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Blood biochemical polymorphisms in rabbits I. Genetic variation and distance among populations of domestic rabbits presently bred in Spain

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SUMMARY - Seventeen blood proteins were studied by electrophoresis, using samples from 598 rabbits belonging to various breeds (Spanish Common, 102; Spanish Giant, 67; Butterfly, 79; Lyoné de Bourgogne, 64; New Zealand White, 105; Californian, 81; and a hybrid combination, obtained from crosses between selected individuals of the latter two, 100). Eight of these markers were monomorphic (*Ak, Sod, Ca-1, Cat, Dia-1, Hb, Tf* and *Cp*); five were each controlled by diallelic loci (*Pgd, Es-1, Es-2, Ca-2* and *Est-7*); three were controlled by triallelic loci (Es-3, Dia-2 and Ada) and one by a tetrallelic locus (*Hx*). Population studies concerning the genetic variation revealed the existence of heterogeneity among populations with respect to gene frequencies of polymorphic loci (with the exception of *Pgd* and *Ca-2*). Similarity among populations was observed for the average degree of heterozygosity (≥ 0.35; ≤ 0.44) and for the percentage of polymorphic loci ($\geq 41\%$; $\leq 53\%$). The Spanish Giant breed showed a slightly lower number of alleles/locus (1.76) than the remaining breeds. Inbreeding coefficients were significantly different from zero in all populations, ranging from 6% (Californian) to 12% (Spanish Common and Lyoné de Bourgogne). Genetic distance estimations revealed that three population groups may be established according to genetic similarities: (i) highly selected, industrially bred populations (New Zealand White and the hybrid combination); (ii) populations under non selective breeding systems (Spanish Common, Butterfly and Lyoné de Bourgogne), and the highly selected Californian breed; and (iii) autochtonous breeds, considered as relics (Spanish Giant). These findings strongly suggest that the present breeding systems are jeopardizing the breed's individuality when favoring crosses between breeds under similar breeding systems or when purposefully incorporating genes from highly selected breeds (e.g. Californian) in the genetic make-up of other breeds, all of them representative of the populations presently found in Spain.

Key words: Biochemical polymorphisms, blood markers, genetic variation, genetic distance, rabbit.

RESUME - "Polymorphismes biochimiques sanguins chez le lapin. I. Variation génétique et distance entre populations de lapins actuellement élevés en Espagne". Dix-sept protéines sanguines ont été étudiées, en utilisant des échantillons de 598 lapins, appartenant à plusieurs races (Commune Espagnole, 102; Géante Espagnole, 67; Papillon, 79; Fauve de Bourgogne, 64; Néo-Zélandaise Blanche, 105; Californienne, 81; et une combinaison hybride, obtenue de croisements entre individus selectionnés des deux dernières races, 100). Huit de ces marqueurs étaient monomorphiques (Ak, Sod, Ca-1, Cat, Dia-1, Hb, Tf *et* Cp); *cinq d'eux étaient contrôlés par loci diallélíques (*Pgd, Es-1, Es-2, Ca-2 *et* Est-7); *trois étaient contrôlés par loci triallélíques (*Es-3, Dia-2 *et* Ada) *et un par un locus tetra-allélíque (*Hx). *Les études sur la variation génétique, ont révelé l'existence d'hétérogéneité entre populations concernant les fréquences aux loci polymorphiques (à l'exception de* Pgd *et* Ca-2). *Le degré moyen d'hétérozygotie ($\geq 0,35$; $\leq 0,44$) et le pourcentage de loci polymorphiques ($\geq 41\%$; $\leq 53\%$) étaient pareils dans les différentes populations. La race Géante Espagnole montrait un nombre d'allèles/locus (1,76) légèrement plus bas que les autres races. Les coefficients de consanguinité étaient significativement différents de zéro dans toutes les populations, en oscilant entre 6% (Californienne) et 12% (Commune Espagnole et Fauve de Bourgogne). Les estimations de distances génétiques ont montré la possibilité d'établir trois groupes de populations, selon les similarités génétiques: (i) populations hautement sélectionnées, avec des croisements industriels (Néo-Zélandais Blanche et combinaison hybride); (ii) populations avec des systèmes d'élevage sans sélection (Commune Espagnole, Papillon et Fauve de Bourgogne), et la race Californienne, hautement sélectionnée; et (iii) races autochtones, considérées comme reliques (Géante Espagnole). Ces observations suggèrent fortement que les systèmes d'élevage actuels sont en train d'éteindre l'individualité des races quand ils favorisent des croisements entre races différentes du même système d'élevage ou quand on incorpore des gènes de races hautement selectionnées (par exemple, Californienne) dans le génome d'autres races, toutes elles, représentatives des populations actuelles de l'Espagne.*

Mots clés: *Polymorphismes biochimiques, marqueurs sanguins, variation génétique, distances génétiques, lapin.*

Introduction

The study of genetic distances among rabbit populations has been mainly carried out in wild rabbits, using one (Coggan et al., 1974) or four biochemical polymorphisms (Richardson, 1980; Richardson et al., 1980).

Given the importance of the preservation of the gene pool in the rabbit species and the increasing use of this species in livestock, we have initiated the genetic distance studies in domestic rabbit populations, using five polymorphic markers (Zaragoza et al., 1986).

Considering the possible application of these studies to breeding programs involving inter and intra-breed crosses, we have pursued this line of research, after these preliminary studies, making a methodological effort to increase the number of genetic markers under study, as well as assuring a representative number of animals from each of the breeds considered.

Accordingly, in this work domestic rabbits from the most representative breeds and breed combinations presently found in Spain were analysed, using 17 blood markers, for the purpose of studying the genetic characteristics, similarities and differences among these populations.

Materials and Methods

A total of 598 adult animals bred in Spain under captivity conditions were used in this study. They belong to 6 breeds: Spanish Common (102), Spanish Giant (67), Butterfly (79), Lyoné de Bourgogne (64), New Zealand White (105), Californian (81) and a hybrid combination (100 F1 individuals of crosses between purposefully selected New Zealand females and highly selected Californian males).

Seventeen blood proteins were studied in this work (Ak, Sod, Ca-1, Cat, Cp. Dia-1, Pgd, Hb, Es-1, Es-2, Ca-2, Est-7, Tf, Es-3, Dia-2, Ada, and Hx). The technical methods and statistical estimations were carried out according to the procedures applied by Larruga et al. (1983), Arana (1985), Zaragoza et al. (1985) and Zaragoza et al. (1987). The technique for identification of the protein Es-2 differs from that described in our previous studies (Zaragoza et al., 1985) in that the substrate used in this work is α -naphtyl propionate (2%). Genetic distances were estimated according to the methods described by Nei (1972) and by Cavalli-Sforza & Edwards (1967) with Edward's modification (1971). Dendrograms were elaborated using the UPGMA method of Sokal & Sneath (1963).

Results

Phenotypes and allelic frequencies

Of the 17 proteins studied eight were found monomorphic (Hb, Ca-1, Dia-1, Ak, Sod, Cat, Tf and Cp). The nine remaining polymorphic proteins were controlled by loci with two alleles (*Pgd, Es-1, Es-2, Ca-2* and *Est-7),* three alleles (*Es-3, Dia-2* and *Ada)* or four alleles (*Hx*).

As shown in Table 1, gene frequencies generally differed among breeds (specially when comparing the New Zealand breed or the hybrid combination with the remaining breeds). However, *Pgd* and *Ca-2* alleles showed similar frequencies among breeds.

A Hardy-Weinberg equilibrium situation was generally found with the exception of the Spanish Common breed, at disequilibrium for the *Es-2* locus ($p < 0.001$).

Inbreeding and genetic variation

Genetic variation was studied by estimating the degree of heterozygosity for each individual locus at each breed (D.H.) and for all loci in each bred or for all breeds at each locus (average degrees of heterozygosity, A.D.H.). D.H. values were low (≤ 0.11) for *Pgd* and *Ca-2,* intermediate (from 0.26 to 0.47) for *Es-1, Es-2* or *Est-7* and it reached high values (≥ 0.60) for the loci with highest number of alleles (*Es-3, Dia-2, Ada* and *Hx*) in several populations (Table 1).

In general, the expected D.H. showed slightly higher values than the observed D.H. (Table 1). Keeping this in mind, together with the observation that for most loci in the majority of the populations there appeared to be a tendency (insufficient to cause disequilibrium) towards an excess of homozygotes over the expected Hardy-Weinberg equilibrium proportions, (data not shown), inbreeding coefficients were estimated (average inbreeding coefficient f; Kidd et al., 1980). The f values were significantly different from zero, ranging from 6% in Californian or 7% in Spanish Giant to 12% in Spanish Common or Lyoné de Bourgogne.

D.H. differed among loci, reaching lower values ($\leq$ 0.07) for *Pgd* and *Ca-2,* intermediate values (≥ 0.33; $\leq$ 0.40) for *Dia-2, Est-7, Es-2* and *Es-1,* and high values (≥ 0.46, ≤ 0.54) for *Es-3, Ada* and *Hx.* On the contrary, D.H. were similar among breeds, ranging from 0.31 (New Zealand) to 0.38 (Spanish Giant). The corresponding standard deviations were high because eight of the 17 loci studied were monomorphic.

Although the percentage of polymorphic loci was similar among breeds, it was in Spanish Giant and Californian breeds slightly lower than in Lyoné de Bourgogne and New Zealand White breeds (see 95%

Table 1.

GENETIC STRUCTURE OF 7 RABBIT POPULATIONS ESTABLISHED WITH 17 GENETIC MARKERS: GENE FREQUENCIES, PARTIAL AND AVERAGE INBREEDING COEFFICIENTS, PARTIAL AND AVERAGE HETEROZYGOSITIES, % POLYMORPHIC LOCI AND AVERAGE NUMBER OF ALLELES/LOCUS.

Poly- ▼ morphic locus	Alleles and esti- mations	Individual populations ¶							Total population
		SC	SG	BU	LB	NZ	CA	HL	Average ± s. error
Pgd	Pgd^1	0.95	0.96	0.96	0.91	0.93	0.96	0.92	–
	Pgd^2	0.05	0.04	0.04	0.09	0.07	0.04	0.08	–
	Obs. het.	0.08	0.04	0.07	0.11	0.09	0.05	0.08	0.07 ± 0.01
	Exp. het.	0.09	0.07	0.07	0.16	0.13	0.07	0.15	0.11 ± 0.01
Es-1	$Es\text{-}1^A$	0.64	0.76	0.60	0.56	0.37	0.52	0.38	–
	$Es\text{-}1^B$	0.36	0.24	0.40	0.44	0.63	0.48	0.62	–
	Obs. het.	0.38	0.33	0.42	0.41	0.41	0.44	0.44	0.40 ± 0.02
	Exp. het.	0.46	0.36	0.48	0.49	0.46	0.50	0.47	0.46 ± 0.02
Es-2	$Es\text{-}2^F$	0.42	0.56	0.28	0.30	0.31	0.40	0.32	–
	$Es\text{-}2^S$	0.58	0.44	0.72	0.70	0.69	0.60	0.68	–
	Obs. het.	0.27	0.45	0.30	0.39	0.36	0.41	0.47	0.38 ± 0.02
	Exp. het.	0.49	0.49	0.40	0.42	0.43	0.48	0.43	0.45 ± 0.02
Ca-2	$Ca\text{-}2^F$	0.97	1.00	0.95	0.95	0.95	0.96	0.97	–
	$Ca\text{-}2^S$	0.03	0.00	0.05	0.05	0.05	0.04	0.03	–
	Obs. het.	0.06	–	0.07	0.08	0.04	0.05	0.06	0.05 ± 0.01
	Exp. het.	0.06	–	0.09	0.09	0.09	0.07	0.06	0.07 ± 0.01
Est-7	$Est\text{-}7^A$	0.68	0.80	0.79	0.64	0.55	0.60	0.46	–
	$Est\text{-}7^B$	0.32	0.20	0.21	0.36	0.45	0.40	0.54	–
	Obs het.	0.40	0.28	0.26	0.34	0.42	0.43	0.40	0.36 ± 0.02
	Exp. het.	0.43	0.32	0.33	046	0.49	0.48	0.49	0.43 ± 0.02
Es-3	$Es\text{-}3^A$	0.59	0.38	0.53	0.51	0.87	0.61	0.73	–
	$Es\text{-}3^B$	0.29	0.22	0.28	0.32	0.08	0.28	0.18	–
	$Es\text{-}3^C$	0.12	0.40	0.19	0.17	0.05	0.11	0.09	–
	Obs. het.	0.46	0.60	0.56	0.53	0.19	0.55	0.35	0.46 ± 0.02
	Exp. het.	0.55	0.65	0.60	0.64	0.23	0.54	0.43	0.51 ± 0.02
Dia-2	$Dia\text{-}2^A$	0.90	0.63	0.88	0.62	0.78	0.75	0.87	–
	$Dia\text{-}2^B$	0.04	0.28	0.08	0.34	0.14	0.16	0.02	–
	$Dia\text{-}2^C$	0.06	0.09	0.04	0.04	0.08	0.09	0.11	–
	Obs. het.	0.15	0.60	0.18	0.42	0.34	0.37	0.22	0.32 ± 0.03
	Exp. het.	0.18	0.52	0.22	0.50	0.36	0.40	0.23	0.34 ± 0.02
Ada	Ada^1	0.46	0.59	0.47	0.47	0.66	0.43	0.65	–
	Ada^2	0.40	0.32	0.39	0.33	0.29	0.42	0.26	–
	Ada^3	0.14	0.09	0.14	0.20	0.05	0.15	0.09	–
	Obs. het.	0.54	0.43	0.59	0.53	0.40	0.66	0.47	0.52 ± 0.02
	Exp. het.	0.61	0.54	0.61	0.63	0.48	0.62	0.50	0.57 ± 0.02
Hx	Hx^{1F}	0.15	0.18	0.16	0.08	0.07	0.13	0.10	–
	Hx^1	0.14	0.16	0.11	0.08	0.03	0.06	0.06	–
	Hx^2	0.42	0.35	0.43	0.59	0.67	0.62	0.69	–
	Hx^3	0.29	0.31	0.30	0.25	0.23	0.19	0.15	–
	Obs. het.	0.62	0.67	0.63	0.47	0.53	0.43	0.39	0.54 ± 0.02
	Exp. het.	0.69	0.72	0.69	0.58	0.49	0.56	0.49	0.60 ± 0.02
Total loci		17	17	17	17	17	17	17	–
Obs.avg.het. ± s.error		0.33 ± 0.03	0.38 ± 0.04	0.34 ± 0.03	0.36 ± 0.04	0.31 ± 0.03	0.37 ± 0.03	0.32 ± 0.03	
Exp.avg.het. ± s.error		0.39 ± 0.03	0.41 ± 0.04	0.39 ± 0.04	0.44 ± 0.04	0.35 ± 0.03	0.41 ± 0.04	0.36 ± 0.03	
Avg.inbreeding (f)		0.12 ± 0.03	0.07 ± 0.03	0.08 ± 0.03	0.12 ± 0.03	0.10 ± 0.03	0.06 ± 0.03	0.09 ± 0.03	
% Polym.loci(99% level)		52.94	47.05	52.94	52.94	52.94	52.94	52.94	
% Polym.loci(95% level)		47.05	41.17	47.05	52.94	52.94	41.17	47.05	
Avg. No. alleles/locus		1.82	1.76	1.82	1.82	1.82	1.82	1.82	

▼ The remaining 8 (*Ak, To, Ca-1, Cat, Día-1, Cp. Hb* and *Tf*) were monomorphic.
¶ SC = Spanish Common; SG = Spanish Giant; BU = Butterfly; LB = Lyoné de Bourgogne; NZ = New Zealand White; CA = Californian; HL = Hybrid combination.

Table 2.

GENETIC DISTANCE MATRIX FOR SEVEN DOMESTIC RABBIT POPULATIONS BRED IN SPAIN. (NEI'S ESTIMATION METHOD, 1972).

Populations	SC	SG	BU	LB	NZ	CA
SG	0,0370					
BU	0,5073	0,0394				
LB	0,0260	0,0497	0,0216			
NZ	0,0451	0,1061	0,0491	0,0377		
CA	0,0130	0,0541	0,0192	0,0113	0,0234	
HL	0,0407	0,1091	0,0488	0,0401	0,0072	0,0210

Table 3.

GENETIC DISTANCE MATRIX FOR SEVEN DOMESTIC RABBIT POPULATIONS BRED IN SPAIN (CAVALLI-SFORZA & EDWARDS ESTIMATION METHOD, 1967).

Populations	SC	SG	BU	LB	NZ	CA
SG	0,3933					
BU	0,1631	0,3775				
LB	0,3440	0,4016	0,2953			
NZ	0,4167	0,5972	0,4262	0,3986		
CA	0,2324	0,4250	0,2527	0,2185	0,3144	
HL	0,3786	0,6010	0,3989	0,4335	0,2414	0,3008

level, Table 1), being the first of these breeds the least polymorphic, due to fixation of the *Ca-2*^{F} allele (see 99% level and average number of alleles/locus, Table 1).

Genetic distances

Tables 2 and 3 illustrate the genetic distances estimated according to the procedures of Nei (1972) and Cavalli- Sforza and Edwards (1967), respectively. These data were used to elaborate the corresponding dendrograms, of which only one is shown (Fig. 1), given the similarity of results obtained by both estimation methods.

The Spanish Giant breed appears in a unique branch of the dendrogram, whereas the remaining breeds are grouped in another branch, which shows two major groupings, differentiating the New Zealand breed and the Hybrid combination from the remaining four populations. The latter also show two groups, one of them with the Lyoné de Bourgogne and Californian breeds and the other with the Butterfly and Spanish Common breeds.

Discussion

Phenotypes and allelic frequencies

The monomorphism observed for *Hb, Ak, Sod, Ca-1, Tf* and *Cp*, has also been observed by other authors (Dayhoff, 1972; Robinson & Osterhoff, 1983; Vergnes et al., 1974; Bernoco, 1969; Binette, 1976: Juneja et al., 1981) and in our previous work (Zaragoza et al., 1986). Other rabbit loci *(Cat* and *Ca-1),* also showing monomorphism, have been studied for the first time in this work.

The results on the electrophoretic variation observed for *Pgd, Ca-2, Es-1, Es-2, Es-3, Est-7, Ada* and *Hx* coincide with those obtained by other authors (Bernoco, 1969; Suzuki & Stormont, 1972; Coggan et al., 1974; Richardson et al., 1980; Peluso et al., 1982; Salermo et al., 1982) are in agreement with our previous findings (Zaragoza et al., 1985).

Also as in our previous studies (Arana & Zaragoza, 1986), evidence for three alleles at the *Dia-2* locus was found. Other authors, however have found either monomorphism (Vergnes et al., 1974) or diallelism (Richardson et al., 1980) at this locus, perhaps due to differences in the electrophoretic procedure applied, or in the gene frequencies of the populations studied.

The gene frequencies estimated for *Es-1* and *Es-3* are similar to those estimated in the work of Suzuki & Stormont (1972) in the New Zealand breed and in our previous studies (Zaragoza et al. 1987), except for the Spanish Giant breed.

This exception as well as the observed *Es-1* allelic frequency differences with respect to those estimated by Schiff & Stormont, (1967), in the New Zealand breed and by Grunder et al., (1965), in the Californian breed or the *Hx* allele frequency differences (with respect to those estimated by Grunder, 1966, or by Hagen et al., 1978) could be explained by the influence of factors such as genetic drift, founder effect, selection or inbreeding, on the gene frequencies of the populations sampled by different authors.

On the contrary, we believe that differences in *Es-2* gene frequencies with respect to those found by other authors (Schiff & Stormont, 1967) and in our previous studies (Zaragoza, et al., 1987) are most likely due to differences in the electrophoretic procedure applied (a different substrate was used in this study in an effort to improve band resolution). These technical differences could also explain that, in this study, lack of genetic equilibrium was only detected for *Es-2* in one breed (Spanish Common) whereas, in our previous studies it was detected in all populations (Zaragoza et al., 1987).

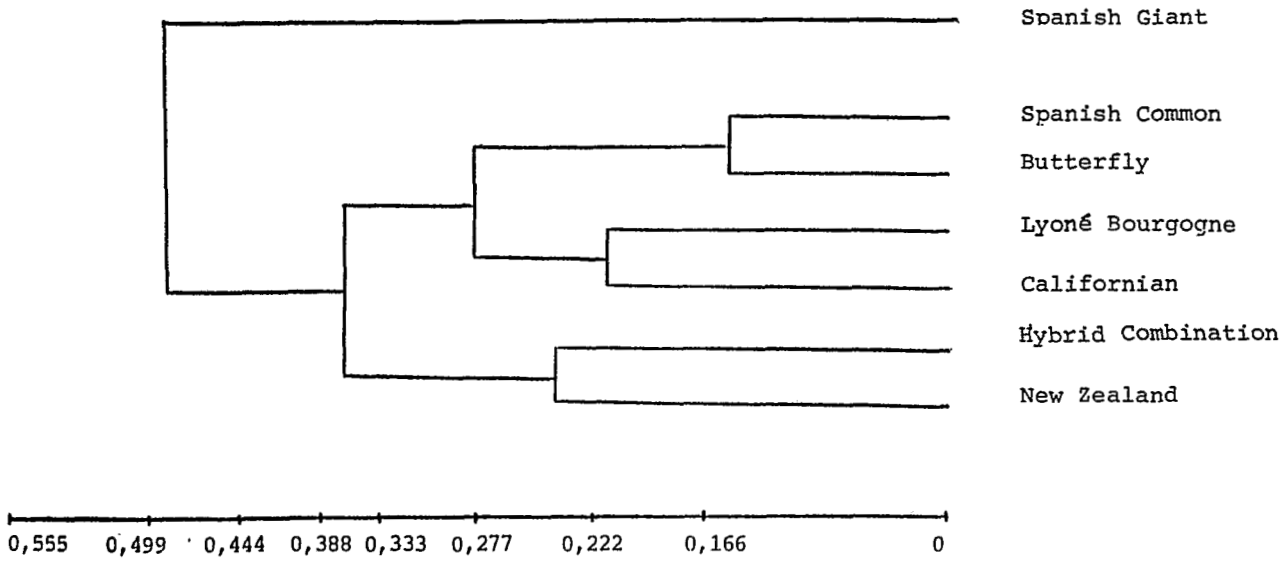

Fig. 1: Genetic dendrogram for 7 domestic rabbit populations bred in Spain (Cavalli-Sforza & Edwards distance estimation method, 1967).

Inbreeding and genetic distances

The fact that inbreeding in populations under non selective breeding systems (e.g. Spanish Common) is higher than that found in other populations, could be due to the smaller number of animals involved in reproduction and to the high frequency of matings among related individuals.

Also, the present breeding strategies appear to play an important role in the establishment of genetic distances. It is clear that, when studying the dendrogram obtained, highly selected, industrially bred populations (New Zealand White or hybrid combination) belong to a group distinct from other no industrially bred populations (Spanish Common, Butterfly, Lyoné de Bourgogne). To the latter group also belongs the highly industrialized Californian breed, which in our previous studies (Zaragoza et al., 1987) was assigned to the former group. The higher number of biochemical polymorphisms considered in this study may reflect the real genetic distances more accurately than that of our previous studies, strongly suggesting that the Californian population studied has been hybridized to non industrially bred populations (Lyoné de Bourgogne). Similarly, inter-breed crosses could explain the proximity of Spanish breeds (Spanish Common) to other breeds (Butterfly) reared under the same breeding system. These data confirm the observations made in our previous studies with regard to the jeopardizing effect on the breed's individuality that the present breeding strategies are originating.

On the other hand, finding a unique dendrogram branch, where the Spanish Giant breed belongs, is encouraging in that this autochtonous breed, presently considered as a relic, appears to be keeping its identity. As a natural breeding strategy, the relatively large size of the Spanish Giant individuals when compared to other individuals may have contributed to this isolation from other breeds.

In conclusion, the information presented in this work contributes to the knowledge of the genetic structure and to the characterization of the populations studied. It also helps identifying some of the genetic differences and similarities among the breeds presently bred in Spain under captivity conditions. These results are applicable to phylogenetic studies and are also of practical use as a warning sign for rabbit breeders, intending to carry out crosses among genetically distant populations, when searching for hybrid vigour.

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