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Biochemical variation in purebred and crossbred strains of domestic rabbits (*Oryctolagus cuniculus* L.)

G. B. HARTL[1] AND H. HÖGER[2]

[1] *Forschungsinstitut für Wildtierkunde der Veterinärmedizinischen Universität Wien, A-1160 Vienna, Savoyenstrasse 1, Austria*
[2] *Versuchstierzucht und -haltung der Universität Wien, A-2325 Himberg, Brauhausgasse 34, Austria*

Summary

Genetic variation in 40 domestic rabbits (*Oryctolagus cuniculus*) from eight different strains was investigated by horizontal starch gel electrophoresis. Twenty nine enzyme systems were examined in different tissues, 10 isoenzymes were found to be polymorphic. Indices of genetic variation show values comparable to those found in most other mammalian species. Thus the unusually high values reported previously by other authors may be due to a limited and not randomly chosen set of enzymes studied.

1. Introduction

Besides mice and rats, in many respects rabbits are well-studied laboratory animals. However, in contrast to the former species little information on their biochemical genetics is available. Extensive studies have been carried out only on esterases (Schiff & Stormont, 1970; Van Zutphen, 1974*a*, *b*; Van Zutphen & den Bieman, 1975; Van Zutphen, den Bieman & Bouw, 1977; Van Zutphen, Fox & den Bieman, 1983; Fox & Van Zutphen, 1977; Bellen *et al.* 1984) and some serum proteins (Juneja, Van de Weghe & Gahne 1981, 1984). From a study of 12 proteins Skow, Fox & Womack (1978) estimated the proportion of polymorphic loci in JAX strains of domestic rabbits to be about 33%, an unusually high value for mammals (Hartl, 1985; Baccus *et al.* 1983). Skow *et al.* (1978) suggest that their data may not give a representative estimate of genetic variation in rabbits for several reasons. One of them is the limited set of enzymes studied. Furthermore, these enzymes were chosen because they revealed polymorphism in other mammalian species. However, for the estimation of genetic variability in populations, ideally a large and randomly chosen sample of the genome should be studied (Gorman & Renzi, 1979; Nei & Roychoudhury, 1974; Hartl, 1985).

To get a more comprehensive picture of biochemical variation in the rabbit in this study we investigated 29 enzyme systems. The results show a considerable amount of polymorphism, but not significantly higher than in other mammalian species (see Nevo, 1983; Baccus *et al.* 1983 for review).

2. Materials and Methods

Three specimens were tested from each of the purebred strains: New Zealand White, Silver Yellow, English Piebalds and Schwarzloh. To increase the chance of finding genetic variation, individuals from different crossbred strains were screened additionally. Eleven animals were hybrids between New Zealand White and Russians, all of unknown F- generation, obtained from a commercial breeder (from two does and one buck, where F1 animals were available, twelve offspring were investigated additionally to test the genetic basis of the enzyme variants). The other five were obtained from a fancy breeder as offsprings from does of unknown ancestry. The strain of the bucks were English Piebalds, Marburger Feh and Schwarzloh. All specimens were adult. Samples of liver, kidney, muscle, brain, heart and lung were taken from freshly killed animals and frozen immediately at −30 °C. Homogenates were prepared with an Ultra Turrax TP 18/10 homogenizer using a 0·01 M phosphate buffer (pH 7·4) containing 0·1 mM-$MgCl_2$. After freezing and thawing the homogenates were centrifuged for 20 min at 4 °C. The clear supernatant was absorbed to filter pads and inserted into gel slots. Horizontal starch gel electrophoresis was performed using the following buffer systems. A continuous phosphate buffer (pH 7·4, buffer system I) and a continuous tris-maleate buffer (pH 7·7, buffer system II), both described in Csaikl, Engel & Schmidtke (1980) and a continuous tris-citrate buffer (pH 8·0, buffer system III) described in Manlove *et al.* (1975).

Buffer system I was used for screening lactate de-

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Table 1. *List of polymorphic enzymes in different strains of rabbits*

E.C. number	Enzyme	Loci	Alleles found NZ p	NZ c	SY p	EP p	EP c	MF c	SL p	SL c
1.1.1.1	Alcohol dehydrogenase (ADH)	1 (L)	*a*	*a*	*a*	*a*	*a*	*a*	*a*	*a*
			b	*b*						
1.1.1.40	Malic enzyme (MOD)	1 (K)	*a*	*a*	*a*		*a*	*a*	*a*	*a*
			b	*b*		*b*	*b*			
		2 (K)	*a*	*a*	*a*		*a*	*a*	*a*	*a*
			b	*b*		*b*	*b*			
1.1.1.47	Glucose dehydrogenase (GDH)	1 (L)	*a*	*a*		*a*	*a*	*a*	*a*	*a*
			b	*b*	*b*	*b*	*b*			
		2 (L)	*a*	*a*	*a*	*a*	*a*	*a*	*a*	*a*
1.1.1.49	Glucose-6-phosphate dehydrogenase (GPD)	1 (K)		*a*	*a*					
			a	*b*	*b*	*b*	*b*	*b*	*b*	*b*
3.1.1.1	Esterases (ES)	I (K)	*a*	*a*	*a*	*a*	*a*	*a*	*a*	*a*
			b	*b*	*b*		*b*	*b*		
		II (K)	*a*	*a*	*a*	*a*	*a*	*a*	*a*	*a*
			b	*b*		*b*	*b*	*b*	*b*	
		III (K)	*a*	*a*	*a*	*a*	*a*	*a*	*a*	*a*
		IV (K)	*a*	*a*	*a*	*a*	*a*	*a*	*a*	*a*
		V (K)				– Unscorable –				
		VI (K)	*a*	*a*	*a*	*a*	*a*	*a*	*a*	*a*
		VII (K)	*a*	*a*	*a*	*a*	*a*	*a*	*a*	*a*
				b						
3.4.11	Peptidases (PEP)	1 (K)	*a*	*a*	*a*	*a*	*a*	*a*	*a*	*a*
		2 (K)	*a*	*a*	*a*	*a*	*a*	*a*	*a*	*a*
			(*b*)							
5.3.1.8	Mannosephosphate isomerase (MPI)	1 (K)	*a*	*a*	*a*	*a*	*a*	*a*	*a*	*a*
				b	*b*				*b*	*b*
			c	*c*		*c*	*c*			

p = purebred, c = crossbred, NZ = New Zealand White, SY = Silver Yellow, EP = English Piebalds, MF = Marburger Feh, SL = Schwarzloh. Besides locus number tissue used for routine screening indicated in parentheses (L = liver, K = kidney).

hydrogenase (LDH, E.C. 1.1.1.27), 6-phosphogluconate dehydrogenase (PGD, E.C. 1.1.1.44), glucose-6-phosphate dehydrogenase (GPD, E.C. 1.1.1.49), superoxide dismutase (SOD, E.C. 1.15.1.1), phosphoglucomutase (PGM, E.C. 2.7.5.1), esterases (ES, E.C. 3.1.1.1), peptidases (PEP, E.C. 3.4.11), mannosephosphate isomerase (MPI, E.C. 5.3.1.8) and glucosephosphate isomerase (GPI, E.C. 5.3.1.9); buffer system II for alcohol dehydrogenase (ADH, E.C. 1.1.1.1), α-glycerophosphate dehydrogenase (GDC, E.C. 1.1.1.8), sorbitol dehydrogenase (SDH, E.C. 1.1.1.14), malate dehydrogenase (MOR, E.C. 1.1.1.37), malic enzyme (MOD, E.C. 1.1.1.40) and aldolase (ALDO, 4.1.2.13); buffer system III for isocitrate dehydrogenase (IDH, E.C. 1.1.1.42), glucose dehydrogenase (GDH, E.C. 1.1.1.47), xanthine dehydrogenase (XDH, E.C. 1.2.3.2), glutamate dehydrogenase (GLUD, E.C. 1.4.1.3), catalase (CE, E.C. 1.11.1.6), glutamate oxaloacetate transaminase (GOT, E.C. 2.6.1.1), hexokinase (HK, E.C. 2.7.1.1), creatine kinase (CK, E.C. 2.7.3.2), adenylate kinase (AK, E.C. 2.7.4.3), acid phosphatases (ACP, E.C. 3.1.3.2), aminoacylase-1 (ACY-1, E.C. 3.5.1.5), adenosine deaminase (ADA, E.C. 3.5.4.4), fumarate hydratase (FH, E.C. 4.2.1.2) and aconitase (ACO, E.C. 4.2.1.3).

After electrophoresis the gels were sliced and stained for: ADH, GDC, SDH, LDH, MOR, MOD, IDH, PGD, GPD, SOD, GOT, PGM, ADA, GPI, ACP, ES, HK and CK according to Shaw & Prasad (1970); GDH and ACO according to Harris & Hopkinson (1976); GLUD and CE according to Brewer & Sing (1970); XDH according to Selander *et al.* (1971); AK, MPI, ALDO and FH according to Siciliano & Shaw (1976); PEP according to Siciliano & Shaw (1976) using glycyl-L-leucine as substrate; ACY-1 according to Qavi & Kit (1980).

The genetic basis of biochemical variants was tested by family studies in crossbred New Zealand Whites. Where this was impossible (all parents homozygous for the same allele), the genetic interpretation of the electrophoretic patterns was based on the principles described by Harris (1980) and Harris & Hopkinson (1976). Furthermore, the published enzyme structure scheduled by Klotz (1967), Darnall & Klotz (1975)

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and Ward (1977) was used for the interpretation of heterozygote banding patterns. The result was compared to homozygotes and heterozygotes of inbred strains of *M. musculus*. Loci and allele designations are according to Lyon (1977) and Staats (1980).

3. Results and Discussion

Screening of 29 enzyme systems revealed polymorphism in 10 isoenzymes: alcohol dehydrogenase (ADH), malic enzyme -1 and -2 (MOD-1, MOD-2), glucose dehydrogenase -1 (GDH-1), glucose-6-phosphate dehydrogenase (GPD), esterase zone -I, -II and VII (ES-Zone I, ES-Zone II, ES-Zone VII), peptidase -2 (PEP-2) and mannosephosphate isomerase (MPI). The alleles found at the corresponding enzyme loci in the different strains are listed in Table 1.

The genetic basis of biochemical variants in ADH, MOD, ES-Zone I and MPI was proven by family studies in crossbred New Zealand Whites. In GDH, GPD and ES Zone II and VII the parents were homozygous for the same allele and so were their F_1 offsprings. Alcohol dehydrogenase exhibits one cathodally migrating band, representing the gene product of one genetic locus. Both homozygotes and the corresponding heterozygous phenotype could be scored in the various strains (Fig. 1). In malic enzyme two anodally migrating fractions, MOD-1 and MOD-2, could be identified, representing the gene products of two different loci. Polymorphism in both isoenzymes in rabbits was detected previously by Skow *et al.* (1978). In our material in each MOD-1 and MOD-2 three phenotypes were found (Fig. 2*a*, *b*). Since this enzyme is assumed to be tetrameric in most species (Harris & Hopkinson, 1976; Darnall & Klotz, 1976) we interpreted the heterozygous phenotypes to consist of five distinct bands, although they could not be clearly resolved on the gels. A five-banded pattern in rabbits is also suggested by Skow *et al.* (1978). On gels stained for glucose dehydrogenase two anodally migrating isoenzymes GDH-1 and GDH-2 were detected and tentatively interpreted as the molecular products of two genetic loci. In our material the locus *Gdh-1* was found to be polymorphic for a second allele (Fig. 3). In glucose-6-phosphate dehydrogenase two different phenotypes were scored. This locus was reported to be monomorphic in rabbits by Skow *et al.* (1978). Since *Gpd* is X-linked (Soulié & de Grouchy, 1983) the gene products of two alleles can be found only in heterozygous females, which did not appear within our samples. As expected, the interpretation of the banding patterns of tissue esterases was rather complicated. In a first approach we scored at least 7 different fractions provisionally called ES-I–ES-VII (Fig. 4). Each fraction is assumed to be the molecular product of one genetic locus with the exception of fraction V, which consists of several bands, not consistently scorable and probably encoded by more than one genetic locus. Polymorphism was detected in ES-Zone I and ES-Zone II. In these isoenzymes heterozygotes for the same rare allele were found in some of the strains.

The cathodally migrating isoenzyme (ES-Zone I) may be identical with Est-5 described by Van Zutphen *et al.* (1983), where again a polymorphism was detected in rabbits. In ES-Zone VII presumed two-banded heterozygote phenotypes were found exclusively in crossbred New Zealand Whites. In peptidase two anodally migrating isoenzymes (PEP-1, PEP-2) were stained, and interpreted as the gene products of two loci. In the more anodal fraction one presumed two-banded heterozygote phenotype was found. Mannosephosphate isomerase exhibits one fast anodally migrating band showing several one- and two-banded phenotypes (Fig. 6). Since this enzyme is monomeric within our material the *Mpi* locus is polymorphic for three alleles. These results are in agreement with the findings of Skow *et al.* (1978), who found three alleles in several JAX strains of domestic rabbits.

The following loci were monomorphic in all strains (L = liver, K = kidney, H = heart, M = muscle, B = brain): *Gdc* (L), *Sdh* (L), *Ldh-1* (K), *Ldh-2* (K), *Mor-1* (K), *Mor-2* (K), *Idh-1* (K), *Idh-2* (K), *Pgd* (K),

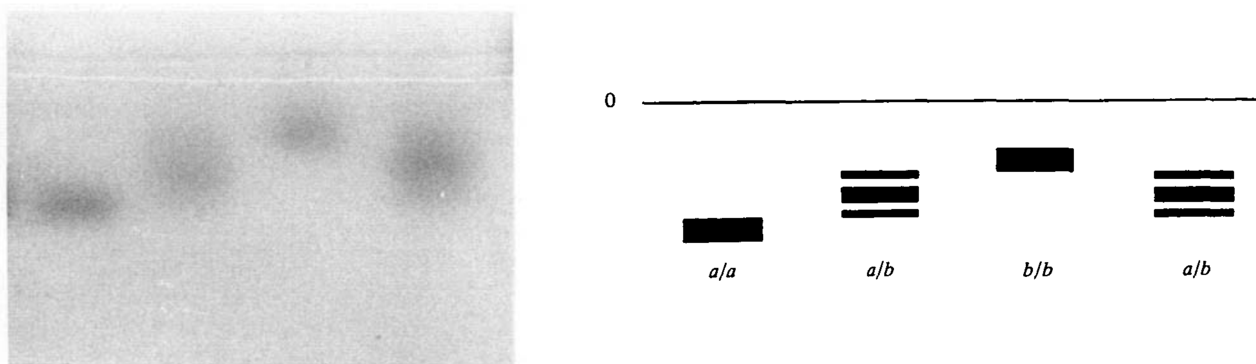

Fig. 1. ADH

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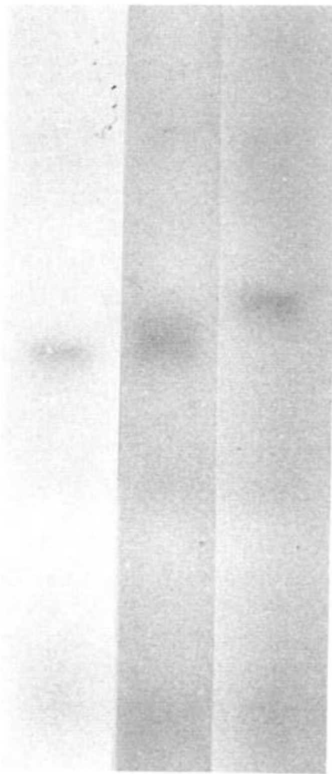


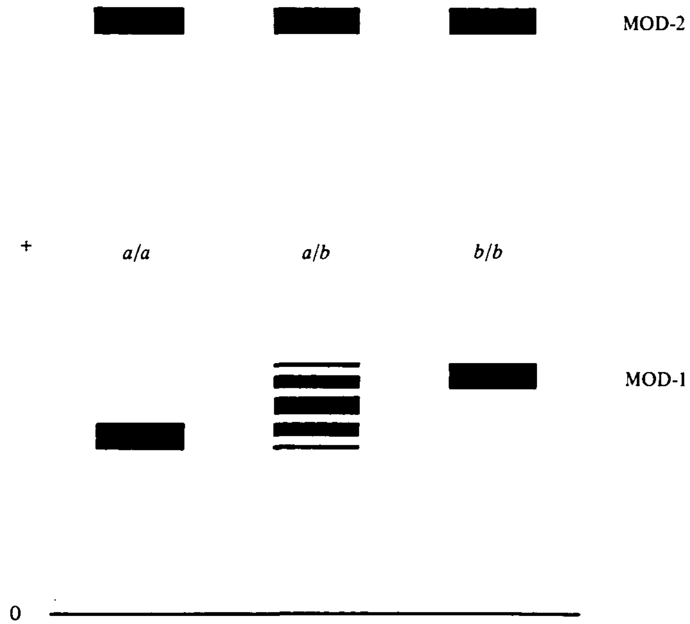
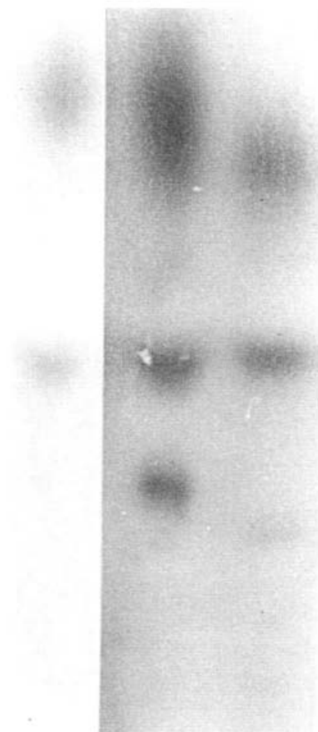


Fig. 2a. MOD-1.



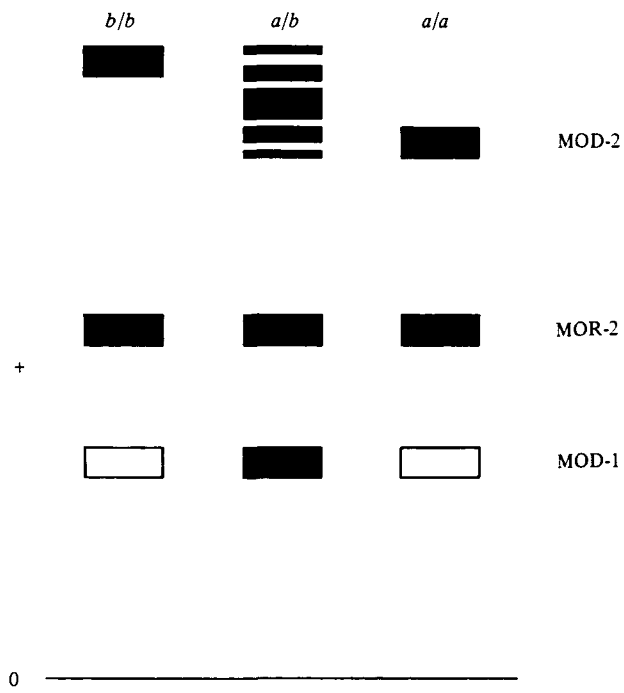


Fig. 2b. MOD-2.

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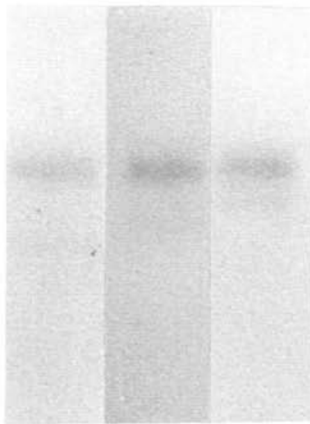


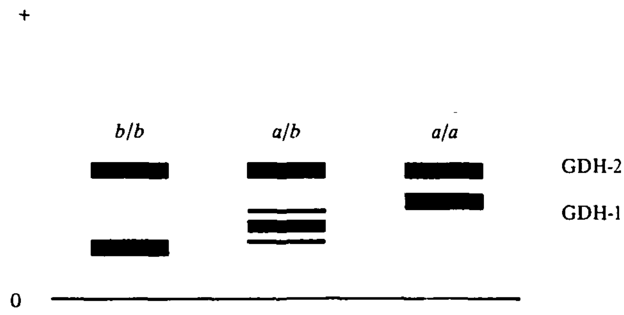
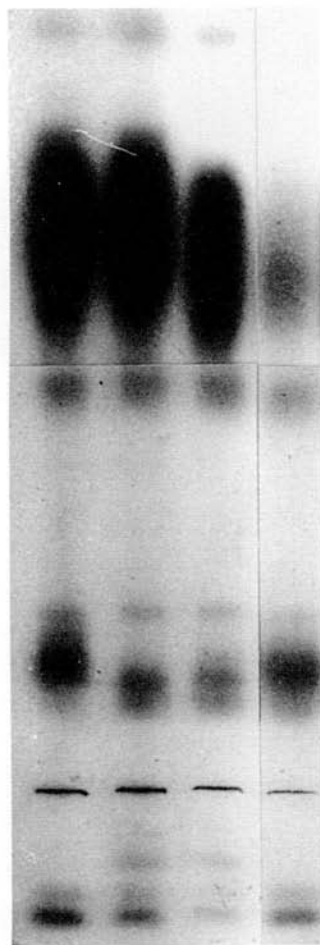


Fig. 3. GDH.



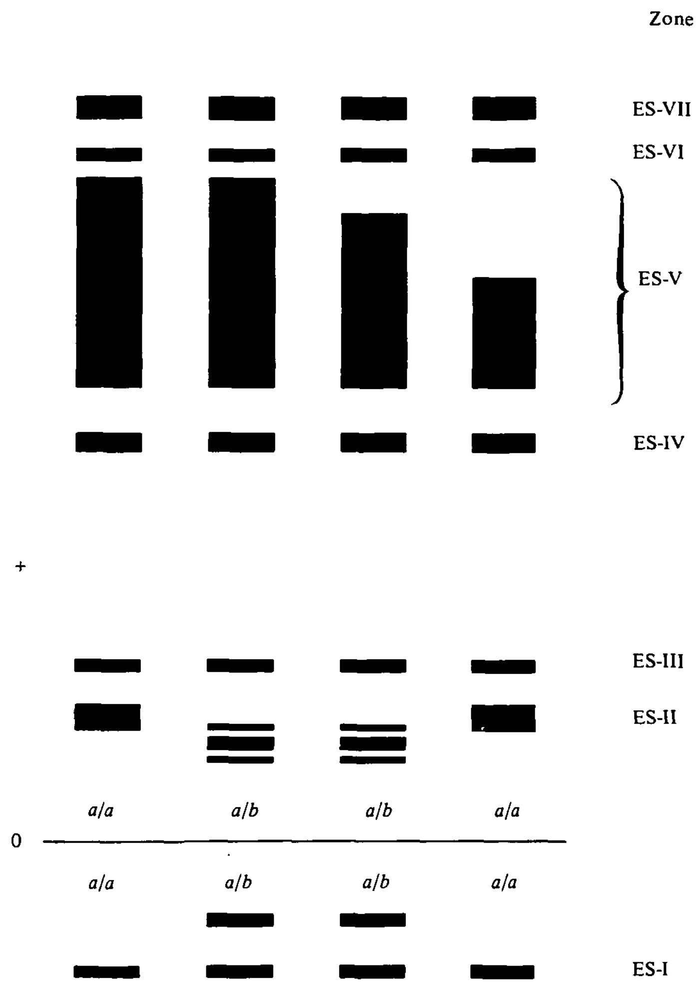
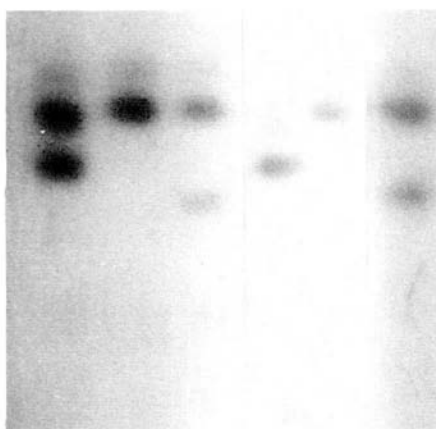


Fig. 4. ES.



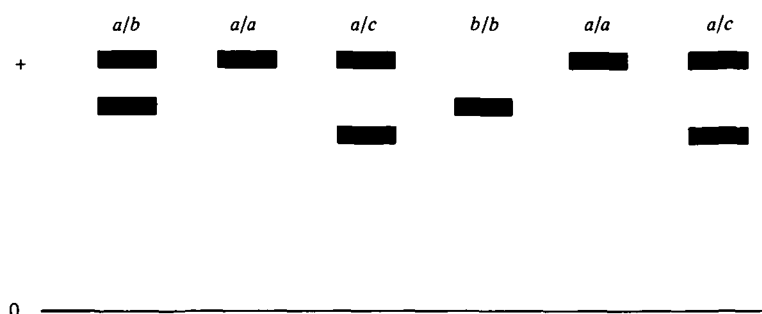


Fig. 5. MPI.

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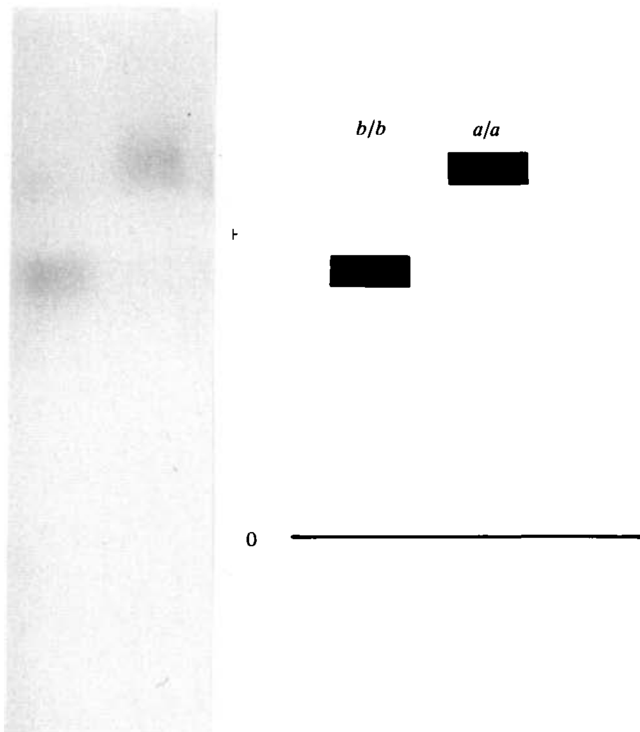

Fig. 6. GPD.

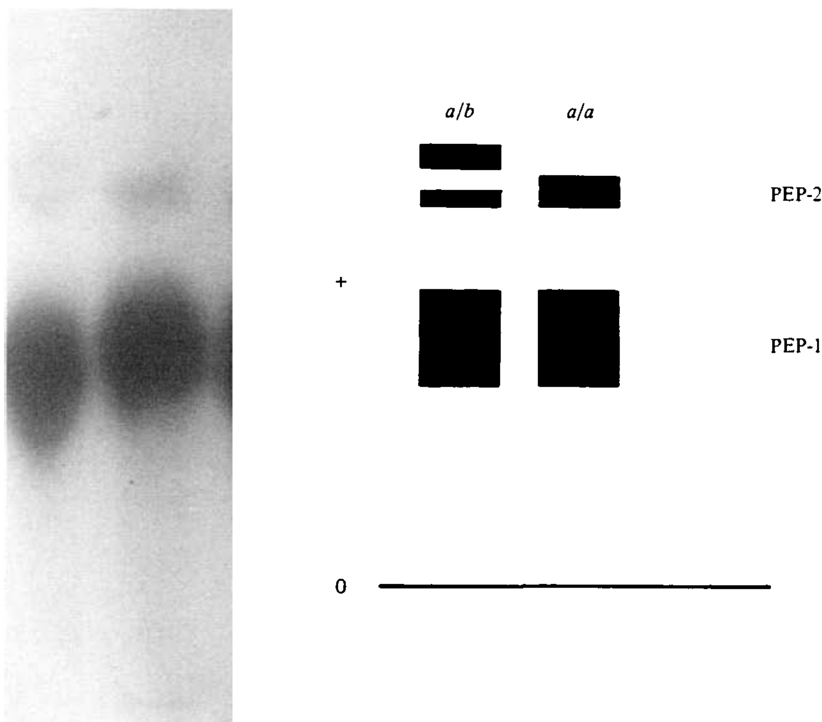

Fig. 7. PEP.

Figs. 1–7. Electrophoretic and diagrammatic representation of alcohol dehydrogenase (ADH), malic enzyme (MOD), glucose dehydrogenase (GDH), esterases (ES), mannosephosphate isomerase (MPI), glucose-6-phosphate dehydrogenase (GPD) and peptidase (PEP).

Gdh-2 (L), *Xdh* (L), *Glud* (L), *Cat* (L), *Sod-1* (K), *Sod-2* (K) *Got-1* (K), *Got-2* (K), *Hk-1* (H), *Hk-2* (H), *Hk-3* (H), *Ck-1* (H), *Ck-2* (H), *Ck-3* (B), *Ak-1* (H), *Ak-2* (L), *Ak-3* (L), *Pgm-1* (L), *Pgm-2* (L), *Es-III*, *-IV*, *-VI* (K), *Acp-1* (K), *Acp-2* (K), *Acp-3* (K), *Pep-1* (K), *Acy-1* (K), *Ada-1* (L), *Ada-2* (unscorable, L), *Aldo-1* (M), *Aldo-2* (B), *Fh* (L), *Aco-1* (K), *Aco-2* (K), *Gpi-1* (L), *Gpi-2* (L).

The isoenzymes always showed a phenotype typical for a homozygous state and could easily be interpreted according to Harris & Hopkinson (1976).

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Table 2. *List of polymorphic loci in crossbred New Zealand Whites*

Enzyme locus	Alleles found	New Zealand White × Russians	
		p	H
Adh	*a*	0·682	0·454
	b	0·318	
Mod-1	*a*	0·773	0·273
	b	0·227	
Mod-2	*a*	0·864	0·273
	b	0·136	
Gdh-1	*a*	0·864	0·090
	b	0·136	
Gpd	*a*	0·636	0·000
	b	0·364	
ES-I	*a*	0·909	0·182
	b	0·091	
ES-II	*a*	0·636	0·545
	b	0·364	
ES-VII	*a*	0·864	0·272
	b	0·136	
MPI	*a*	0·455	0·636
	b	0·318	
	c	0·227	

$\bar{P} = 0{\cdot}167$ $\bar{H} = 0{\cdot}050$

p = allele frequency, H = heterozygosity, $\bar{H}$ = average heterozygosity, $\bar{P}$ = proportion of polymorphic loci, $n = 11$ (offspring omitted because of close relationship). $\bar{H}$ and $\bar{P}$ are calculated according to the formulae given by Ayala (1977), assuming a total of 54 genetic loci investigated.

To get an idea of the genetic variability in domestic rabbits the proportion of polymorphic loci and average heterozygosity were calculated in the crossbred strain of New Zealand White and Russians because it provided the largest sample size (Table 2). Although considerable genetic variation was detected, the $\bar{P}$ (= proportion of polymorphic loci) and $\bar{H}$ (= average heterozygosity) values are within the range commonly found in mammals (Baccus *et al.* 1983; Hartl, 1985). The $\bar{P}$ value of purebred New Zealand Whites is much higher than in all other purebred strains. This could be explained by the fact that it is the only strain of economic importance where selection for good reproduction and growth is carried out. Thus, if heterotic effects are involved, by selection for these criteria a high amount of heterozygosity may be preserved even within purebred strains. A similar effect may also be responsible for residual heterozygosity within inbred strains of laboratory animals. As seen in New Zealand Whites, morphological homogeneity is not necessarily correlated with uniformity in biochemical marker systems. On the other hand, our results indicate that the obvious differentiation in coat colour and other morphological characters between the strains is accompanied by a partial fixation of different genes determining isoenzyme systems, which may therefore be a powerful tool for identification of certain rabbit strains in mixed breeds.

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