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Blood Biochemical Polymorphisms in Rabbits Presently Bred in Spain: Genetic Variation and Distances amongst Populations

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Abstract

A total of 816 rabbits, belonging to breeds presently bred in Spain (Spanish Common, Spanish Giant, Butterfly, Lyoné de Bourgogne, New Zealand White, Californian and a hybrid line obtained from crosses between selected individuals of the latter two), were tested using nine blood electrophoretic markers: Hb, Ak, Co, Tf, Es-1, Es-2, Es-3, Ada and Pgd. The latter five proteins were found polymorphic, each being controlled by one locus and showing autosomal co-dominant Mendelian inheritance. Three of these loci (*Es-1*, *Es-2* and *Pgd*) have two alleles, and the remaining two (*Es-3* and *Ada*) have three alleles. These polymorphic loci were used to study the population structure and breed similarities genetically.

Gene frequencies for *Es-1*, *Es-3* and *Ada* clearly differed amongst populations. Intra-breed variation was found for *Es-3* in the Spanish Common breed. Lack of Hardy–Weinberg equilibrium was only found for *Es-2* in most populations. Average inbreeding coefficients were not significantly different from zero, except in Spanish Giants ($f = 0{\cdot}09$) and New Zealand Whites ($f = -0{\cdot}04$). To estimate the genetic variability the average degree of heterozygosity was calculated and gave values of 0·40–0·56 for *Es-1*, *Ada* or *Es-3*, 0·82–0·84 for *Es-2*, due to an excess of heterozygotes, and 0·08–0·10 for *Pgd*, due to an excess of homozygotes. No association was found between genotype and sex, or between loci.

Contingency χ^2 tests and genetic distance estimations revealed that two population groups may be established according to genetic similarities: (i) populations under non-selective breeding systems (Spanish Common, Spanish Giant, Butterfly and Lyoné de Bourgogne); and (ii) highly selected, industrially bred populations (New Zealand White, Californian and the hybrid line). The genetic distances reached are greater for the latter group than for the former, where apparent cross-breeding may jeopardize breed purity.

Introduction

The study of genetically controlled biochemical polymorphisms of blood proteins is at present a useful tool to characterize livestock breeds and populations genetically; hence it contributes to the knowledge of genetic similarities and distances amongst them (Zetner *et al.* 1970; Kidd and Pirchner 1971; Kidd and Sgaramella-Zonta 1971).

These studies are not only of genetic and phylogenetic interest; they may also be of practical application in livestock improvement. They provide information to the breeder in programs where a hybrid vigour is aimed for and an estimation of the genetic distances amongst the parental breeds is desired. The electrophoretically detected blood proteins have been widely studied in many livestock species (see Manwell and Baker 1970). However, this line of research is still at its early stages in rabbits (Grunder *et al.* 1965; Johnson 1968; Coggan *et al.* 1974; Richardson 1980; Juneja *et al.* 1981; Zaragoza 1984). Given the great diversity existing within this species and its increasing value in the livestock industry of

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many countries, a genetic characterization of breeds, lines and populations can now be carried out smoothly, with the help of the information and mathematical methodology available at present (Cavalli-Sforza and Edwards 1967; Kidd and Sgaramella-Zonta 1971).

Within this context we report in this paper results on the electrophoretic study of different blood (erythrocyte and plasma) proteins in rabbit populations presently bred in Spain, the genetic characterization of these populations and the study of the genetic divergences and similarities amongst them.

Materials and Methods

A total of 816 rabbits (≈ 2 months old, 1800–2000 g liveweight) were used in this study. They belong to populations of six breeds (Spanish Common, 287 individuals; Spanish Giant, 53; Butterfly, 50; Lyoné de Bourgogne, 50; Californian, 50; New Zealand White, 226) and a hybrid line (100 individuals of the F_1 of crosses between purposefully selected New Zealand females and highly selected Californian males).

With regard to the availability of animals, it was difficult to find pure-bred individuals, due to the invasion in Spain of highly productive imported lines. Two of the breeds (Spanish Giant and Spanish Common) were chosen because they are autochthonous; and the remaining four breeds and the hybrid line, because they are widely used in this country even though they originated from abroad. The New Zealand females and Californian males were highly selected to become the parental generation for the hybrid line individuals. However, the F_1 cross (hybrid line) between these parents may not be the simple average of the two unselected parental breeds. This line has been included in the study, given its high representation amongst the rabbit populations in Spain.

Two of the six breeds, Spanish Common and New Zealand White, representative of different breeding systems (non-selective *v.* industrial selective systems), were also included in the study to examine possible differences between them for intra-breed differentiation. Spanish Common rabbits (287) were distributed into three groups, according to their place of origin: 143 individuals from eastern Spain (Vallés Oriental, Barcelona); 52 from northern Spain (Oscoz, Navarra); and 92 from central Spain (Ribera del Huerva, Zaragoza). Also, New Zealand White rabbits (226) were obtained from two areas: 51 from eastern Spain (Vallés Oriental, Barcelona) and 175 from northern Spain (Oscoz, Navarra).

Samples (about 4 ml of heparinized blood; 750 heparin units/tube) were collected from the jugular vein of each animal at slaughter. After centrifugation (≈ 1500 *g*, 15 min), plasma was stored at -30°C for a maximum of 6 months. Erythrocytes were resuspended (0·9% w/v NaCl solution, pH 7–7·4), centrifuged (≈ 1500 *g*, 10 min) and the supernatant discarded. This washing procedure was repeated twice or three times until a colorless transparent supernatant was obtained. Haemolysis of the sedimented compacted erythrocytes was produced by freezing the cells at -30°C for at least 24 h.

Nine proteins were studied in this work (haemoglobin, Hb; adenylate kinase, Ak; cytochrome *c* oxidase, Co, formerly called indophenol oxidase; esterases, Es-1, Es-2, Es-3; adenosine deaminase, Ada; phosphogluconate dehydrogenase, Pgd; and transferrin, Tf). Of these, eight were found in erythrocytes and one (transferrin) in plasma. Starch-gel electrophoresis was carried out for these proteins according to the procedures applied by Zaragoza *et al.* (1985*a*).

Gene frequencies were estimated by direct gene counting. A contingency χ^2 test was applied to verify whether differences in gene frequencies were statistically significant (Cabrera *et al.* 1980). Hardy–Weinberg equilibrium was studied using χ^2 tests ($\chi^2_{eq.}$). The Yates correction was systematically applied (Fisher and Yates 1963), in tests with one degree of freedom. The inbreeding coefficients were estimated considering each locus ($\bar{f}$) and all loci (average inbreeding coefficient, f), were estimated according to the methods applied by Kidd *et al.* (1980). When more than one population was sampled in a single breed, Wright's (1943) method based on Wahlund's principles (1928) was applied for estimating the inbreeding coefficient for each locus (F) and for each breed ($\bar{F}$). A Student's *t*-test was applied to verify if the inbreeding values were significantly greater than zero.

To study the genetic variability for each population and for each locus, heterozygosity was estimated according to Rendel (1967). The proportion of polymorphic loci was calculated according to Cabrera *et al.* (1980), establishing three levels: 99, 95 and 75%. Specifically, a particular locus is, for example, polymorphic at the 99% level, if the frequency of the most common allele at this locus is $<0{\cdot}99$. The proportion of polymorphic loci at the 99% level will thus equal the number of loci polymorphic at the 99% level, divided by the total number of loci analysed. To detect homogeneity or divergence among genotypic distributions of two or more populations χ^2 tests were applied (χ^2_h: Lutz 1978).

The possible association amongst polymorphic loci, breeds or genotypes and sexes was studied by contingency χ^2, according to procedures of Snedecor and Cochran (1969).

'E' genetic distances were estimated by the procedure of Cavalli-Sforza and Edwards (1967) with Edwards' modifications (1971). Dendrograms were elaborated using the additive model of Cavalli-Sforza and Edwards (1967) with the modifications described by Kidd and Sgaramella-Zonta (1971), Zonta and Kidd (1973, 1974) and Altarriba and Lamuela (1983).

Results

Phenotypes and Allelic Frequencies

As shown in Table 1, the electrophoretic results revealed that four of the nine proteins studied are monomorphic in all populations tested (Hb, Ak, Co and Tf), three show two variants (Es-1, Es-2 and Pgd) and two show three variants (Es-3 and Ada). Each protein is controlled by a single locus and the variants are inherited in a simple Mendelian co-dominant manner (Zaragoza 1984; Zaragoza *et al.* 1985*b*).

Table 1. Monomorphic and polymorphic blood proteins found in the studied populations

Monomorphic marker		Polymorphic marker		
Protein	Locus	Protein	Locus	Alleles
Hemoglobin	*Hb*	Esterase-1	*Es-1*	$Es\text{-}1^A$
Adenylate kinase	*Ak*			$Es\text{-}1^B$
Cytochrome *c* oxidase	*Co*	Esterase-2	*Es-2*	$Es\text{-}2^F$
Transferrin	*Tf*			$Es\text{-}2^S$
		Esterase-3	*Es-3*	$Es\text{-}3^A$
				$Es\text{-}3^B$
				$Es\text{-}3^C$
		Adenosine deaminase	*Ada*	Ada^1
				Ada^2
				Ada^3
		6-Phosphogluconate dehydrogenase	*Pgd*	Pgd^1
				Pgd^2

Table 2 summarizes the estimations on gene frequencies, equilibrium χ^2 ($\chi^2_{eq.}$), inbreeding coefficient (f), and degree of heterozygosity observed and expected at each locus and each population. Also, estimations on the average degree of heterozygosity and of inbreeding ($\bar{f}$) in each breed considering all loci, the proportion of polymorphic loci at the 99, 95 and 75% levels, and the mean number of alleles per locus in each population, are shown in this table.

Genetic Characteristics of the Breeds

Considering the polymorphic loci, data in Table 2 reveal that the differences between breeds in gene frequencies for *Es-2* and *Pgd* are very small, since in all breeds there is an excess of heterozygotes for the first locus ($Es\text{-}2^F$ frequency, 0·48–0·52) and an excess of homozygotes for the second locus (Pgd^1 frequency, 0·91–0·96). In contrast, for *Es-1*, *Es-3* and *Ada*, there are two groups of populations with regard to similarities on gene frequency values: (*a*) Spanish Common, Spanish Giant, Butterfly and Lyoné de Bourgogne; and (*b*) New Zealand White, Californian and hybrid line.

Table 3 illustrates that significant gene frequency differences occur for only four loci (*Es-1*, *Es-3*, *Ada* and *Pgd*) ($P < 0{\cdot}05$–$P < 0{\cdot}001$) in comparisons between groups (*a*) and (*b*). Non-significant differences were observed amongst group (*a*) breeds, although some differences were found amongst group (*b*) breeds (though at low significance levels, $P < 0{\cdot}05$). Based on these contingency χ^2 tests, it is clear that the relative contribution

Table 2. Genetic structure [gene frequencies, equilibrium χ^2, partial (f) and average ($\bar{f}$) inbreeding coefficients, partial and average heterozygosities, percentage polymorphic loci and average number of alleles per locus] of seven rabbit populations, established with nine genetic markers

*$P<0.05$; **$P<0.01$; ***$P<0.001$; χ^2 values not accompanied by asterisks represent equilibrium situations. Also, average inbreeding values without asterisks are non significantly different from 0

Polymorphic locus[A]	Alleles and estimations	Individual populations[B] SC	SG	BU	LB	NZ	CA	HL	Total population: Av. ± s.e. (s.d.)
Es-1	*Es-1*^{A}	0·58	0·56	0·60	0·52	0·40	0·40	0·38	
	Es-1^B	0·42	0·44	0·40	0·48	0·60	0·60	0·62	
	$\chi^2_{eq.}$	0·92	0·15	0·13	1·42	0·43	0·05	0·25	
	f	0·08	−0·14	0·08	0·20	0·04	0·00	0·06	
	Obs. het.	0·45	0·56	0·44	0·40	0·46	0·48	0·44	0·46 ± 0·02 (0·05)
	Exp. het.	0·49	0·49	0·48	0·50	0·48	0·48	0·47	0·48 ± 0·02 (0·01)
Es-2	*Es-2*^{F}	0·50	0·48	0·51	0·50	0·48	0·57	0·52	
	Es-2^S	0·50	0·52	0·49	0·50	0·52	0·43	0·48	
	$\chi^2_{eq.}$	44·15***	13·25**	23·85**	39·61***	73·57***	2·74	44·57***	
	f	−0·68	−0·76	−0·72	−0·92	−0·58	−0·26	−0·68	
	Obs. het.	0·84	0·88	0·86	0·96	0·79	0·62	0·84	0·83 ± 0·01 (0·10)
	Exp. het.	0·50	0·50	0·50	0·50	0·50	0·49	0·50	0·50 ± 0·02 (0·04)
Es-3	*Es-3*^{A}	0·54	0·46	0·56	0·47	0·80	0·67	0·74	
	Es-3^B	0·31	0·38	0·25	0·35	0·16	0·28	0·18	
	Es-3^C	0·15	0·16	0·19	0·18	0·04	0·04	0·08	
	$\chi^2_{eq.}$	5·48	1·95	2·16	0·96	1·18	0·77	4·79	
	f	0·08	0·21	0·22	0·23	−0·03	0·32	0·14	
	Obs. het.	0·54	0·48	0·46	0·48	0·34	0·32	0·35	0·42 ± 0·02 (0·08)
	Exp. het.	0·59	0·61	0·59	0·62	0·33	0·47	0·41	0·52 ± 0·02 (0·11)
Ada	*Ada*^{1}	0·40	0·52	0·44	0·48	0·55	0·37	0·66	
	Ada^2	0·44	0·32	0·38	0·33	0·31	0·41	0·26	
	Ada^3	0·16	0·16	0·18	0·19	0·14	0·22	0·08	
	$\chi^2_{eq.}$	7·09	3·26	0·23	0·99	10·63	4·12	0·42	
	f	0·09	0·26	0·08	0·13	0·08	0·04	0·02	
	Obs. het.	0·56	0·44	0·58	0·54	0·53	0·62	0·48	0·54 ± 0·02 (0·06)
	Exp. het.	0·62	0·60	0·63	0·62	0·58	0·65	0·49	0·60 ± 0·02 (0·05)
Pgd	*Pgd*^{1}	0·93	0·92	0·92	0·91	0·94	0·96	0·93	
	Pgd^2	0·07	0·08	0·08	0·09	0·06	0·04	0·07	
	$\chi^2_{eq.}$	n.e.[C]	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	
	f	0·15	0·58	0·46	0·38	0·18	−0·25	0·46	
	Obs. het.	0·11	0.08	0·08	0·10	0·09	0·10	0·07	0·09 ± 0·01 (0·01)
	Exp. het.	0·13	0·19	0·15	0·16	0·11	0·08	0·13	0·13 ± 0·01 (0·03)
Obs. av. het.		0·28	0·27	0·27	0·28	0·25	0·24	0·24	0·26 ± 0·01 (0·30)
± s.e.		±0·03	±0·06	±0·06	±0·06	±0·03	±0·06	±0·04	
(s.d.)		(0·32)	(0·32)	(0·32)	(0·34)	(0·30)	(0·27)	(0·30)	
Exp. av. het.		0·26	0·25	0·26	0·27	0·22	0·24	0·22	0·25 ± 0·01 (0·26)
± s.e.		±0·03	±0·06	±0·06	±0·06	±0·03	±0·06	±0·04	
(s.d.)		(0·28)	(0·28)	(0·28)	(0·29)	(0·25)	(0·27)	(0·24)	
$\bar{f}$		−0·01	0·09	0·06	0·05	−0·04	0·03	0·02	
± s.e.		±0·00	±0·03*	±0·03	±0·03	±0·01*	±0·02	±0·01	
Polymorphic loci (%)									
99 and 95% level		55·55	55·55	55·55	55·55	55·55	55·55	55·55	55·55
75% level		44·44	44·44	44·44	44·44	33·33	44·44	44·44	42·85
Av. No. of alleles per locus		1·78	1·78	1·78	1·78	1·78	1·78	1·78	1·78
(s.d.)		(0·83)	(0·83)	(0·83)	(0·83)	(0·83)	(0·83)	(0·83)	(0·79)

[A] Total number of loci = 9; the remaining four loci (*Hb*, *Ak*, *Co* and *Tf*) were monomorphic.
[B] SC, Spanish Common; SG, Spanish Giant; BU, Butterfly; LB, Lyoné de Bourgogne; NZ, New Zealand White; CA, Californian; HL, hybrid line.
[C] Not estimated; see explanation in text.

Table 3. Genetic structure in populations of Spanish Common and New Zealand White breeds (gene frequencies, equilibrium χ^2, homogeneity χ^2, total inbreeding, average heterozygosities, percentage of polymorphic loci and average number of alleles per locus), established with nine genetic markers

See Table 1 for polymorphic *v.* non-polymorphic loci. $*P<0{\cdot}05$; $**P<0{\cdot}01$; $***P<0{\cdot}001$

Polymorphic locus[A]	Alleles and estimations	Spanish Common			New Zealand White		Spanish Common	New Zealand White
		Eastern	Northern	Central	Eastern	Northern	Av. ± s.e. (s.d.)	Av. ± s.e. (s.d.)
Es-1	*Es-1*^{A}	0·59	0·48	0·60	0·43	0·39		
	Es-1^B	0·41	0·52	0·40	0·57	0·61		
	$\chi^2_{eq.}$	1·63	0·09	0·04	0·40	1·38		
	χ^2_h	←	2·29	→	← 1·32	→		
	F (%)	←	0·80	→	← 0·09	→		
	Obs. het.	0·43	0·50	0·48	0·56	0·43	0·47 ± 0·03 (0·03)	0·49 ± 0·03 (0·09)
	Exp. het.	0·48	0·49	0·48	0·49	0·48	0·48 ± 0·03 (0·00)	0·48 ± 0·03 (0·01)
Es-2	*Es-2*^{F}	0·50	0·50	0·49	0·55	0·47		
	Es-2^S	0·50	0·50	0·51	0·45	0·53		
	$\chi^2_{eq.}$	77·83***	17·12**	23·32***	14·18***	59·27***		
	χ^2_h	←	5·86	→	← 1·91	→		
	F (%)	←	n.e.	→	← n.e.	→		
	Obs. het.	0·87	0·95	0·75	0·80	0·78	0·86 ± 0·02 (0·10)	0·79 ± 0·02 (0·01)
	Exp. het.	0·50	0·50	0·50	0·50	0·50	0·50 ± 0·02 (0·00)	0·50 ± 0·02 (0·01)
Es-3	*Es-3*^{A}	0·56	0·38	0·52	0·83	0·79		
	Es-3^B	0·36	0·55	0·30	0·17	0·16		
	Es-3^C	0·14	0·07	0·18	0·00	0·05		
	$\chi^2_{eq.}$	2·66	1·11	0·05	2·17	4·43		
	χ^2_h	←	29·43***	→	← 2·64	→		
	F (%)	←	0·40	→	← 0·14	→		
	Obs. het.	0·43	0·50	0·69	0·34	0·34	0·54 ± 0·02 (0·12)	0·34 ± 0·03 (0·00)
	Exp. het.	0·57	0·56	0·59	0·28	0·36	0·57 ± 0·02 (0·01)	0·32 ± 0·03 (0·05)
Ada	*Ada*^{1}	0·45	0·16	0·38	0·32	0·60		
	Ada^2	0·42	0·80	0·40	0·30	0·32		
	Ada^3	0·13	0·04	0·22	0·38	0·08		
	$\chi^2_{eq.}$	2·89	n.e.	3·21	2·89	0·89		
	χ^2_h	←	15·24	→	← 34·48***	→		
	F (%)	←	1·00	→	← 4·00	→		
	Obs. het.	0·57	0·40	0·57	0·59	0·52	0·51 ± 0·02 (0·08)	0·55 ± 0·03 (0·05)
	Exp. het.	0·60	0·33	0·65	0·56	0·53	0·53 ± 0·02 (0·17)	0·55 ± 0·03 (0·02)
Pgd	*Pgd*^{1}	0·95	0·93	0·89	1·00	0·93		
	Pgd^2	0·05	0·07	0·11	0·00	0·07		
	$\chi^2_{eq.}$	n.e.[B]	n.e.	n.e.	n.e.	n.e.		
	χ^2_h	←	3·50	→	← 4·23	→		
	F (%)	←	1·20	→	← 1·00	→		
	Obs. het.	0·07	0·15	0·15	0·00	0·11	0·12 ± 0·01 (0·04)	0·06 ± 0·01 (0·08)
	Exp. het.	0·10	0·14	0·19	0·00	0·13	0·14 ± 0·02 (0·03)	0·07 ± 0·01 (0·09)
$\bar{F}$ (%) ± s.e.		←	0·85 ± 0·02	→	← 1·30 ± 0·04 →			
Obs. av. het.		0·26	0·28	0·24	0·25	0·24	0·28 ± 0·03 (0·31)	0·25 ± 0·03 (0·24)
± s.e.		± 0·03	± 0·06	± 0·04	± 0·06	± 0·03		
(s.d.)		(0·32)	(0·33)	(0·32)	(0·32)	(0·29)		
Exp. av. het.		0·25	0·22	0·27	0·20	0·22	0·25 ± 0·03 (0·26)	0·21 ± 0·03 (0·24)
± s.e.		± 0·03	± 0·04	± 0·04	± 0·05	± 0·03		
(s.d.)		(0·28)	(0·24)	(0·28)	(0·25)	(0·24)		
Polymorphic loci (%)								
99 and 95% level		55·55	55·55	55·55	44·44	55·55	55·55	49·99
75% level		44·44	33·33	44·44	33·33	33·33	40·73	33·33
Av. No. of alleles								
per locus		1·78	1·78	1·78	1·66	1·78	1·78	1·72
(s.d.)		(0·78)	(0·78)	(0·78)	(0·84)	(0·78)	(0·78)	(0·81)

[A] Total number of loci = 9; the four remaining loci (*Hb*, *Ak*, *Co* and *Tf*) were monomorphic.

[B] Not estimated either because the population is not at equilibrium (*Es-2*), or because of a low (< 5) number of expected individuals and impossibility of phenotype grouping (*Ada*).

to the establishment of differences amongst breeds is 37·5% for *Es-3*, 33·3% for *Es-1*, 25% for *Ada*, and 4·2% for *Pgd*. (Percentages have been estimated from the formula n_i/T, where n is the number of significant contingency χ^2 values for the i locus, and T is the total number of significant χ^2 values found for all loci; Table 3.)

Considering the Hardy–Weinberg genetic equilibrium, an equilibrium situation was observed for the loci studied, with the exception of *Es-2*, only at equilibrium in the Californian breed. This lack of equilibrium at *Es-2* is attributable to an excess of heterozygotes (data not shown).

Although for *Pgd* χ^2 calculations could not be applied (minimum number of expected individuals <5, Lutz 1978), an equilibrium situation is presumably present in this breed, given the apparent adequacy between the observed and expected numbers in each genotypic class.

Inbreeding and Genetic Variation

Because for most loci in most populations there appears to be a tendency towards an excess of homozygotes (over the expected Hardy–Weinberg equilibrium proportions; data not shown), inbreeding coefficients (f and $\bar{f}$) were estimated (see Methods). Obviously, inbreeding expected values should not be high, since most populations for the majority of the loci are in equilibrium.

In general the inbreeding coefficient (f) values were positive (Table 2), with some exceptional loci for which negative values were found: *Es-1* in Spanish Giant, *Es-3* in New Zealand White, *Pgd* in Californian, and *Es-2* in all breeds (this was due to an excess of heterozygotes at this locus). For the latter locus the f values, ranged from $-0{\cdot}58$ in New Zealand White, to $-0{\cdot}92$ in Lyoné de Bourgogne with the less negative value ($-0{\cdot}26$) corresponding to the only breed in Hardy–Weinberg equilibrium for this locus (Californian). As excepted, the average inbreeding coefficient ($\bar{f}$) was generally not significantly different from zero. There were, however, two exceptions: New Zealand White ($f = -0{\cdot}04$); and Spanish Giant ($f = 0{\cdot}09$).

To study the genetic variability, the degree of heterozygosity was estimated, not only individually, for each locus within each breed, but also for each locus, considering the populations altogether, and for each breed considering all loci (Table 2).

When considering the heterozygosity for each locus, a high genetic variability (i.e. intermediate degree of heterozygosity, D.H.) for *Es-1* and *Ada* was observed in all breeds. However, for *Es-3*, there was a slightly slower D.H. in three of the seven populations (New Zealand White, Californian and hybrid line; D.H. = 0·32–0·35). Finally, there was a low D.H. for *Pgd* (0·07–0·11) due to an excess of Pgd^1 homozygotes, and a high D.H. for *Es-2* (0·62–0·96) due to an excess of heterozygotes. It is clear that the values of observed and expected heterozygosity are very close to each other with the exception of *Es-2* in most breeds. Only the Californian breed, at equilibrium for this locus, showed closer observed *v.* expected values (0·62 *v.* 0·49).

Estimations on the average D.H. for each breed revealed that these values were similar amongst breeds, ranging from 0·22 to 0·27. It is not surprising that the corresponding standard deviations where high, given that four of the nine studied loci are monomorphic. On the other hand, there was a wide range of variation on the average heterozygosity for each locus when considering the breeds altogether (from 0·09 for *Pgd* to 0·83 for *Es-2*). In this case, the standard deviations were low.

With respect to the percentage of polymorphic loci at the 75% level, while in most breeds it reaches the value of 44%, in New Zealand White it is lower (33·3%). Similarly, at the 95% level, while in most breeds it reaches the value of 55·5%, in the Californian breed it is lower (44·4%). However, these differences in percentage amongst breeds are not observed at the 99% level (55·5% in all breeds).

Finally, there is a remarkable similarity amongst breeds with respect to the average number of alleles per locus (1·78).

Intra-breed Genetic Diversity

As shown in Table 3, several populations were compared within the Spanish Common and New Zealand White breeds, for the purpose of studying the intra-breed variability and differentiation, as well as estimating the inbreeding coefficient (F) related to the Wahlund effect (Wahlund 1982). Concerning the Spanish Common populations, there are no differences between them when considering *Es-2* and *Pgd*; however, with respect to *Es-1*, the eastern and central populations ($Es\text{-}1^A$ frequency $\approx 0{\cdot}60$) appear to slightly differ from the northern population (frequency of 0·48 for $Es\text{-}1^A$). This difference is more obvious for *Es-3*, since both eastern and central populations have frequencies of $>0{\cdot}52$, $>0{\cdot}36$ and $>0{\cdot}14$ for the alleles $Es\text{-}3^A$, $Es\text{-}3^B$ and $Es\text{-}3^C$, respectively, whereas the corresponding frequencies in the northern populations are 0·38, 0·57 and 0·07, respectively. Similarly, for *Ada* the allelic frequencies in the eastern and central populations ($>0{\cdot}38$ for Ada^1, $<0{\cdot}42$ for Ada^2 and $>0{\cdot}13$ for Ada^3) differ from those of the northern populations (0·13, 0·80 and 0·04, respectively).

Hardy–Weinberg equilibrium estimations ($\chi^2_{eq.}$) revealed that in the three Spanish Common populations there is equilibrium for most loci, except for *Es-2*. χ^2 tests were not applied to *Pgd* since the excepted number of individuals was <5 (Lutz 1978; Weir 1979).

Table 4. Contingency χ^2 values to evaluate gene frequency differences amongst seven rabbit populations, considering five polymorphic loci

See Table 3 for breed abbreviations and for polymorphic loci. $*P<0{\cdot}05$; $**P<0{\cdot}01$; $***P<0{\cdot}001$; n.s., not significant ($P>0{\cdot}05$)

Breed	SG	BU	LB	NZ	CA	HL
SC	n.s.	n.s.	n.s.	*Es-1* (28·05)***	*Es-1* (8·78)*	*Es-1* (20·08)***
				Es-3 (60·44)***	*Es-3* (15·46)***	*Es-3* (17·22)**
				Ada (20·94)***		*Ada* (38·29)***
SG		n.s.		*Es-3* (20·72)***	*Ada* (6·24)	n.s.
BU			n.s.	*Es-1* (12·46)**	*Es-1* (7·03)*	*Es-1* (12·73)**
				Es-3 (16·62)**	*Es-3* (6·52)*	*Ada* (18·87)**
LB				*Es-1* (6·14)*	*Es-1* (14·51)***	*Es-3* (16·48)**
				Es-3 (32·21)**	*Es-3* (11·24)*	
NZ					*Ada* (12·51)*	*Pgd* (8·73)*
CA						*Ada* (9·43)*

Concerning homogeneity studies (χ^2_h), the difference in gene frequencies observed in the northern population reflects the lack of homogeneity amongst the three Spanish Common populations, only with respect to *Es-3* ($\chi^2_h = 29{\cdot}43$; Table 4). This lack of homogeneity was further studied by comparing the three subpopulations two by two, revealing highly significant differences in all cases ($\chi^2_h \geq 23$; $P < 0{\cdot}001$).

All Spanish Common populations were, however, similar to each other with respect to other parameters (average heterozygosity, percentage polymorphic loci and average number of alleles per locus). In contrast to the Spanish Common breed, when the New Zealand White populations were analysed, an intra-breed homogeneity was observed, as expected from a highly standardized and selected breed. Also, as shown in Table 4, there is lack of equilibrium at both breeds only for *Es-2*, due to an excess of heterozygotes at this locus.

Association Studies and Genetic Relationships

Contingency χ^2 tests reveal that there is no association between genotype and sex of the individual in any of the breeds, and that there is no association amongst loci in any of the pair-wise combinations.

The study of genetic differences and similarities amongst breeds was carried out by two methods: contingency χ^2 (already illustrated in Table 3) and 'E' genetic distance (Cavalli-Sforza and Edwards 1967). Both methods yield similar estimations, although the former establishes the significance of the differences and the latter quantifies them.

Table 5. 'E' distance matrix for seven rabbit populations
Estimations involving five polymorphic loci (*Es-1*, *Es-2*, *Es-3*, *Ada* and *Pgd*) are given. See Table 3 for breed abbreviations

Populations	SG	BU	LB	NZ	CA	HL
SC	0·1028	0·0656	0·0970	0·2474	0·1946	0·2556
SG		0·1092	0·0486	0·2653	0·2373	0·2468
BU			0·0955	0·2467	0·2236	0·2467
LB				0·2597	0·2188	0·2455
NZ					0·1720	0·1076
CA						0·2283

Based on the estimated gene frequencies an 'E' distance matrix was elaborated (Table 5). There are smaller distances (from 0·0486 to 0·1092) between the Lyoné de Bourgogne, Butterfly, Spanish Common and Spanish Giant breeds, than between these breeds and the New Zealand White, Californian and hybrid line populations. The distances can be observed between the latter three breeds range from 0·1076 to 0·2283.

Table 6. Characteristics of the four entry trees and best fitting tree obtained for elaboration of the dendrogram
Based on the distance matrix of Table 5, the best adjustment tree, without negative segments, was obtained by least squares. The number of iterations to reach this final solution is indicated for the first three entry models, elaborated with logical criteria, as well as for the fourth model, established at random

Entry model	Σ Segment lengths	Σ (error^2)^A	Negative segments	Number of iterations
1	0·56587	0·00480	2	4
2	0·70195	0·02233	2	4
3	0·69998	0·02998	2	7
4	0·87134	0·05782	3	11
Best adjustment	0·49623	0·00234	0	

^{A} $\Sigma(D-D_a)^2$, D = total distance, and D_a = absolute distance.

For a graphical illustration of these results, a dendrogram was elaborated from this matrix (Table 6; Fig. 1). Four entry trees were used (three established with logical criteria and one at random) and of the 30 derived trees, one of them showing no negative segments. This tree represents the minimal evolution (0·49623) and the best adjustment by minimum squares (0·00234), thus being the best solution for graphically representing the genetic divergences and similarities amongst breeds.

As illustrated in Fig. 1, four breeds appear associated in a common branch (Spanish Common, Spanish Giant, Butterfly and Lyoné de Bourgogne), while the remaining three breeds (New Zealand White, Californian and hybrid line), at greater genetic distances, are associated in a different branch. There is a remarkable proximity between breeds of the first group, whereas in the second group Californian breed has reached a significantly smaller distance than the hybrid line or the New Zealand White breed.

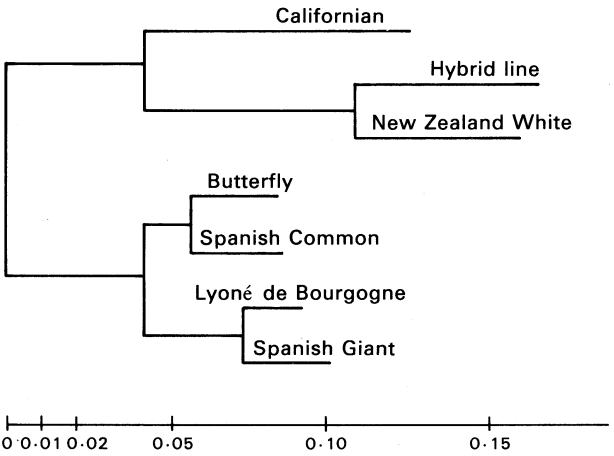

Fig. 1. Genetic distance dendrogram corresponding to the best solution obtained from the E distance matrix. The numerical data used are given in Tables 4 and 6.

Discussion

Phenotypes and Allelic Frequencies

The fact that intact rabbit haemoglobin molecules are not polymorphic in starch-gel electrophoresis is not surprising (Dayhoff 1972; Robinson and Osterhoff 1983), since before the run, α- and β-chains have not been chromatographically separated and thus the untreated Hb dimers may not reflect the electrophoretic variation possibly existing in individual chains (Barnabas and Müller 1962; Ishibashi *et al.* 1968; Luppis and Conconi 1970; McIlwaine *et al.* 1973; Garrick *et al.* 1974). Only Ramos *et al.* (1972) found two intact haemoglobin variants in the Spanish Giant breed, without separating the chains chromatographically. However, the differences in electrophoretic mobility observed by these authors may not reflect the genetic variation proposed, but small variations in electrophoretic conditions occasionally appearing. This would explain the observations made by these authors on the low frequency of the slow variant, as well as on the lack of heterozygotes (only a single sample was run in each cellulose acetate strip).

On the other hand, the results obtained on adenylate kinase and indophenol oxidase coincide with those of Vergnes *et al.* (1974) in the Petit Russe breed, but differ from those of Coggan *et al.* (1974), who found two Ak variants, perhaps due to the greater gene pool of the Australian rabbits used in their study.

As in this work, lack of transferrin polymorphism was also observed by Binette (1979) in the New Zealand White breed; however, in other genetically richer breeds like Petit Russe and Chinchilla (Markovich and Pomitko 1977) or Spanish wild rabbits (Arana 1985) two alleles have been observed.

The electrophoretic results obtained for *Es-1*, *Es-2* and *Es-3* coincide with the findings of Suzuki and Stormont (1972) and Richardson *et al.* (1980), but not with those of two earlier publications: Grunder *et al.* (1965) found monomorphism for *Es-2* and *Es-3*; and Schiff and Stormont (1970), besides observing higher gene frequencies in New Zealand White for $Es\text{-}1^{B}$ (0·73) and $Es\text{-}2^{F}$ (0·64), only found three *Es-3* phenotypes (two alleles).

The phenotypic variation and gene frequencies observed for *Ada* and *Pgd* has also been found recently by other authors (Coggan *et al.* 1974; Richardson *et al.* 1980; Peluso *et al.* 1982; Salermo *et al.* 1982).

The excess of heterozygotes for *Es-2* could be more likely attributed to the electrophoretic technique applied (Schiff and Stormont 1970) with regard to the substrate type and concentration rather than to the more attractive hypothesis of a heterozygote advantage.

Inbreeding and Genetic Variation

With regard to inbreeding, the estimated average inbreeding ($\bar{f}$) was low in most breeds, except in the Spanish Giant and New Zealand White breeds ($\bar{f}$ significantly different from 0; $P < 0{\cdot}05$). In Spanish Giants, this may be due to the limited number of animals tested and existing in the breed at present and to the small number of individuals that originated the breed. However, in the New Zealand White breed, this may be attributable to the high selective pressure to which it is being submitted, thus increasing the probability of finding two alleles identical by descent in a given individual.

Concerning the genetic variation within breeds (estimated by the average degree of heterozygosity, percentage of polymorphic loci, or average number of alleles per locus), similar results have been obtained for most breeds, due to the high standard deviation from the presence of four monomorphic loci amongst the nine loci.

Concerning the variation of *Es-3* different population groups (i.e. bred under non-selective *v.* industrial selective systems) show different variation. Possibly, industrialized populations, highly selected, have an excess of homozygotes for particular loci (like *Es-3*), some of which may have selective advantage for production traits.

Intra-breed Genetic Diversity

As far as the intra-breed genetic diversity is concerned, it appears that in the Spanish Common breed (but not in the New Zealand White breed), the allelic frequencies at three loci (*Es-1*, *Es-3* and *Ada*) differ between the northern, eastern and central populations. Whether or not these differences are real or attributable to the sampling method is unknown. The fact that at least one locus (*Es-3*) reveals heterogeneity, points out the possibility that the Spanish Common breed is undergoing intra-breed differentiation, as expected, considering the local type of its breeding system.

With respect to the Wahlund effect on inbreeding (*F*) upon the breed subdivision in populations, this effect is observed in the Spanish Common breed but not in New Zealand White breed, likely due to genetic drift and isolation existing in the first but faded away in the second because of artificial selection and industrial breeding.

Genetic Distances

Keeping in mind that shared dendrogram branches are indicative of genetic similarities, it is clear that there are two branches, i.e. two population groups amongst those studied: those bred under selective systems (New Zealand White, Californian and the hybrid line); and those bred under non-selective systems (Spanish Common, Spanish Giant, Butterfly and Lyoné de Bourgogne). The genetic distances that the first group has reached are greater (when compared with those of the second group), with the maximum value for the hybrid line, likely due to an intensive selection process, of both parental breeds to create this line. It is obvious that the prize of this selection process is the loss of genes, not necessarily detrimental, which may be still present in the less-selected breeds of the second population group.

Although it may apparently be surprising to find autochthonous breeds, Spanish Common and Spanish Giant, at short distances from other imported breeds like Butterfly and Lyoné de Bourgogne, there is a logical interpretation to this finding, considering that cross-breeding may have occurred amongst these populations, all of them bred under non-selective systems in private farms. This observation may also represent warning signs of pure breed extinction.

Some of these appreciations may be confirmed by increasing the number of degrees of freedom for the elaboration of the dendrogram by incorporating more loci to the study. Also, the analysis of a higher number of individuals may re-affirm the present observations, for example, with regard to the distance findings on the Californian breed in relation to the two other breeds of the same group.

Altogether, the data presented in this work provide information on inbreeding, intra-breed

genetic differentiation and on genetic distances amongst populations, and may thus contribute to the genetic knowledge of the world's rabbit genetic reserve and of the rabbit population structure and characterization. The knowledge of the genetic structure and intra- or inter-breed differentiation may be of practical use for maintaining the rabbit genetic reserve to be used in studies on the genetic and productive potential of unselected breeds and for research involving cross-breeding between different populations or breeds. Experimentation on crosses between genetically distant *v.* close populations for these biochemical polymorphic traits to obtain hybrid vigour may be of relevance for the improvement of the rabbit livestock production.

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