences in gene frequency on the mainland makes it unlikely that the high levels of variation found there have arisen through multiple separate releases.

The significant differences found in gene frequencies between mainland populations are the result of the net effects of drift, migration, selection and founder effects. While the roles of these factors cannot be determined with any accuracy, it is possible to examine their significance in a little more detail. For example, there is no relationship between the genetic distances and the geographical distances separating populations in Australia. This supports the idea that migration has not played a significant role in reducing the level of between-population variation, at least on this scale of geographical distances.

Rabbits spread throughout Australia by a series of colonizing events and consequently, because of founder effects, rabbit populations further from Geelong should have gone through more bottlenecks in the size of the gene pool than those closer to Geelong. However, when the genetic distances of each population from the British populations are plotted against geographical distance from Geelong there is no increase in genetic distance with increasing geographical distance. Clearly if such an effect is present it is masked by variation due to other factors.

The relative roles of selection and drift can be examined because the dates of divergence of the various populations can be estimated. Cavalli-Sforza and Bodmer (1971) state that the genetic distance between populations determined by angular transformation is proportional to f, the kinship coefficient. Moreover $-\log(1-f)$ is proportional to time under situations of drift while $f^{\frac{1}{2}}$ is proportional to time for differentiation due to local differences in selection intensity. In fact there is no apparent increase in genetic distance between populations separated for different periods of time so neither explanation can be simply tested. There seems to be an increase in variance in gene frequency with time but this could be an artefact of the distribution of the data, since the number of comparisons increases progressively with more populations separated for longer periods.

The simplest explanation of the interpopulation variation is, then, drift due to the small effective size of local populations. The reduced effective population size may well be due to the severe reductions in local population size that occur at irregular intervals due, for example, to drought, myxomatosis or pest-control

programs. Founder effects and migration seem to have played little effective part in producing the present distribution of gene frequencies.

Taylor *et al.* (1977) calculated the Mahalanobis generalized distance between rabbit populations in Australia, using a series of metrical characters and it might be predicted that these Mahalanobis distances and the Rogers genetic distances determined during this study would be correlated. We have already seen that there is no geographical component either in the between-population comparison or in the distance from Geelong comparison. Taylor *et al.* (1977) argue that there is a correlation between geographical distance from Geelong and biological distance, which would be at variance with the biochemical data; however, this result depends on the interpretation by Taylor *et al.* (1977) of their data from Tero Creek in north-western New South Wales, and ignores the data from Western Australia. An alternative explanation of their data would suggest that the populations studied, including those from Western Australia, were about equally divergent. The only exception to this pattern would be the animals from Tero Creek which have diverged quite markedly from all other populations studied. This could be an effect of drift, though selection in this extreme desert environment might also be responsible. Such an interpretation would bring the morphological data into line with the biochemical data with both sets of data showing much between-population divergence but no relationship between distance and divergence. Small effective population size leading to drift would again be the simplest explanation of the data.

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