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Blood biochemical polymorphisms in rabbits II. Genetic variation and distance among populations of Spanish wild rabbits and relationships with other rabbit populations

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SUMMARY - Blood samples from 412 Spanish wild rabbits belonging to six populations located in the Northern (Navarra 1), North-Eastern (Zaragoza 1, Zaragoza 2) and Central Spain (Toledo 2, Toledo 3) were tested for 17 genetic markers in this study, using starch and polyacrylamnide gel electrophoresis. Population studies revealed a higher level of genetic polymorphism for some of these 17 loci in wild rabbit populations, when compared to non wild rabbit populations. Only six loci (*Ak, To, Ca-1, Cat, Dia-1* and *Cp)* were monomorphic; seven *(Hb, Pgd, Es-1, Es-2, Ca-2, Es-7,* and *Tf)* were diallelic; two (*Dia-2* and *Est-3)* were triallelic; and the remaining two (*Ada* and *Hk)* were tetraallelic. Thus, the percentage of polymorphic loci (≥ 47; ≤ 58.8) is higher than in populations bred under captivity, specially in the Northern and Central wild rabbit populations (where the average number of alleles/locus is 1.94). The inbreeding coefficient in the total population, as estimated by Wright's method (1943), indicative of the Wahlund's effect (F = 5.66), was lower than that estimated by Kidd et al's. method (1980), attributable to consanguineous matings within the population (f = 15.62), the latter being higher than in other rabbit populations. The study of genetic distances among the five populations reveals that factors other than geographic distance (e.g. founder effect, geographic barriers such as rivers, mountains, etc.) may explain the findings that the Northern population can be grouped with two of the Central populations (Toledo 2 and Toledo 3) whereas the North-Eastern populations (Zaragoza 1 and Zaragoza 2) can be grouped with another Central population (Toledo 1). The former group shows more genetic similarities with populations bred under captivity than the latter.

Key words: Biochemical polymorphisms, blood markers, genetic variation, genetic distance, Spanish wild rabbits.

*RESUME - "Polymorphismes biochimiques sanguins chez le lapin. II. Variation génétique et distances entre populations de lapins sauvages espagnols et relation avec d'autres populations de lapins". On a obtenu des échantillons sanguins de 412 lapins de la race Sylvestre Espagnole, appartenant à six populations localisées dans le Nord (Navarra 1), Nord-Est (Zaragoza 1, Zaragoza 2) et Centre de l'Espagne (Toledo 1, Toledo 2, Toledo 3). On a étudié 17 marqueurs génétiques, par electrophorèse (amidon et polyacrylamide). Les études révèlent un niveau de polymorphisme génétique haut dans quelques loci quand on compare ces populations sylvestres avec des populations domestiques. Seulement six loci (*Ak, To, Ca-1, Cat, Dia-1 *et* Cp) *étaient monomorphiques; sept (*Hb, Pgd, Es-1, Es-2, Ca-2, Est-7 *et* Tf) *étaient dialléliques; deux (*Dia-2 *et* Es-3) *étaient trialléliques; et deux (*Ada *et* Hk) *étaient tétraalléliques. C'est pour cela que le pourcentage de loci polymorphiques (≥ 47;≤ 58,8) est plus haut que dans des populations domestiques, spécialement dans les populations sylvestres du Nord et du Centre (oœ le nombre moyen d'allèles/ locus est 1,94). Le coefficient de consanguinité dans la population totale, estimé par la méthode de Wright (1943) indicative de l'effet de Wahlund (F=5,66), était plus bas que l'estimé par la méthode de Kidd et al. (1980), comme mesure de la consanguinité (f= 15,62). Ce dernier coefficient était plus haut que l'observé dans d'autres populations de lapins. Les estimations des distances génétiques entre cinq populations montrent l'existence de facteurs (effet fondateur, barrières géographiques comme des rivières, montagnes, etc.) qui pourraient, plutôt que les distances géographiques, expliquer le fait que la population du Nord peut être groupée avec deux populations Centrales (Toledo 2 et Toledo 3) pendant que les populations du Nord-Est (Zaragoza 1 et Zaragoza 2) peuvent être groupées avec la population Centrale Toledo 1. Le premier groupe montre plus de similitude génétique avec des populations domestiques que le dernier groupe.*

***Mots clés:** Polymorphismes biochimiques, marqueurs sanguins, variation génétique, distances génétiques, lapins sylvestres espagnols.*

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Introduction

Spanish wild rabbits represent an important part of the total rabbit populations of the Iberian Peninsula, being increasingly used for meat production.

These wild rabbits have contributed to the origins of other rabbit breeds, not only in Spain (Spanish Giant and Spanish Common breeds) but also in other countries, where they have been established by natural migration or other means.

Studies on the genetic structure and characteristics of wild rabbits have been initiated in some countries (Richardson et al., 1980). In Spain we have also initiated the study of wild rabbit genetic markers (Arana & Zaragoza, 1986). In this work, data on the genetic characteristics of the Spanish wild rabbit populations and on the distances among them and other populations of this species are provided.

Material and methods

A total of 412 wild rabbits were tested in this work. They belonged to populations isolated from each other by geographical barriers (rivers, mountains, etc.) and located in various points within the Iberian Peninsula (Fig. 1): Northern Spain (Navarra 1, 53 animals), North-Eastern Spain (Zaragoza 1, 53 animals; Zaragoza 2, 111 animals) and Central Spain (Toledo 1, 50 animals; Toledo 2, 48 animals; Toledo 3, 97 animals). A special care was taken in that the populations sampled had not been manipulated (transportation of animals, breeding, etc.) by man. Animals were captured by live trapping or shooting. Blood samples were obtained from the ear's marginal vein, jugular, liver or heart and were collected in heparinized tubes (110 heparin units/ml.).

Seventeen blood proteins were studied in this work (*Ak, Sod, Ca-1, Cat, Cp, Dia-1, Pgd, Hb, Es-1, Es-2, Es-3, Est-7, Ca-2, Tf, Dia-2, Ada* and *Hx).*

Statistical estimations and electrophoretic procedures were done according to the procedures used by Larruga et al. (1983), Zaragoza et al. (1987) and Arana et al. (1989).

Inbreeding coefficients in the total population, estimated according to Kidd et al.'s (1980) procedure, will be referred to as f, whereas the inbreeding coefficient reflecting the Wahlund's effect (Wright, 1943) will be designated as F.

Genetic distances were estimated according to the methods described by Nei (1972) and Cavalli-Sforza & Edwards (1967) with Edwards's modification (1971). Dendrograms were elaborated using the UPGMA method of Sokal & Sneath (1963).

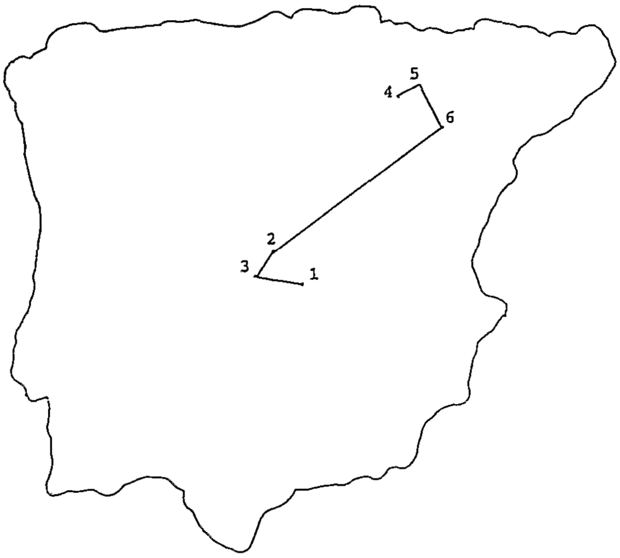

Fig. 1: Localization of populations analyzed in the Iberian Peninsula.

1 = Toledo 1.
2 = Toledo 2.
3 = Toledo 3.
4 = Navarra 1.
5 = Zaragoza 1.

Distances: from 1 to 3 = ~ 150 km.
from 3 to 2 = ~ 50 km.
from 2 to 6 = ~ 450 km.
from 6 to 5 = ~ 120 km.
from 5 to 4 = ~ 50 km.

Results

Genetic structure of populations

As shown in Table 1, of the 17 proteins analysed, 11 are controlled by polymorphic loci. Of the latter, seven are diallelic (*Pgd, Hb, Es-1, Es-2, Ca-2, Est-7* and *Tf),* two are triallelic (*Dia-2 and Es-3)* and two are tetraallelic (*Ada* and *Hx).* The remaining six proteins show monomorphism (*Ca-1, Dia-1, Ak, Sod, Cat* and *Cp).*

Hb 1, Tf 2 and Ada 4 variants, specific to wild rabbit populations (Arana et al., 1989), showed different distributions: Hb1 was only present in the Northern population (Navarra 1), Tf 2 was present in all six populations and Ada 4 was only present in the three Central populations (Toledo 1, Toledo 2 and Toledo 3). This explains why the level of polymorphism (average number of alleles per locus) was particularly high in the Northern and Central populations. Other variants, controlled by the tetraallelic loci (*Ada* and *Hx)* or by the triallelic loci (*Es-3* and *Dia-2),* also differed in frequencies among populations (Table 1).

Table 1.

GENETIC STUCTURE OF 6 SPANISH WILD 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

Polimorphic▼ locus	Alleles and estimations¶	Individual populations						Total population		
		Toledo 1	Toledo 2	Toledo 3	Navarra 1	Zaragoza 1	Zaragoza 2	Average±s.error	F(%)±s.error	f(%)±s.error
Pgd	*Pgd*^{1}	0.95	0.80	0.86	0.85	0.92	0.96			
	Pgd^{2}	0.05	0.20	0.14	0.15	0.08	0.04			
	Obs. het.	0.06	0.14	0.22	0.15	0.11	0.01	0.11 ± 0.01	2.53 ± 0.007	4.2 ± 0.00
	Exp. het.	0.09	0.32	0.24	0.25	0.15	0.08	0.19 ± 0.03		
Hb	*Hb*^{1}				0.11					
	Hb^{2}	1.00	1.00	1.00	0.89	1.00	1.00			
	Obs. het.				0.18			0.03 ± 0.02	N.E.	N.E.
	Exp. het.				0.19			0.03 ± 0.02		
Es-1	*Es-1*^{A}	0.41	0.49	0.64	0.48	0.53	0.37			
	Es-1^{B}	0.59	0.51	0.36	0.52	0.47	0.63			
	Obs. het.	0.38	0.35	0.46	0.39	0.37	0.48	0.40 ± 0.02	4.25 ± 0.009	16 ± 0.01
	Exp. het.	0.48	0.50	0.46	0.50	0.50	0.47	0.48 ± 0.02		
Es-2	*Es-2*^{F}	0.31	0.36	0.49	0.38	0.41	0.41			
	Es-2^{S}	0.69	0.64	0.51	0.62	0.59	0.59			
	Obs. het.	0.38	0.35	0.40	0.37	0.39	0.41	0.38 ± 0.02	1.37 ± 0.005	19 ± 0.01
	Exp. het.	0.42	0.46	0.50	0.47	0.48	0.48	0.47 ± 0.02		
Ca-2	*Ca-2*^{F}	0.92	0.97	0.97	0.98	1.00	1.00			
	Ca-2^{S}	0.08	0.03	0.03	0.02					
	Obs. het.	0.12	0.06	0.05	0.03			0.04 ± 0.01	3.75 ± 0.009	20 ± 0.01
	Exp. het.	0.15	0.06	0.06	0.04			0.05 ± 0.01		
Est-7	Est-7^{A}	0.40	0.49	0.32	0.47	0.55	0.39			
	Est-7^{B}	0.60	0.51	0.68	0.53	0.45	0.61			
	Obs. het.	0.40	0.37	0.37	0.41	0.41	0.40	0.40 ± 0.02	2.36 ± 0.007	16 ± 0.018
	Exp. het.	0.48	0.50	0.43	0.50	0.50	0.47	0.48 ± 0.02		
Tf	*Tf*^{1}	0.82	0.95	0.86	0.92	0.92	0.88			
	Tf^{2}	0.18	0.05	0.14	0.08	0.08	0.12			
	Obs. het.	0.28	0.10	0.24	0.15	0.13	0.21	0.18 ± 0.02	2.29 ± 0.007	5 ± 0.009
	Exp. het.	0.29	0.09	0.24	0.15	0.15	0.21	0.19 ± 0.02		
Es-3	*Es-3*^{A}	0.27	0.32	0.50	0.29	0.40	0.40			
	Es-3^{B}	0.46	0.40	0.32	0.27	0.38	0.33			
	Es-3^{C}	0.27	0.28	0.18	0.44	0.22	0.27			
	Obs. het.	0.52	0.31	0.45	0.30	0.62	0.48	0.45 ± 0.02	N.E.	29 ± 0.022
	Exp. het.	0.64	0.66	0.61	0.65	0.65	0.61	0.64 ± 0.05		
Dia-2	*Dia-2*^{A}	0.68	0.80	0.93	0.89	0.44	0.46			
	Dia-2^{B}	0.28	0.05	0.03	0.05	0.54	0.52			
	Dia-2^{C}	0.04	0.15	0.04	0.06	0.02	0.02			
	Obs. het.	0.38	0.18	0.14	0.13	0.30	0.62	0.29 ± 0.02	19.8 ± 0.019	19 ± 0.019
	Exp. het.	0.46	0.33	0.13	0.20	0.51	0.52	0.36 ± 0.02		
Ada	*Ada*^{1}	0.27	0.63	0.65	0.52	0.51	0.49			
	Ada^{2}	0.55	0.35	0.29	0.41	0.44	0.43			
	Ada^{3}	0.13	0.10	0.03	0.07	0.05	0.08			
	Ada^{4}	0.05	0.02	0.03						
	Obs. het.	0.50	0.54	0.31	0.52	0.54	0.58	0.50 ± 0.02	5.34 ± 0.009	12 ± 0.018
	Exp. het.	0.60	0.68	0.49	0.51	0.54	0.57	0.57 ± 0.02		

Table 1. (Continued)

Polimorphic▼ locus	Alleles and estimations¶	Individual populations						Total populations		
		Toledo 1	Toledo 2	Toledo 3	Navarra 1	Zaragoza 1	Zaragoza 2	Average±s.error	F(%)±s.error	f(%)±s.error
Hx	Hx^{1F}		0.19	0.11	0.13		0,04			
	Hx^{1}		0.18	0.14	0.13		0,10			
	Hx^{2}	0.57	0.49	0.49	0.56	0.83	0,55			
	Hx^{3}	0.43	0.14	0.26	0.18	0.17	0,31			
	Obs. het.	0.50	0.45	0.56	0.47	0.22	0,66	0.48 ± 0.02	4.57 ± 0.009	16 ± 0.018
	Exp. het.	0.49	0.72	0.66	0.62	0.28	0,59	0.56 ± 0.02		
Total loci		17	17	17	17	17	17			
Obs. avg. het. ± s.error		0.32 ± 0.04	0.26 ± 0.04	0.29 ± 0.04	0.28 ± 0.04	0.28 ± 0.04	0.35 ± 0.03			
Exp. avg. het. ± s.error		0.37 ± 0.04	0.39 ± 0.04	0.35 ± 0.03	0.34 ± 0.04	0.37 ± 0.04	0.36 ± 0.05			
Avg. inbreeding $(\overline{F})$±s.error							5.66 ± 0.010			
Avg. inbreeding $(\overline{f})$±s.error							15.62 ± 0.017			
% Polym.loci(99% level)		58.82	58.82	58.82	64.70	52.94	52.94			
% Polym.loci(95% level)		58.82	52.94	52.94	58.82	52.94	47.05			
Avg.No. alles/locus		1.82	1.94	1.94	1.94	1.70	1.82			

▼ The remaining 6 loci (*Ak, To, Ca-1, Cat, Día-1 and Cp*) were monomorphic.
¶ Standard errors of gene frequencies were not included since they vere < 0.03.
N.E. = Non estimated, given all the populations were not at equilibrium.

Table 2.

X^2 VALUES FOR DIFFERENCES BETWEEN OBSERVED PHENOTYPIC FREQUENCIES AND THOSE EXPECTED AT HARDY-WEINBERG EQUILIBRIUM (ONLY LOCI WITH SIGNIFICANT DIFFERENCES).

Locus	Population	d.f.	X^2_{eq}
Es-3	Toledo 2	3	32.75***
	Toledo 3	3	20.55***
	Navarra 1	3	25.23***
	Zaragoza 2	3	27.26***

***P < 0.001

Although heterozygosity varied among loci (e.g. *Pgd*, *Ca-2* and *Tf* showed a lower average degree of heterozygosity, ≤ 0.19), similar average degrees of heterozygosity were observed among populations, when considering all loci (≥ 0.34, ≤ 0.39; Table 1).

The value of the inbreeding coefficient (F = 5.66%) due to the Wahlund effect (genetic drift upon total population subdivision) was lower than that reflecting consanguinity within each population (f= 15.62%). Both, F and f estimated values differed significantly from zero (Table 1).

Table 3.

GENE FREQUENCIES AND X^2_{EQ} IN THE TOTAL WILD RABBIT POPULATION TESTED (N=412), CORRESPONDING TO FOUR POLYMORPHIC LOCI SHOWING HOMOGENEITY AMONG POPULATIONS.

Locus	Allele	(Frequencies)	Allele	(Frequencies)	X^{o2}_{eq}
Es-2	$Es\text{-}2^F$	(0.40)	$Es\text{-}2^S$	*(0.60)*	*13.96****
Ca-2	$Ca\text{-}2^F$	(0.98)	$Ca\text{-}2^F$	*(0.02)*	*N.E.*
Est-7	$Est\text{-}7^A$	(0.43)	$Est\text{-}7^B$	*(0.57)*	*15.86****
Tf	Tf^1	(0.88)	Tf^2	*(0.12)*	*0.02(N.S.)*

***P < 0.01
N.E. = Non estimated, given the low number of homozygous Ca-2-S individuals.
(N.S.) = Non significant; P > 0.05.

Genetic disequilibrium was detected for *Es-3* (Table 1) in four populations, due to an excess of homozygous individuals (Table 2). It was also detected in the total population for *Es-2* and *Est-7*. According to homogeneity X^2 tests (data not shown), both of these loci, together with *Ca-2* and *Tf* showed similar gene frequencies and phenotype distributions in all six populations; Tables 1 and 3).

Table 4.

GENETIC DISTANCE MATRIX (NEI'S ESTIMATION METHOD, 1972) FOR SIX SPANISH WILD RABBIT POPULATIONS.

Populations	Toledo 2	Toledo 3	Navarra 1	Zaragoza 1	Zaragoza 2
Toledo 1	0.0434	0.0511	0.0332	0.0353	0.0203
Toledo 2		0.0190	0.0160	0.0493	0.0440
Toledo 3			0.0224	0.0625	0.0531
Navarra 1				0.0490	0.0422
Zaragoza 1					0.0158

Tabla 5.

GENETIC DISTANCE MATRIX FOR SIX SPANISH WILD RABBIT POPULATIONS (CAVALLI-SFORZA & EDWARD'S ESTIMATION METHOD, 1967).

Populations	Toledo 2	Toledo 3	Navarra 1	Zaragoza 1	Zaragoza 2
Toledo 1	0.6315	0.5964	0.5976	0.4523	0.4453
Toledo 2		0.3177	0.3208	0.6643	0.5422
Toledo 3			0.3822	0.6568	0.5499
Navarra 1				0.6299	0.5185
Zaragoza 1					0.3559

Table 6.

GENETIC DISTANCE MATRIX (NEI'S ESTIMATION METHOD, 1972) AMONG SIX SPANISH WILD RABBIT POPULATIONS AND SEVEN DOMESTIC RABBIT POPULATIONS.

Populations	Toledo 2	Toledo 3	Navarra 1	Zaragoza 1	Zaragoza 2	Spanish Common	Spanish Giant	Butterfly	Lyoné Bourg.	New Zealand	Californian	Hybrid Combination
Toledo 1	0.0556	0.0682	0.0397	0.0457	0.0266	0.0678	0.1080	0.0683	0.0400	0.0856	0.0435	0.0733
Toledo 2		0.0236	0.0114	0.0672	0.0594	0.0365	0.0660	0.0414	0.0362	0.0571	0.0329	0.0336
Toledo 3			0.0269	0.0837	0.0714	0.0346	0.0760	0.0591	0.0551	0.0565	0.0383	0.0354
Navarra 1				0.0637	0.0538	0.0378	0.0663	0.0418	0.0407	0.0611	0.0320	0.0387
Zaragoza 1					0.0211	0.0785	0.0753	0.0790	0.0239	0.0710	0.0387	0.0736
Zaragoza 2						0.0822	0.0888	0.0873	0.0342	0.0676	0.0455	0.0675
Spanish Common							0.0370	0.5073	0.0260	0.0451	0.0130	0.0407
Spanish Giant								0.0394	0.0497	0.1061	0.0541	0.1091
Butterfly									0.0216	0.0491	0.0192	0.0488
Lyoné Bourgogne										0.0377	0.0113	0.0401
New Zealand											0.0234	0.0072
Californian												0.0210

Genetic relationships

Genetic distances among the six populations were estimated using the data presented in Table 1 on 11 polymorphic loci (a total of 28 alleles). Tables 4 and 5 illustrate the distances obtained by the methods of Nei (1972) and Cavalli-Sforza & Edwards (1967), respectively.

Similar dendrograms are obtained by both methods and therefore only one of them is illustrated. As shown in Fig. 2, there are two major groups of populations. One of them is formed by the North-Eastern population (Zaragoza 1 and Zaragoza 2) and one of the Central populations (Toledo 1). The other is formed by the Northern population (Navarra 1) and two of the Central populations (Toledo 2 and Toledo 3).

Wild rabbit populations were also compared with domestic rabbit populations to establish the genetic differences among them, using data on 9 polymorphic loci (Table 1 and Arana et al., 1989; Zaragoza et al., 1990). Tables 6 and 7 show the estimated genetic distances which are geographicaly represented in Fig. 3 (dendrogram on distances estimated by the method Cavalli-Sforza & Edwards, 1967). Three of the six wild rabbit populations, (Navarra 1, Toledo 2 and Toledo 3) showed more genetic similarities with non wild rabbit populations than others,

Table 7.

GENETIC DISTANCE MATRIX (CAVALLI-SFORZA & EDWARDS'S ESTIMATION METHOD, 1967) AMONG SIX SPANISH WILD RABBIT POPULATIONS AND SEVEN DOMESTIC RABBIT POPULATIONS.

Populations	Toledo 2	Toledo 3	Navarra 1	Zaragoza 1	Zaragoza 2	Spanish Common	Spanish Giant	Butterfly	Lyoné Bourg.	New Zealand	Californian	Hybrid Combination
Toledo 1	0.6168	0.5954	0.5491	0.4419	0.4421	0.6024	0.6825	0.5764	0.4555	0.6108	0.4980	0.6300
Toledo 2		0.3014	0.2347	0.6631	0.5360	0.3674	0.4805	0.3970	0.4166	0.5124	0.3800	0.3713
Toledo 3			0.3098	0.6539	0.5446	0.3484	0.5242	0.4342	0.4739	0.4670	0.4030	0.3620
Navarra 1				0.5920	0.4697	0.3512	0.4531	0.3635	0.4036	0.4959	0.3606	0.3791
Zaragoza 1					0.3533	0.6481	0.5998	0.6236	0.4221	0.5604	0.4909	0.6330
Zaragoza 2						0.5411	0.4850	0.5276	0.3400	0.5179	0.4120	0.5656
Spanish Common							0.3933	0.1631	0.3400	0.4167	0.2324	0.3786
Spanish Giant								0.3775	0.4016	0.5972	0.4250	0.6010
Butterfly									0.2953	0.4262	0.2527	0.3989
Lyoné Bourgogne										0.3986	0.2185	0.4339
New Zealand											0.3144	0.2414
Californian												0.3008

Table 8.

GENETIC DISTANCE MATRIX (NEI'ESTIMATION METHOD, 1972) AMONG WILD RABBIT POPULATIONS FROM SPAIN (6) AND FROM TASMANIA, GREAT BRITAIN, FRANCE AND AUSTRALIAN (3).

Populations	Toledo 2	Toledo 3	Navarra 1	Zaragoza 1	Zaragoza 2	Tasmania	Great Britain	France	South East. Aust.	West Aust.	North West Aust.
Toledo 1	0.0554	0.0666	0.0238	0.0222	0.0144	0.0905	0.0254	0.1988	0.0359	0.0377	0.0282
Toledo 2		0.0107	0.0884	0.0154	0.0234	0.0421	0.0407	0.1505	0.0168	0.0235	0.0277
Toledo 3			0.0158	0.0138	0.0388	0.0393	0.0315	0.1200	0.0122	0.0098	0.0556
Navarra 1				0.0027	0.0083	0.0557	0.0253	0.1617	0.0106	0.0137	0.0178
Zaragoza 1					0.0097	0.0545	0.0174	0.1545	0.0073	0.0057	0.0255
Zaragoza 2						0.0637	0.0301	0.1847	0.0164	0.0259	0.0056
Tasmania							0.0344	0.0520	0.0273	0.0450	0.0707
Great Britain								0.1133	0.0172	0.0111	0.0540
France									0.0984	0.1359	0.1925
South East Aust.										0.0099	0.0276
West Aust.											0.0505

Table 9.

GENETIC DISTANCE MATRIX (NEI'ESTIMATION METHOD, 1972) AMONG WILD RABBIT POPULATIONS FROM SPAIN (6) AND FROM TASMANIA, GREAT BRITAIN, FRANCE AND AUSTRALIA (3).

Populations	Toledo 2	Toledo 3	Navarra 1	Zaragoza 1	Zaragoza 2	Tasmania	Great Britain	France	South East. Aust.	West Aust.	North West Aust.
Toledo 1	0.3077	0.3327	0.2715	0.3058	0.2806	0.4200	0.2778	0.5594	0.3089	0.3259	0.2691
Toledo 2		0.1509	0.1579	0.2349	0.2681	0.3683	0.3814	0.5379	0.2721	0.2740	0.2344
Toledo 3			0.1955	0.2208	0.2973	0.3929	0.3822	0.4872	0.2233	0.2435	0.2926
Navarra 1				0.1236	0.1712	0.4011	0.3359	0.5402	0.2107	0.1995	0.1624
Zaragoza 1					0.1241	0.4542	0.3481	0.5921	0.2332	0.1178	0.2171
Zaragoza 2						0.4451	0.3329	0.6186	0.2596	0.1773	0.1616
Tasmania							0.2563	0.3444	0.3165	0.4128	0.3914
Great Britain								0.4486	0.2754	0.2896	0.3453
France									0.3696	0.5778	0.5357
South East Aust.										0.2483	0.2162
West Aust.											0.2865

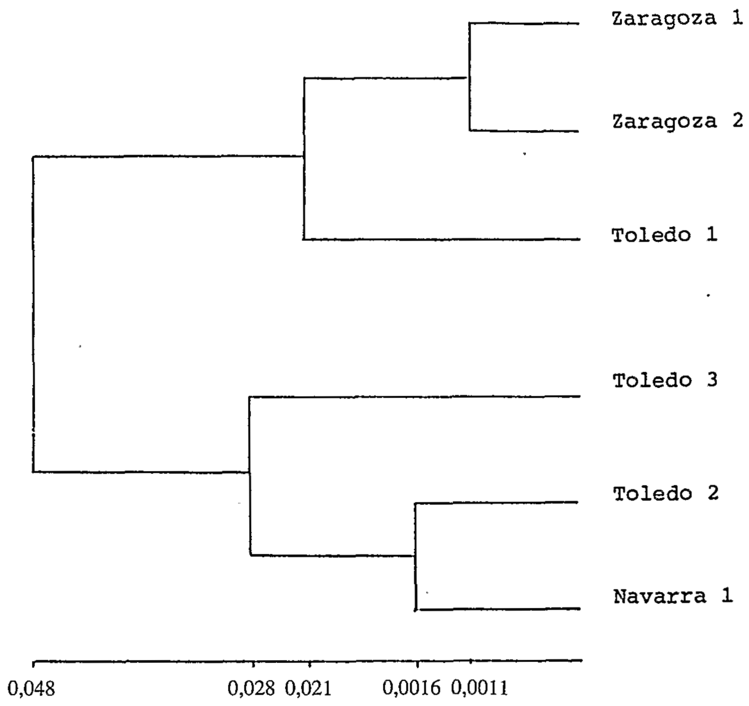

Fig. 2: Genetic distance dendrogram for six Spanish wild rabbit populations, obtained from the distance matrix (Nei's distance estimation method, 1972).

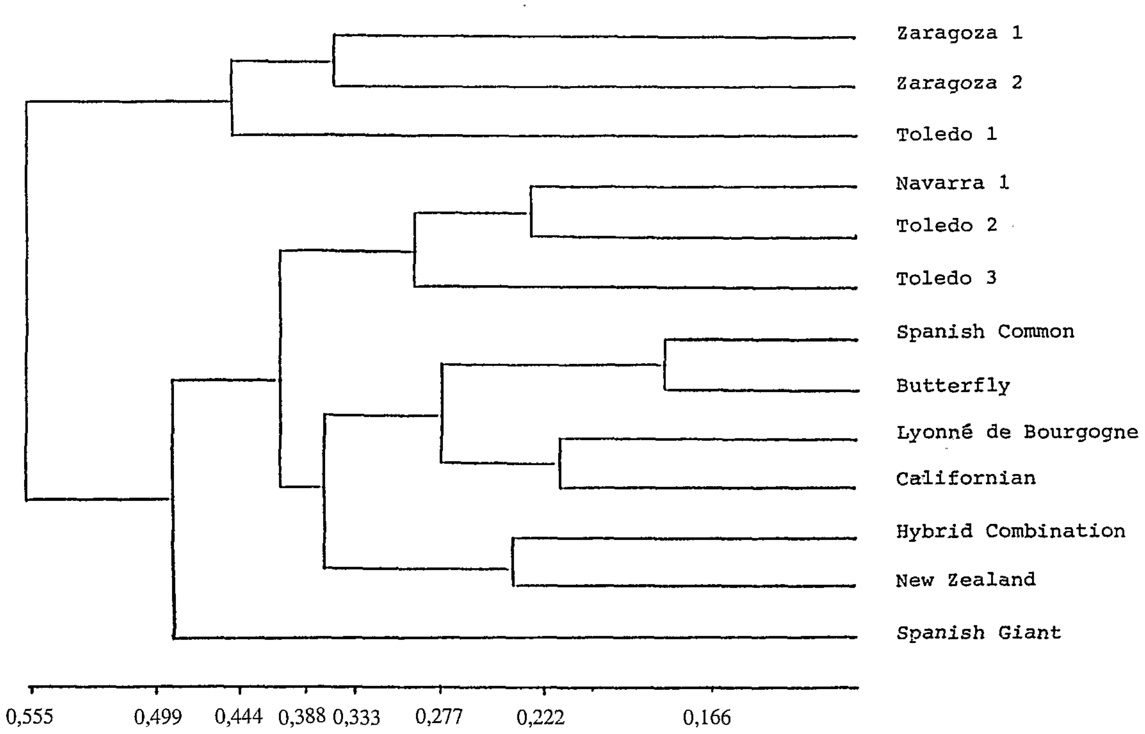

Fig. 3: Genetic distance dendrogram from six rabbit populations and seven domestic rabbit populations, obtained from the distance matrix (Cavalli-Sforza & Edward's distance estimation method, 1967).

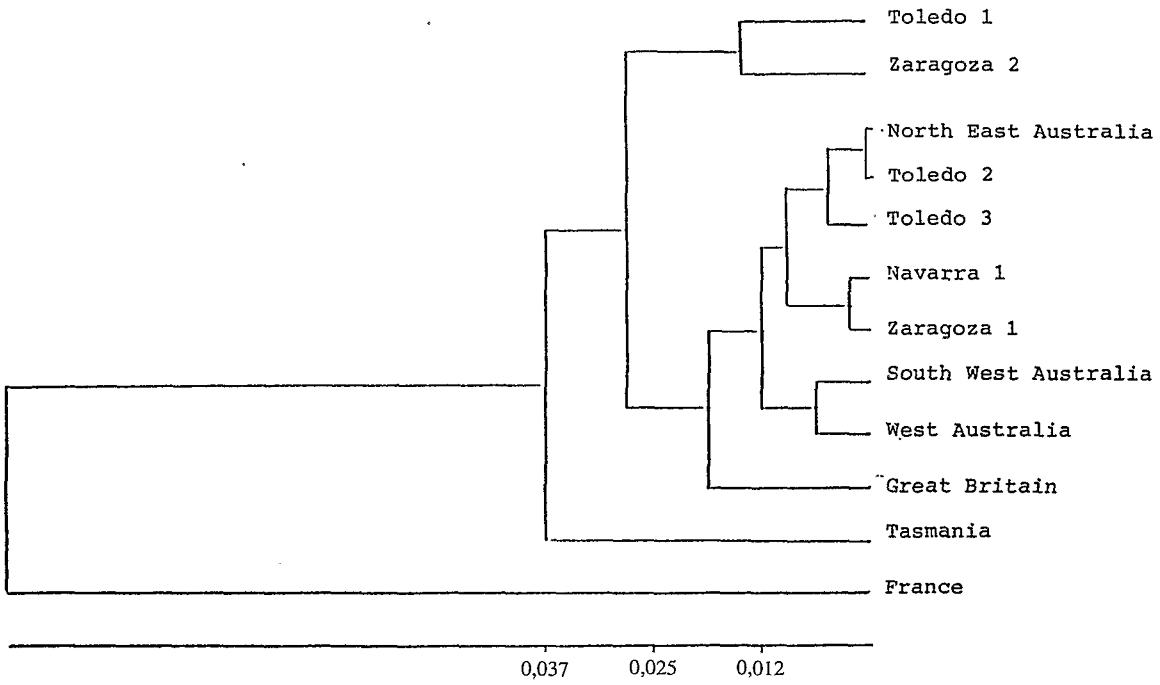

Fig. 4: Genetic distance dendrogram for wild rabbit populations from Spain (6) and Tasmania, Great Britain, France and Australia (3) (Nei's, 1972 distance estimation method).

sharing dendrogram branches, although they can be recognized as a group.

All of the groups of wild rabbit populations which were identified when considering wild rabbits alone, can still be identified in this overall study. Similar to the results obtained when studying non wild populations (Zaragoza et al. 1990), the Spanish Giant breed appeared as the most differentiated population. Using Nei's distances, this breed even appeared in an independent branch (data not shown).

Spanish wild rabbit populations were also compared to wild rabbit populations from other countries, for which the genetic distances had been estimated using 4 biochemical polymorphisms by Richardson et al., (1980). Thus, the study had to be restricted to these polymorphisms (Tables 8 and 9) and hence these results may be considered as preliminary. Dendrograms in Figs. 4 and 5 represent the genetic distances obtained by different estimation methods. In both cases, Spanish and Australian populations are genetically more similar to each other, when compared to British, Tasmanian and French populations.

Discussion

Phenotypes, allelic frequencies and inbreeding

The data presented in this work clearly show that the Spanish wild rabbit populations studied have analogous gene and genotype frequencies for particular loci (*Es-2, Est-7, Ca-2, Tf*). This conclusion cannot be made extensive to domestic breeds (Zaragoza et al., 1990). On the other hand, differentiation within the total Spanish wild rabbit population, is evidenced by other polymorphic loci considered in this work (*Hb, Ada, Hx, etc.*), whose gene frequencies differ among the populations analysed. These differences could be attributable to genetic drift, founder effect, isolation, mutation or natural selection.

When comparing the gene frequencies observed in this work with those estimated in wild rabbit populations from other countries (Coggan et al., 1974; Richardson et al., 1980) there are similarities in the results obtained (high variation) for *Ada* and *Dia-2* but there are also differences (low variation in frequencies) for *Pgd, Ca-2* and *Es-1*.

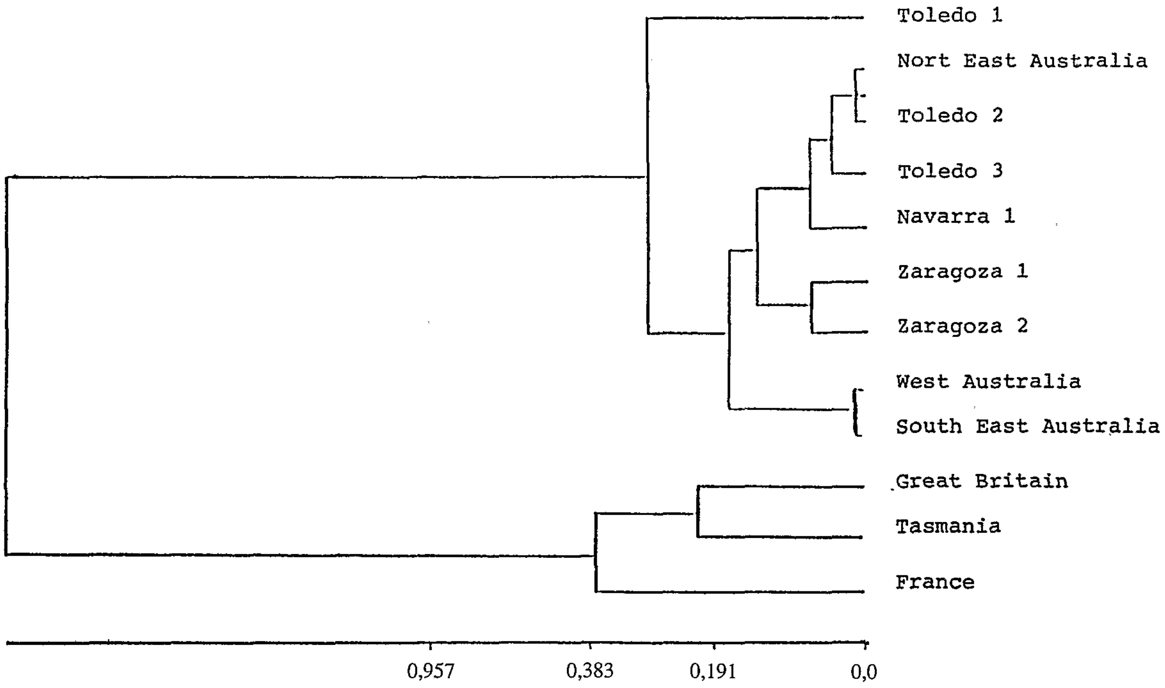

Fig. 5: Genetic distance dendrogam for wild rabbit populations from Spain (6) and from Tasmania, Great Britain, France and Australia (3) (Cavalli Sforza & Edward's, 1967 distance estimation method).

Genetic disequilibrium at the *Es-2* locus could be explained by the factors (electrophoretic procedure) described in domestic populations (Zaragoza et al., 1990). On the contrary, disequilibrium at the *Es-3* and *Est-7* loci (only occurring in wild rabbit populations) is unlikely due to methodological procedures. Conditions such as genetic drift, or inbreeding are more likely. In this sense, other authors have attributed disequilibrium in wild rabbit populations at the *Ada, Es-1* and *Ca-2* loci to a Wahlund effect, this effect was also observed in this work (F = 5.66%).

In the wild rabbit populations analysed, the inbreeding coefficient (f = 15.76%) appears to be higher than in other populations (≤ 12%; Zaragoza et al., 1990), probably because, in the natural environment, matings often occur between related individuals, specially when geographical barriers separate populations.

Also, when compared to other populations bred in Spain, the wild rabbit populations analysed show a higher genetic variation (average degree of heterozygosity/population, percentage of polymorphic loci and average number of alleles/locus). As indicated in our previous work (Arana et al., 1989), some of the allelic variants at the *Hb, Ada* and *Tf* loci have only been detected so far in wild rabbits. These findings strongly suggest that there is a high genetic potential in wild rabbits. Whether this is relevant to production, reproduction, disease resistance, etc., is at present unknown.

Genetic similarities among populations

From the data presented in this work, it is clear that two groups of Spanish wild rabbit populations, formed according to genetic distances, do not correspond to those expected according to their geographical distribution (distances) within the Iberian Peninsula. These results indicate that the degree of migration and the number of individual exchanges among populations is relatively small, when geographical barriers (mountains, rivers, etc.) are present. It is likely that conditions like hunting, mixomatosis, etc., affected the population as genetic drift factors. At the same time, natural selection might also be reponsible for the prevalence of certain alleles, affecting the individuals either randomly or through a possible selective (physiological) advantage of a particular genetic make-up within the ecological environment.

With regard to the origin of variants which cause major differences between wild and domestic rabbit populations (i.e. those that only appear in the wild populations tested), it is likely that they were already present in the ancestral rabbit populations, and that factors like founder effect or the mentioned genetic drift or natural selection (or artificial selection of domestic breeds) have eliminated them either within the wild population (i.e. Hb 1 variant is only present in the Northern population, and Ada 4 variant in the Central populations), or in the process of creation of domestic breeds. It is however unlikely that these new variants have appeared as spontaneous mutations after the domestic breeds have been obtained and are only detectable in wild rabbits. This latter possibility could be explained if a selective disavantage (concernig the productive and reproductive traits) was associated with these new variants, which were thus automatically eliminated from breeds subjected to man-driven selection (domestic breeds). Experimental approaches to these observations may elucidate which of these explanations reflects reality.

With regard to genetic similarities among wild rabbit populations from Spain and from other countries, it is rather surprising that Spanish populations are genetically closer to Australian populations than to British populations. Historical data (Rolls, 1969) reveal that rabbits were introduced in Australia through British populations.

Considering that only the genetic markers (four) used by Richardson et al. (1980) to study British, Tasmanian, French and Australian wild rabbit populations were considered for this study, it is likely that the apparent relationships found change when increasing the number of genetic markers compared among all these populations. The fact that this latter comparative study should be taken as preliminary (few markers involved) is suggested by the noticeable differences obtained in these dendrograms when distances have been estimated by different methods (Nei's vs. Cavalli-Sforza and Edwards'). On the other hand, it is also possible that factors like founder effect, genetic drift, etc., may have affected the gene frequencies so that some of the results obtained could still be compatible with the historical data on the world's wild rabbit populations.

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