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ELECTROPHORETIC MARKERS OF ANDALUSIAN HORSES: COMPARISON OF SPANISH AND LUSITANIAN LINEAGES

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Abstract—1. Genetic variants at eight blood loci were analysed, disclosing in Andalusian breed six rare markers: variants J of transferrin, H of esterase, D and S of Xk, M and W of prealbumin.

2. Two of these, Tf^J and Pr^M appear as characteristic markers of Andalusian breed.

3. Allelic frequencies showed minor differences between Spanish (300 horses) and Lusitanian (100 horses) populations.

4. Comparison was established with historically related breeds, Thoroughbreds or Connemara, and with Arab horses because of a presumed relationship.

5. No visible similarities in genetic profiles were found with two former breeds, nor with Arab horses.

6. Unpredicted similarities were found however between Tarpans and Andalusian horses, appearing rather as convergences than witnessing a common ancestry.

INTRODUCTION

The investigations on likeliness or distinctiveness of various horse breeds have been carried out in our laboratory for the past 10 years with respect of the "genetic structure" based on electrophoretic variants of blood proteins (Kaminski, 1978, 1982, 1984). The array of studied breeds is seldom fruit of a deliberate choice commanded by scientific reasons; mostly it is imposed by the availability of experimental material. Studies on the origin or relatedness of breeds are thus often jeopardized or else are limited.

The case of Andalusian horses joins scientific interest to availability of animal material. Investigations on this breed have been initiated by one of the authors as the object of a doctoral thesis concerning 7 loci in 115 horses (Andres Cara and Rodero, 1985). A preliminary note on 194 Spanish horses disclosed the presence of three genetic variants, uncommon or rare (Andres Cara and Kaminski, 1984). The distribution of one of these was compared in Andalusian and other horse breeds (Kaminski and Andres Cara, 1984).

The present work reports complete data on 8 loci in 300 Spanish and 100 Lusitanian horses, both populations belonging to Andalusian breed. Besides phenotype distribution, family data are reported to bring evidence of the allelic character of a fourth rare variant, found in this breed.

Comparisons of "genetic profiles" of this breed with others are discussed on the basis of data obtained in this laboratory; thus Tarpans share with Andalusians two rare genetic markers, while Arab, Barb, Thoroughbred horses and Connemara ponies are thought to have been related to Spanish horses in one way or another.

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Prehistorical and historical background

The present day horse breeds are evidently not natural populations, still less the original ones. Since the old times when horses began to be mounted, then domesticated, human needs and fancies modified the genetic pools of ancestral populations through migrations and invasions. Further on, with progress of civilization, the needs and fancies changed in time and from one human group to another. Importations of horses bearing desired particular characteristics, then breeding habits with arbitrary matchings and crosses weighed heavily on ancestral horse populations, many of which having been destroyed and probably all having been modified.

The admixture of ancient local breeds with imported lines on the one hand and the lack of precise information on the initial stock of several newly created equine types on the other hand greatly confuse the patterns of distribution of the current breeds today.

Only a few present day horse populations of Europe, North Africa and the Arabian peninsula can be traced back to prehistorical equine forms. Some pony breeds of north-western Europe seem to have preserved the ancestral morphology owing to almost natural breeding conditions in little modified environment during many centuries; it is believed that they derive from the primitive wild horses of the European continent (Kaminski and Urbanska-Nicolas, 1979).

The most renowned and relatively ancient "pure blood" breed, the Arab horses, previously called Oriental, are believed to originate from "Aryan" type horses as opposed to the "Mongolian" type (Blomac et Bogros, 1978). Diverse nomadic pastoral groups brought these two types of horses from Central Asia to Fertile Crescent. The Mongolian type equids have invaded Europe, especially its north-western part, while the Aryan type spread to the Caucasian area, then to Greece. In Mesopotamia, the Semitic or

Chamitic peoples used Aryan type horses for hunting and fighting; thus Assyrian warriors mounted horses very much like the later "Arabian" breed. The Arabian peninsula has been gradually invaded by diverse Arabian populations bringing horses with them, while the previous inhabitants bred camels; in ancient Libya however it seems that horses and camels were bred simultaneously. In the southern regions, desertic or semi-arid, to keep horses was a very hard task demanding privations from the owners; thus they were rare and precious. Bedouin family groups bred female lineages, each line being probably quite in-bred. Horses were used for "razzias", rapid and short plundering expeditions, or for races during festive days; for both, speed performances were necessary. Thus horses have undergone a severe selection, both due to rough and frugal environment and to the owners requirements. The current Arab horse emerged slowly, although it appears that there has never existed a single homogenous population; on the contrary, various subtypes were selected together with low quality products without recognized lineage. After Hegira the Arab populations proceeded westward along the north African coast; in these countries were bred horses of probably ancient African roots, later on called "Barb". Between "Arab" and "Barb" horses there are morphological differences, the latter being heavier than the former; during centuries they coexisted and were used for similar purposes, battles, races and "fantasias". Some Barb lines were bred pure, but many were and still are, cross-bred with Arabs of low quality.

A different horse was sheltered south of the Iberic peninsula (Andrade, 1979). The "Iberic" and "Barb" horses are believed to be descended from the same ancestors, brought by a population coming from Egypt, related to the Hittites. These people settled in north Africa then passed to the Iberic peninsula sometime around the thirteenth century before our era; they were further called Berbers in Africa by Arabs and Iberians in Europe by Greeks. According to other sources, Iberian horses have been mounted as early as 5000 years before our era and were the most anciently known warriors' horses. They were prized by Greeks; for Romans the Iberic cavalry was the best of all, and their horses were used to improve other breeds.

Among the aptitudes of Iberic horses were speed and a unique behaviour developed during fight. This latter particularity, maintained and developed by training, led eventually to the use of Spanish horses for parades and bull-fighting. The Iberic breed has probably been somewhat cross-bred with Barb horses, present on the peninsula; as to Arab blood, on the contrary, there was no or only little admixture, since "oriental" horses were not as widely distributed in old times. Under the names of Andalusian or Spanish horses in Europe, Genet d'Espagne (from Ginesta, designating particular battle display) in France, they went their way until the seventeenth century, used for military or festive purposes, and to improve other breeds. Thus the Irish ponies, Connemara, would be of Andalusian descent. Later on, the Lippizaner or Kladruber were created with the sole contribution of Spanish breed-founders; in general it is believed that Andalusian blood is present in all the so-called warm blood European horses. The most glorious example is the Thoroughbred horse, created in England in the early eighteenth century by breeding 40 "Royal Mares" selected for speed from local stocks to three oriental-type imported stallions: among the mares a good proportion were either pure Spanish or of Spanish descent. With time the military strategy evolved and the artillery needed strong carrier horses instead of the majestic pace of Andalusians, the breeding of which declined in Europe. Simultaneously, for mounting and hunting as well as breed improvers the Arab horses penetrated upward in the European breeding centres.

MATERIALS AND METHODS

Blood samples from the following breeds and populations were analysed in this laboratory for the present work or previously.

Andalusian breed

The horses studied originated from two breeding areas: Spain and Portugal.

Spanish population. This comprises exclusively horses belonging to National Studs; all were purebred, many being of Cartujanes lineages i.e. the oldest purebred lineages available. Two groups were obtained for study. (a) 115 horses bled in 1981, analysed at 7 loci for doctorate thesis (Andres Cara and Rodero, 1985) and (b) 194 blood samples taken in 1983 (Andres Cara and Kaminski, 1984); some of the latter were already analysed previously, while some sera or haemolysates stored frozen were analysed for new polymorphic systems. Globally, 232 horses were analysed afresh in 1983–84, while for 68 we lack data on prealbumin and Xk loci, and on Tf^F subtypes.

There were no complete families in these groups and only a few pairs mare-progeny or stallion-progeny: most of the horses belonged to a single generation.

Horses of Lusitanian breeding, imported or born in France. Several private breeders or owners contributed to this study; numbers of horses ranged from 3 to 44 and the global number reached 101. Horses from one stud were sometimes related to those of other studs, as parents or progeny; the family relationships showed common ancestors 3, 4 or 5 generations upward. Several sire families or pairs mare-progeny were available, as well as groups of full-sibs or half-sibs.

The genealogies of Spanish horses were taken from the Stud Book; these of French horses were provided by L. Fabre, the official keeper of genealogic records of Andalusian horses in France; only for a few horses were the genealogical data lacking.

Other breeds

1. Arabs and mixed-breed population were described in detail (Kaminski and Andres Cara, 1984).
2. Barb horses (Podliachouk *et al.*, 1978).
3. Connemara ponies (Kaminski and Urbanska-Nicolas, 1979; Kaminski 1984).
4. Thoroughbreds from France (Kaminski, 1984).
5. Data for Thoroughbreds from the UK were obtained in 1982 by personal communication from A. M. Scott.

Electrophoretic techniques

Acid prealbumin gels. The method used was that of Scott (1979), derived from the original technique of Braend (1970). The first author recognized in Norwegian horses the 7 allelic variants F, I, L, N, S, T and W, while Scott observed in Arab horses F, G, I, L, N, S, U and Z. Two more alleles, D_1 and D_2 have been described by Braend (1980).

Table 1A. Compared phenotypes distribution of albumin, esterase and Xk in Andalusian and other breeds of horses

	Andalusian		Arab		Barb	Connemara	Tarpan	Mixed breed	Selle	Thoroughbred
N	300 Sp.	101 Fr.	898		36	278	190	89	Fr. 100	Fr. 5948
Albumin										
F	96	19	180		5	70	34	15	69	268
FS	154	48	458		21	143	73	42	401	2018
S	50	34	260		10	65	83	32	530	3662
Esterase										
F	—	—	—		1	12	17	3	6	19
FG	3	2	6		—	17	24	2	9	—
FH	—	1	—		—	—	8	—	—	—
FI	7	13	4		21	48	31	14	125	640
FS	—	1	3		—	2	—	—	3	22
G	20	7	9		—	18	12	3	6	—
GH	2	—	—		—	—	—	—	—	—
GI	100	22	13		113	60	26	14	110	—
GS	—	1	—		—	1	—	—	1	—
HI	14	1	—		—	—	16	4	—	—
I	153	51	672		22	115	55	48	678	4939
IS	1	2	35		—	4	—	—	62	328
Xk				Arab					n.d.	Th. U.K.
			France	Tunisia	Poland	n.d.				
N = 232			111	38	810		*N* = 68			*N* = 500
D	—	—	—	—	—		1	—		—
DF	5	3	1	1	—		19	3		—
DS	1	—	—	—	—		—	—		—
F	216	87	101	37	705		48	73		500
FS	10	11	9	—	104		—	8		—
S	—	—	—	—	1		—	1		—

Table 1B. Albumin, esterase and Xk allelic frequencies

	Andalusian		Arab		Barb	Connemara	Tarpan	Mixed breed	Selle Français	Thoroughbred
Al										
F	0.576	0.425	0.455		0.430	0.510	0.371	0.404	0.270	0.210
S	0.423	0.575	0.545		0.570	0.490	0.629	0.596	0.730	0.790
Es										
F	0.016	0.084	0.028		0.014	0.164	0.255	0.123	0.070	0.058
G	0.242	0.183	0.086		0.180	0.205	0.195	0.123	0.070	—
H	0.027	0.010	—		—	—	0.063	0.022	—	—
I	0.713	0.703	0.864		0.805	0.615	0.481	0.719	0.830	0.914
S	0.001	0.020	0.021		—	0.012	—	—	0.030	0.028
Xk				Arab					n.d.	Th. U.K.
			France	Tunisia	Poland	n.d.				
D	0.013	0.015	0.012	0.013	—		0.154	0.017		—
F	0.963	0.925	0.958	0.987	0.935		0.845	0.882		1.00
S	0.023	0.060	0.030	—	0.065		—	0.056		—

The band M was observed and named by Scott during the International Comparison Test of 1979; this author has not observed bands T nor W (1982, personal communication). In our laboratory T was not encountered, but W was found in one Spanish horse. The same band was named Q by Bell, during the International Comparison Test of 1983.

Altogether, various horses tested in our laboratory disclosed the 11 patterns of bands: F, G, I, L, M, N, S_1, S_2, U, W and Z.

For typing of prealbumins, gels of 13 × 22 cm are used, containing 11.6% of starch. The gel buffer of pH 4.5 contains 116 ml of 0.05 M citric acid and 48 ml of 0.2 M Tris for 1 l. The electrode buffer is 0.03 M boric acid and 1 M NaOH. Fifteen serum samples are run on each gel on a cooling plate at −5°C under a constant voltage of 300 V for 30 min; the inserts being removed, the voltage is increased to 400, then to 500 V. In 4 hr the boundary has migrated 7 cm. One slice is revealed for esterase activity, the second for proteins. The genetic systems phenotyped are Pr, Xk and acid Es.

Alkaline polyacrylamide gels. The original method of Gahne *et al.* (1977) of horizontal electrophoresis with a discontinuous gradient of porosity was adapted by Scott (personal communication, 1982) and used with further minor modifications.

A step gradient of acrylamide concentration of 8, 5 and 12% was used. The gel buffer of pH 7.8 is Tris-sulphate, 0.09375 M of Tris; the electrode buffer, pH 9, is Tris-borate, 0.065 M of Tris.

The gel dimensions are 26 × 20 cm; 60 samples are run simultaneously, on a cooling plate at −5°C. The current is at first set at 60 mA for 6 min; after removing the paper slips the current is 60 W constant during 5–6 hours. The gels are revealed for esterase activity, then stained in Coomassie blue.

Three genetic systems are phenotyped: alkaline Es, Xk and Tf (separation of F into F_1 and F_2).

The remaining systems, Al and the enzymes of haemolysate were analysed as in the previous work.

RESULTS

Phenotypes were recorded at eight loci in various experimental conditions. Tables 1–6 report data on both populations of Andalusian horses compared with a set of other breeds.

The distribution of phenotypes and allelic frequencies for horse populations studied previously were reported for Barb horses (Podliachouk *et al.*,

Table 2A. Compared phenotype distribution and allelic frequencies of transferrin[1]

Breed phenotypes	Andalusian Spain $N = 300$		Andalusian France $N = 101$		Arab Poland $N = 898$	
D	32 D_1	D_1 0.343	12	D_1 0.317	73	D 0.323
	3 D_1D_2	D_2 0.014				
DF	66 D_1F_2		12 DF_2		386 DF_2	
	1 D_2F_2					
	1 D_1H_2					
DH	36 D_1H_1		6		31	
	3 D_2H_1					
DJ	9		5		—	
DO	21 D_1O		9		17	
	1 D_2O					
DR	6		7		—	
F	36 F_2	F_2 0.312	9	F_2 0.245	289 F_2	F_2 0.592
FH	35 F_2H		3 F_2H		54 F_2H	
FJ	3 F_2J		2 F_2J			
FO	8 F_2O		8 F_2O		45 F_2O	
FR	2 F_2R		7 F_2R		—	
H	15	H 0.206	—	H 0.100	2	H 0.050
HJ	7		2		—	
HO	10		7		—	
HR	3		2		—	
J	1	J 0.066	—	J 0.060	—	
JO	1		3		—	
O	—	O 0.068	2	O 0.176	—	O 0.034
OR	—		5		—	
R	—	R 0.018	—	R 0.103	—	

(1) In addition to the 6 alleles first described, namely D, F, H, L, O, R, certain breeds or populations possess other allelic variants, internationally recognized such as D_2, F_3, H_2 (various International Comparison Tests). The variant J, although described in 1974, has not yet had acquired the international recognition, lacking of family studies. In this laboratory D_1 and D_2, F ($F_1 + F_2$) and F_3 are separated on starch gels at pH 7.4. On acrylamide gels are separated F_1 and F_2, H_1 and H_2.

Table 2B. Compared phenotype distribution and allelic frequencies of transferrin

Breed phenotypes	Barb (1) (2) Morocco $N = 36$		Connemara pony France $N = 278$		Saddle or draught imported Eastern Europe $N = 89$	
D	1	D 0.222	15 D_1	D_1 0.203	2 D_1	D_1 0.185
				D_2 0.010	2 D_2	D_2 0.022
DF	10		52 $D_1F_{1,2}$		2 D_1F_1	
			5 $D_2F_{1,2}$		14 D_1F_2	
			14 D_1F_3			
DH	3		2 D_1H		2 D_1H	
DJ	—		—		—	
DO	1		5 D_1O		4 D_1O	
			1 D_2O			
DR	—		10 D_1R		7 D_1R	
F	6		78 $F_{1,2}$	$F_{1,2}$ 0.514	1 F_1	F_1 0.061
			22 $F_{1,2}F_3$	F_3 0.106	4 F_1F_2	F_2 0.404
			9 F_3		12 F_2	
FH	3		7 F_12H		3 F_1H	
			1 F_3H		11 F_2H	
FJ	—		—		—	
FO	3		13 $F_{1,2}O$		13 F_2O	
					1 F_2M	
FR	2		31 $F_{1,2}R$		5 F_2R	
			4 F_3R			
H	1	H 0.138	—	H 0.030	1	H 0.101
HJ	—		—		—	
HO	1		3		—	
HR	1		3		—	
J	—		—		—	
JO	—		—		1 M	M 0.005
O	3	O 0.168	—	O 0.039	1	O 0.106
OR	1		—		—	
R	—	R 0.055	3	R 0.097	5	R 0.123

(1) Population not phenotyped at 7.4. (2) Population not phenotyped on acrylamide gel.

Table 2C. Compared phenotype distribution and allelic frequencies of Transferrin

Breed phenotypes	Selle Français (1) $N = 1\,000$		Thoroughbred (1) France $N = 5\,948$		Thoroughbred (2) UK $N = 532$	
D	97 D_1	D_1 0.350	587 D	D 0.325	55	D 0.322
	1 D_1D_2	D_2 0.013				
	1 D_2					
DF	269 $D_1F_{1,2}$		1859 $DF_{1,2}$		112 DF_1	
	17 $D_2F_{1,2}$				61 DF_2	
	3 D_1F_3					
DH	64 D_1H		157		10	
	2 D_2H					
DJ	—		—		—	
DO	39 D_1O		400		36	
	2 D_2O					
DR	130 D_1R		270		14	
	3 D_2R					
F	212 $F_{1,2}$	$F_{1,2}$ 0.418	1280 $F_{1,2}$	F 0.469	44 F_1	F_1 0.400
	7 $F_{1,2}$	F_3 0.007			54 F_1F_2	F_2 0.177
	1 F_3				15 F_2	
FH	88 $F_{1,2}H$		226		13 F_1H	
					12 F_2H	
FJ	2 $F_{1,2}J$	J 0.045	—		—	
FO	91 $F_{1,2}O$		551		35 F_1O	
	2 F_3O				22 F_2O	
FR	27 $F_{1,2}R$		382		11 F_1R	
	1 F_3R				10 F_2R	
H	9	H 0.100	9 H	0.040	—	H 0.042
HJ	—		—		—	
HO	19		49		7	
HR	9		24		3	
J	—		—		—	
JO	—		—		—	
O	6	O 0.084	53	O 0.100	6	O 0.116
OR	4		81		12	
R	2	R 0.089	25	R 0.068		R 0.047

(1) Population not phenotyped on acrylamide gel. (2) Personal communication from A. M. Scott.

Table 3. Prealbumin phenotypes and allelic frequencies in Andalusian horses

Spain $N = 232$		France $N = 101$	
FL	0	FL	1
FS	0	FS	1
G	1	G	0
GL	1	GL	0
GM	1	GM	0
GS	5	GS	1
GU	1	GU	0
I	1	I	0
IL	1	IL	0
IM	4	IM	1
IS	3	IS	0
L	25	L	4
LM	4	LM	10
LN	0	LN	1
LS	35	LS	26
LU	5	LU	5
M	3	M	2
MS	27	MS	9
MU	2	MU	3
MW	1	MW	0
N	1	N	0
NS	4	NS	4
S	92	S	19
SU	15	SU	14
5 most frequent phenotypes (in %)			
S	0.396	LS	0.257
LS	0.151	S	0.188
MS	0.116	SU	0.138
L	0.107	LM	0.099
SU	0.064	MS	0.089
Total	0.834		0.771

1978), Tarpans (Tomaszewska-Guszkiewcz and Kaminski, 1980) and Selle Français (Kaminski and Nicolas, 1981); for others, data have been recently completed, either because a new series of samples was made available, or because new techniques permitted to observe variants that have gone undetected previously: Arabs from Poland, Connemara ponies, mixed-breed group (Kaminski and Andres Cara, 1984), Thoroughbred horses; finally, data on English horses, Thoroughbreds and Arabs are due to Scott (1980 and unpublished results).

Tables 7 and 8 report genealogical data for some variants present in the Lusitanian population.

Albumin

Only two allelic variants have been observed in both populations of Andalusian horses; there are differences in frequencies, the Spanish group having more of the fast variant. In general, frequencies are around 0.5 for Andalusian, Arab, Connemara and the mixed-breed horses, while Tarpans, Selle Français and most Thoroughbreds show higher proportions of Al^s. The heterozygous phenotype FS is predominant in all tested populations, except in Thoroughbreds.

Transferrin

The eleven variants observed in this laboratory are detected either in starch gels or on acrylamide sheets;

Table 4. Prealbumins: compared allelic frequencies

Allelic variant	Breed Andalusian ref. this paper Spain	France	Thoroughbred Scott, 1982; Pollitt and Bell, 1980 UK	Australia	Arab Scott, 1979 UK	Norwegian Braend, 1970 Norway	Döle
$N =$	232	101	1219	750	386	111	122
F	—	0.010	0.068	0.062	0.16	0.032	0.102
G	0.021	0.005	0.031	0.019	0.11	—	—
I	0.021	0.005	0.068	0.071	—	0.045	0.004
L	0.207	0.252	0.461	0.427	0.35	0.207	0.070
M	0.097	0.133	—	—	—	—	—
N	0.013	0.025	0.173	0.200	0.01	0.203	0.635
S	0.588	0.460	0.078	S_1 0.033 S_2 0.058	0.14	0.374	0.156
T	—	—	—	—	—	0.063	0.008
U	0.050	0.109	0.122	0.130	0.17	—	—
W	0.002	—	—	—	—	0.077	0.025
Z	—	—	—	—	0.06	—	—

they are D or D_1, D_2, F_1, F_2, F_3, H or H_1, H_2, J, M, O and R. The Andalusian horses disclosed 9 variants, lacking F_3 and M. One horse has shown H_2 and 7 sera contained D_2; the former does not seem established in the population, while the latter is shared, at similar frequency, with Connemara, Selle Français and the group of mixed-breed horses from Eastern Europe. The distribution of Tf^{D_2} does not seem to be breed specific.

Concerning the five most widespread variants of transferrin, frequencies of D ($D_1 + D_2$) in Andalusian breed are superior to those of Connemara, close to those of Arab, Thoroughbred or Selle Français.

In general, Tf^F is the most frequent variant; in both populations of Andalusian horses it is however significantly less common than in compared breeds. When F is split into F_1 and F_2, their distribution appears breed dependent; thus Thoroughbreds carry both variants, with higher frequency of the former, while Standardbreds lack the F_1 (McGuire and Weitkamp, 1980). Further studies confirmed the quantitative preponderance of Tf^{F_1} in Thoroughbreds (Scott, 1982). Population data of this laboratory disclosed that in both groups of Andalusian horses as well as in Arab populations from Poland and Tunisia the sole variant detected was F_2; among 81 Arab horses from France tested recently, 69 carried transferrin F, out of which 3 were F_1 F_2, none had F_1 alone and 66 had F_2 alone. Therefore in these two breeds F_2 is largely predominant. F_1 was found in the mixed-breed horses; in this group 11.2% horses carrying F displayed it against 69% in the Thoroughbred population. During the International Comparison Test of 1982–83 organized by the Australian laboratory only four out of 40 horses carried Tf^{F_1}; two were Thoroughbreds, one a cross Thoroughbred × Arab, the last was indicated as Standardbred. Since McGuire and Weitkamp state that 750 Standardbred horses were tested and none carried F_1, it appears astonishing that there were no comments on the above case of the Comparison Test. Concerning further the presence and the proportions of F_1 and F_2 among other breeds, recent data on 67 horses belonging to Connemara, Dartmoor, Fjording, Haflinger, Icelandic, Merens, New Forest, Pottok and Welsh ponies, disclosed among 55 sera containing transferrin F, 50 carrying F_2 alone, 3 heterozygotes F_1F_2 and 2 carrying F_1 alone. Finally, the variant F_3 was found in 3 pony breeds (Kaminski and Urbanska-Nicolas 1979, the designation F_2 used in that work corresponds actually to F_3) mainly Connemara, and in low amounts in Selle Français (Kaminski and Nicolas 1981).

Frequencies of variants Tf^H, Tf^O and Tf^R, although variable in different breeds, generally do not exceed 0.15. The Andalusian horses from Spain show more than usual of Tf^H, while those of France display notable level of Tf^O. Tf^R is very poorly represented among Spanish populations, a little more in France.

The most interesting transferrin variant present among Andalusian horses is Tf^J discovered in an Andalusian stallion in USA (Trommershausen-Smith, 1974); the electrophoretic band J was located between those of H and M. No population studies of registered breeds, nor inheritance analysis were reported, but in this laboratory band J was observed in 31 sera out of nearly 40,000 examined between 1975 and 1983. Horses bearing J were in majority of "unknown origin" or registered as "saddle" with no mention of a breed, a few of them were Selle Français or Trotteur Français.

Using some of these sera it could be demonstrated that, immunochemically, J belongs to the subgroup of "fast-migrating" variants i.e. D, F and H, all four sharing the same antigenic determinants, while the "slow-migrating" variants, M, O and R have a different antigenic structure: the locus of transferrin synthesizes two subgroups of allelic variants (Kaminski *et al.*, 1981).

In Andalusian breed the observed phenotypes were DJ, FJ, HJ, JO and JR, as well as one homozygous animal J in the spanish population (Andres Cara and Kaminski, 1984) (Fig. 1). The French group contained four pairs mare-progeny, both animals carrying J in their phenotype, the second variant being different. Such overall phenotype distribution on the one hand and the phenotypes observed in mare-progeny cases prove that Tf^J is inherited in simple Mendelian way.

In spite of a higher frequency of Tf^J, the French group did not disclose the homozygous J phenotype. In fact, analysis of phenotypes of stallions and mares available in various breeding centres showed that such an event was highly improbable if not impossible. In the Spanish group, studies of genealogical records demonstrated that the 20 analysed horses bearing Tf^J belong to one lineage, having in common, as ascendant up to five generations upward, the

Table 5. Phenotypes of haemolysate loci and allelic frequencies

	Andalusian				Arab		Barb		Connemara		Tarpan		Selle Français		Thoroughbred	
	300		101		891		36		278				169		1000	4500
6-PGD			D 0.01						D 0.014						D 0.01	
DF	—			2	—	—				5	—			17		
DS										3				3		PGD^F 0.57
F	226	F 0.870	74	F 0.091	115	F 0.393	30	F 0.092	215	F 0.876	130	F 0.882	632	F 0.800		
FS	69		16		471		6		52		38		309			PGD^S 0.43
S	5	S 0.131	—	S 0.08	305	S 0.607	0	S 0.08	3	S 0.110	1	S 0.118	39	S 0.190		
PGM																
F	9	F 0.146	1	F 0.034	134	F 0.389	0	F 0.01	9	F 0.207	1	F 0.131	5	F 0.050		
FS	70		5		425		1		97		41		91			PGM^S 1.0
S	221	S 0.854	95	S 0.966	332	S 0.611	35	S 0.99	140	S 0.793	127	S 0.869	903	S 0.950		
SV	—		—								—		1			
PHI																
F	1	F 0.070		F 0.054		F 0.016	0	F 0.010		F 0.001		F 0.021	1	F 0.01		
FI	40		11		28		1		1		7		20	I 0.99		PHI^I 1.0
I	258	I 0.930	76	S 0.946	863	I 0.884	35	I 0.990	243	I 0.996	176	I 0.973	977			
IS	1	S 0.001	—		—		0		2	S 0.003	2	S 0.006	2			

Table 6. Distribution of some rare allelic variants in different breeds of horses

	Andalusian	Arab	Connemara	Thoroughbred	Tarpan
Tf^J	+	−	−	−	−
Tf^{F1}	−	−	−	+	n.d.
Tf^{F2}	+	+	+	+	
Es^H	+	−	−	−	+
Es^S	+	+	+	+	−
Xk^S	+	+	n.d.	−	−
Pr^M	+	−	n.d.	−	+
Xk^D	+	+	n.d.	−	+

renowned stallion Lebrero (born in 1934). Among his 127 descendants studied the frequency of Tf^J amounts to 0.157, indeed above that of the total population. Among Andalusian horses in France, Tf^J is distributed along 2 lineages; one mare on the one hand and one stallion on the other have transmitted this variant to their 2 and 4 descendants respectively. It is worth notice that the descendants of one full-sib and one half-sib of the above stallion, both carrying J, have not inherited it (Table 7).

Concerning the breed distribution Tf^J appears in the present state of analysis as highly characteristic of Andalusian horses, being absent in most European breeds. As to its occurrence in France, sprinkled among half-blood or no-breed animals, it could conceivably be a remnant of the former periods where the large popularity of Andalusian horses led to their contribution to horse populations in Europe, except to the pure-bred lines.

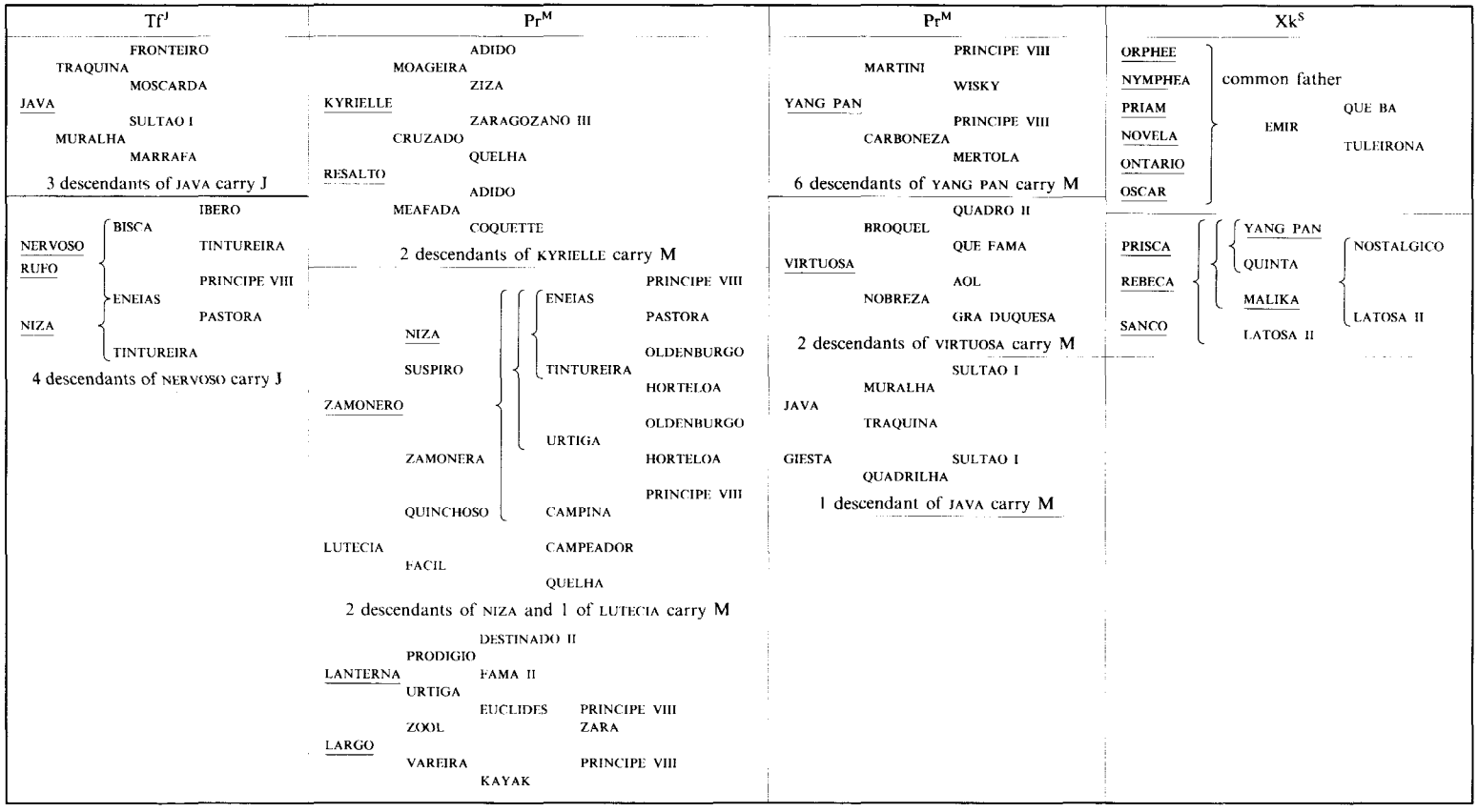
Esterase

Many variants at this locus are distributed with some degree of breed, or rather type of horse specificity. Thus, "light" or "warm blood" horses are characterized by a very large preponderance of Es^I, asociated with moderate amounts of Es^S and with the fast migrating variant F. The second fast-migrating variant G appears in Arabs and in half-blood breeds, its frequency increasing sharply in draught horses or ponies, often lacking Es^S.

Among Andalusian horses Es^G reaches a high frequency, outnumbering those of Connemara and Tarpans. Conversely, Es^F is unfrequent in Spanish population but it is found more often in the French. Its distribution covers many breeding centres but only two lineages contribute to its inheritance (Table 8). The frequency of Es^F in Andalusian horses in France is much lower than in Connemara and Tarpans. As to Es^S there is a difference between two populations, as this variant was present in only a single horse in the Spanish group, while in the French its frequency is comparable to that of breeds "rich" in Es^S. In fact, all horses carrying Es^S are grouped in two close breeding centres, sharing the same reproductory animals (Table 8); three lineages descending from one or two common ancestors in the fourth generation are carriers of this variant.

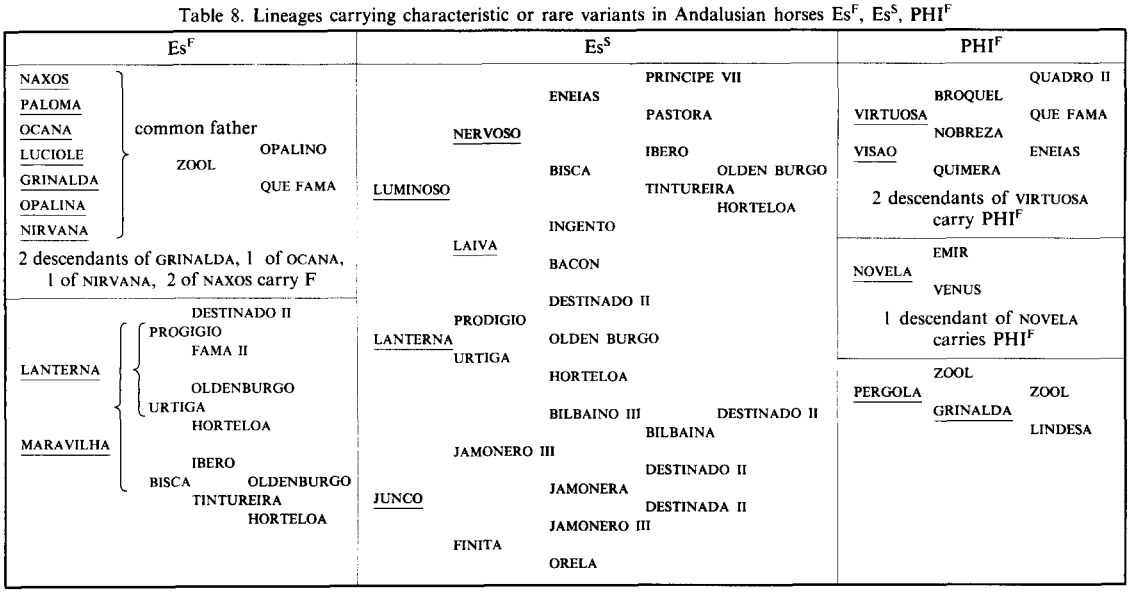
A fourth variant at esterase locus Es^H is rare. In Andalusian horses it was observed in both populations; three phenotypes contained it, HI, FH and GH; the two latter are quite uncommon. Among the breeds studied in this laboratory, only Tarpans disclosed this variant but it was present also in the mixed-breed group. Its distribution in Andalusian

Table 7. Lineages carrying characteristic or rare variants in Andalusian horses Tf^J, Pr^M, Xk^S



The names of analyzed horses are underlined.

Table 8. Lineages carrying characteristic or rare variants in Andalusian horses Es^F, Es^S, PHI^F



The names of analyzed horses are underlined.

horses is not due to a particular lineage; in fact it appears dispersed in the population. A family case in the Spanish group yielded horses carrying phenotypes GH (2 individuals) and HI (one), all three descending from the same ascendant; one is offspring of a stallion HI, the two others are half-sibs. In no case did we observe "irregular" inheritance, not conforming to simple Mendelian pattern, such as was claimed concerning Es locus in Tarpans (Tomaszewska-Guszkiewicz and Didkowski, 1980, 1983). Even at low frequency Es^H can be considered as a second "breed-marker" of Andalusian horses.

Prealbumin Xk

This three-allelic system is weakly polymorphic: in many breeds only the phenotype F is observed. The Andalusian horses contain the two less frequent variants, Xk^D and Xk^S. The former was also observed in Arab horses from France and Tunisia, Tarpans and the mixed-breed group; the latter, studied in Arab horses from Poland, was present in the mixed-breed horses but was lacking in Tarpans (Andres Cara and Kaminski, 1984). The andalusian group in France showed a higher frequency of Xk^S than Spanish horses; the distribution involved two lineages, a stallion and a dam having transmitted it to their descendants; two generations at least could be analysed (Table 8). In the Spanish group several descendants of one dam carry Xk^S, but no parent-progeny pairs were available. No horse carrying the homozygous phenotype S was found, but the Spanish group disclosed a rare phenotype DS.

Prealbumin Pr (or Pi)

Only a few breeds have been examined for this locus and comparable data are scarce the more so as techniques vary with laboratories. The distribution of variants appears breed-dependent; not only the minor variants are concerned but the main ones as well. The first report (Braend, 1970) shows considerable variation in frequency of the three predominant alleles, Pr^N, Pr^S and Pr^L between Dole horses and Norwegian Trotters. Data of Scott (1980, 1982) show differences between English Arabs and Thoroughbreds for the main variant Pr^L; also there are differences in frequencies of fast-migrating variants Pr^F and Pr^G, and for Pr^S; Pr^I and Pr^N are important components in Thoroughbreds, while they appear to lack in Arabs. It is not possible therefore to assign quantitative preponderance to a single variant of prealbumins as is the case with other polymorphic loci for most analysed breeds (Tf^F, Es^I, etc.).

Among 300 Andalusian horses nine variants at prealbumin locus have been detected, seven of which are common or abundant in other breeds: Pr^F, Pr^G, Pr^I, Pr^L, Pr^N, Pr^S, Pr^U and two rare ones Pr^M and Pr^W. Table 3 discloses that altogether 32 horses carry the variants F, G, I and N, which is about 10% of the whole Andalusian population studied, while about 80% of this population is constituted by five phenotypes built up exclusively with variants L, M, S and U. It appears therefore that the genetic structure of Andalusian horses at prealbumin locus is relatively homogeneous, at any rate more than that of Thoroughbreds.

The major variant in Andalusian breed is clearly Pr^S; both variants S_1 and S_2 occurred and the global frequency not only outnumbered largely the corresponding values in Thoroughbreds or Arabs but also those of the main variant in these breeds, Pr^L. This appears as a characteristic feature of Andalusian horses. Secondly, Andalusian horses differ from Thoroughbreds and especially from Arabs by their low amounts of the fast-migrating variants, Pr^F and Pr^G. The third difference is the low or trace amounts of Pr^N in Andalusian breed, compared with its second rank in Thoroughbreds.

Finally, Andalusian horses disclosed a fair amount of Pr^M. This variant has first been observed by Scott during the International Comparison Test of 1979 when 12 out of 20 sera of Tarpans disclosed a new pattern, the main band of which was located between

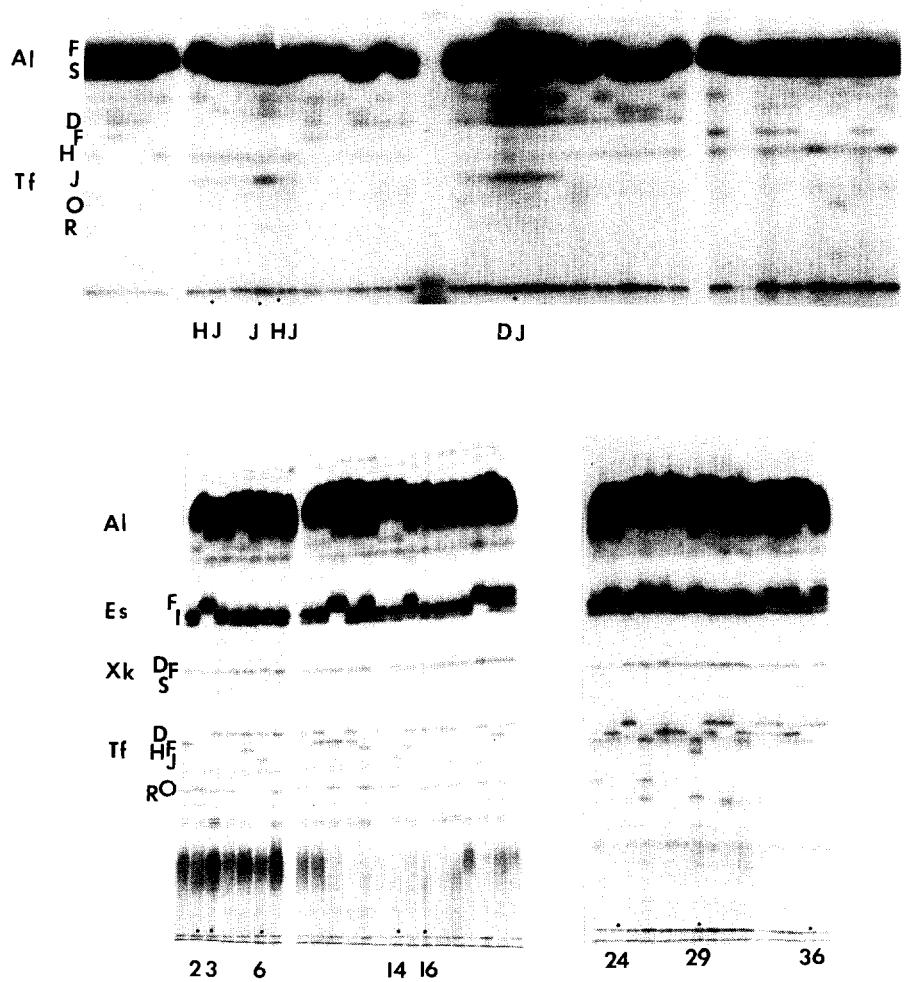


Fig. 1. Transferrin phenotypes of Andalusian horses (anode is upward). (A) Polymorphism of albumin and transferrin in starch gel at pH 7.4. Transferrin phenotypes are indicated for samples with dots. (B) Polymorphism of albumin, alcaline esterase, Xk and transferrin on acrylamide sheets. Samples with a dot, bearing: No. 2 is F in esterase, F in Xk and OR in transferrin; 3 is I in esterase FS in Xk and DR in transferrin; 6 is I in esterase, DF in Xk and DJ in transferrin; 14 is I in esterase, DF in Xk and JO in transferrin; 16 is I in esterase, FS in Xk and DR in transferrin; 24 is FI in esterase, FS in Xk and F_2 in transferrin; 29 is FI in esterase, F in Xk and HJ in transferrin; 36 is I in esterase, FS in Xk and DH in transferrin.

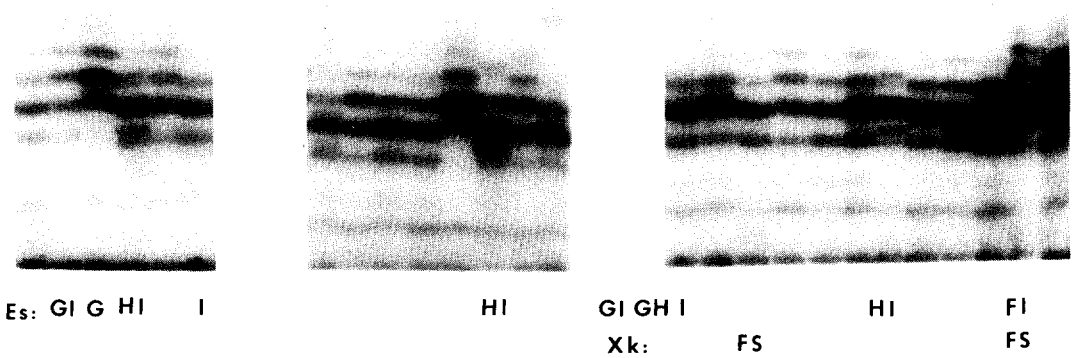


Fig. 2. Polymorphism of acid esterase and of Xk in Andalusian horses (anode is upward). Starch gel slides were first revealed for esterase activity and next stained for proteins; between and above esterase bands are visible ghosts of prealbumin phenotypes.

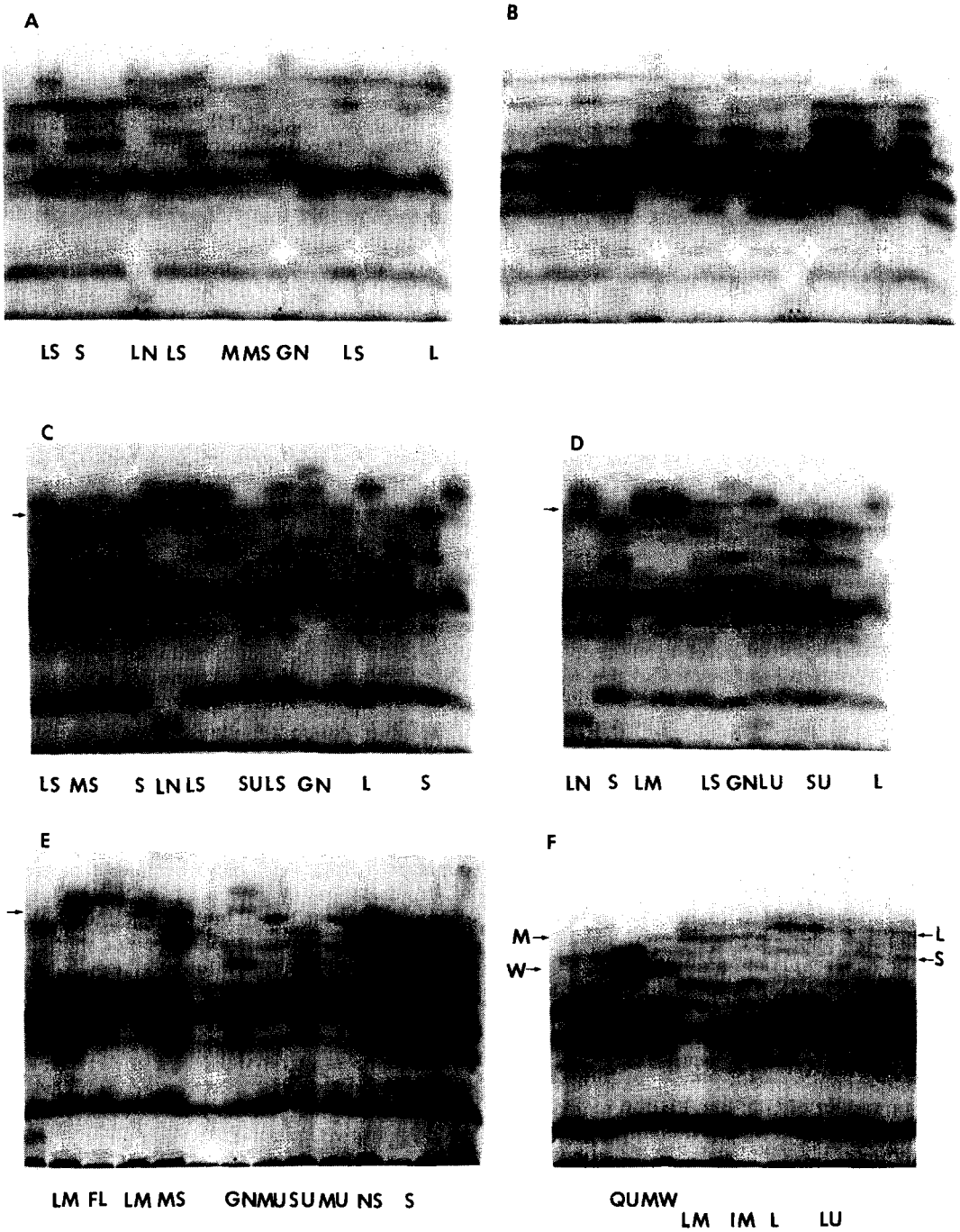


Fig. 3. Starch gel separation of prealbumins: identification of bands M (anode is upward). Samples with dots are controls, single dot Pr GN, double dot Pr LN and Xk S. A and B. Mirror images of two slices of the same gel, left stained for proteins only, right revealed for esterase activity, then stained for proteins. Phenotypes of prealbumins are seen between and above esterase bands. This gel contains the serum of one of the three individuals homozygous for M; this horse is one of 2 full-brothers sired by a stallion bearing phenotype LM in the Spanish population. C, D and E. Gels showing various phenotypes containing M. F. Gel showing identity of a band designated "W" by us (a horse of Spanish group, phenotype MW) and "Q" by Bell (two samples from the International Comparison Test of 1983, here sample No. 35, phenotype QU).

bands of L and N. (Fig. 3). No inheritance data being published concerning band M, it had not been officially recognized yet as an allele of Pr locus.

Since the technique used for typing prealbumins of Andalusian horses was the same as Scott's, the band located between L and N and migrating as the third band of I was called M. Both populations contained horses carrying this band and five horses out of 303 showed only bands M with no other allelic products. They were considered as homozygotes, but neither

parents nor progeny were available. In the French group were two such horses unrelated; in the Spanish group two were full-sibs and the third was unrelated. Considering the global population, the frequency of Pr^M is 0.108 and the probability of the occurrence of homozygous animals amounts to 3.9.

Analysis of lineages and families shows that in the Spanish population, out of 42 horses carrying M, 38 belong to 14 sire families where either one or the two parents have not been available. In France, horses with variant M originate from various breeding centres, some yielding few, others many carriers of Pr^M. Several lineages appear to convey this variant, some of them sharing common ancestors 3, 4 or 5 generations upward (Table 7). The availability of several sire or dam families with progeny having received the parental M permits its Mendelian pattern of inheritance to be stated.

In terms of allelic frequency Pr^M is the third most important component of Pr system in Andalusian horses, 20% of the global population carrying M in their phenotype. Not being encountered in other horse breeds except Tarpans, it appears as a good breed-marker.

Another rare variant has been observed in the prealbumin system among the Spanish group; according to the nomenclature of bands proposed by Braend (1970) and its location on acid gels, we called it W. In fact, this band has been further identified with a band qualified as Q in a phenotype QU, by Bell during the Comparison Test 1983 (Fig. 3). The phenotype of the Spanish horse carrying W was MW. Unfortunately, in the Spanish group we had no complete families and this horse was the offspring of non-analysed parents.

Enzymes of the hemolysate 6-PGD, PGM and PHI

The frequencies of PGD^S, higher in Spanish than in French population, are altogether much lower than in Arab or Thoroughbred horses. At PGM locus the divergence between two populations is even larger, but the mean frequency of PGM^F (0.118) is much lower than that of Arab or Connemara, while it is much higher than in Selle Français. Finally, the PHI^F in Andalusians outnumbers all the compared breeds.

The rare variants, PGD^D and PHI^S were present in only three horses altogether. Globally, Andalusian horses appear at these three loci far apart from Arab and Thoroughbreds, and their profiles are unlike those of other compared breeds.

DISCUSSION AND CONCLUSIONS

The study of Andalusian horses was not meant only as a description of their variants and frequencies, but as a basis for discussion on their origin and relationship. The historical background delineated in the Introduction led us to choose four racial groups, among these analysed in this laboratory, as comparison landmarks. Two breeds of antique origins, Arab and Barb horses, should yield some information on the parallel or divergent evolution of genetic profile in horses bred in similar climate and requirements; two breeds historically descending in part from Spanish horses, Thoroughbreds and Connemara, should disclose in their present genetic profiles the blood influx received centuries ago. Other groups were also enclosed, Tarpans, because of the shared rare variants and the mixed-breed group from eastern Europe as control of the "regional" genetic profile of Tarpans.

It was postulated that if there are variants allowing distinction between compared breeds, or conversely, if there are rare variants shared by two or more breeds, these facts correspond to ancestral divergencies or relationships.

From the methodological point of view, our comparison is based on a restricted number of loci, yet the global number of recognizable electrophoretic variants appeared sufficiently large for an adequate resolution between breeds.

Several problems of origin or relationship between breeds through transmission or sharing of certain variants arise at some loci (Table 6).

Considering transferrin there are at least three such problems. The first question deals with the present distribution of Tf^{F_1} and Tf^{F_2} variants among Thoroughbreds, Andalusian and Arab horses. According to available data, frequency of Tf^{F_1} is about three times higher than that of Tf^{F_2} in Thoroughbreds, while F_1 is rare in the two latter breeds. The creation of Thoroughbreds being based on a large infusion of oriental, mainly Arab, blood into a stock strongly impregnated by Spanish contribution, one wonders what horses brought the variant F_1? Were they the horses from Great Britain, some genes of which remained in the Royal Mares? Although our data concerning ponies are limited, the observed rarity of F_1 would rather plead against a large distribution of this variant in old days. Were they some of the imported stallions? Apart from Arabs, they were known as Barb or Turk, but little is known of populations from which they originated. The number of founders carrying F_1 must be inferior than that of founders of the breed, but it cannot be evaluated. In any case, Tf^{F_1} appears as a gene not affected in a reductive way by selection for speed nor by genetic drift; on the contrary, it seems to have proliferated among Thoroughbreds. An alternative hypothesis would be that of a relatively recent mutation.

The contribution of Andalusian or Arab horses to Thoroughbreds cannot therefore be estimated with respect to Tf^F genes, since it was rather as "diluants" than as positive influence.

The second problem is the occurrence of Tf^J variant in Andalusian horses. It qualifies as a true breed-marker, since it was found in none of the 23 registered breeds in France, 5 in Poland and 4 in north Africa. Its scattered presence among the "no breed" horses results most probably from crossbreeding of Spanish horses and their descendants. As to Selle Français, it does contain trace amounts of Tf^J, but it is still an open breed, and its creation was recent; Tf^J has rather penetrated into it through individual horses than with a definite population.

Considering now the origin of Tf^J among the present day Andalusian horses themselves, it appears unlikely that it arrived with horses from the south, that eventually have been admixed to ancient Iberic stock: neither Arab nor Barb horses do not disclose the J. As to Barb horses that we have analysed (Podliachouk *et al.*, 1978), the group was small, but all horses were certified pure bred, and they were the

selected national stallions. Until new data are available, Tf^J appears therefore as part of the ancient genetic pool of Iberic horses. This variant has not been transmitted by Andalusian horses to Connemara nor to Thoroughbreds, or else it has already been eliminated by genetic drift or due to a low number of founders.

A third variant of transferrin, Tf^R, was often discussed with respect to other breeds. In spite of an accumulation observed among the French group of Andalusian horses, due to one leading stallion in one breeding centre carrying Tf^R, the global frequency of this variant, 0.04, is lower or equal to that of Thoroughbreds and about half of that of Connemara. Again the genic contribution of Andalusian horses to the two latter breeds was not a positive one. The case is even more marked for Arabs which lack Tf^R altogether, as has been recognized from the very beginning of electrophoretic typing of horses; their contribution to Thoroughbreds was therefore like a dilution of the initial stock of genes. In fact, compared with most breeds that have been, for the purpose of breed improvement, more or less crossed with Arabs, the genic pool of the latter is the poorest since they carry only the most common variants in the tested loci except for Pr and Xk. The genic input of Arab horses to other breeds can therefore be defined as the mirror image of the founder effect or a "negative" contribution.

The esterase locus contains also some variants present or lacking in considered breeds. The two fast-migrating variants Es^F and Es^G can be described as "noble" and "common": the former being present in the aristocracy of light breeds, the latter being characteristic of draught horses or ponies. In some breeds of course they are both present, and then there is generally more of Es^G than of Es^F. Another pair of variants, Es^F and Es^S, seem to occur in parallel; in any case, a high level of Es^G is not accompanied by an increase of Es^S above the mean level due to the presence of Es^F. The Tarpans seem an exception, having more Es^F than Es^G without Es^S at all.

The frequency of Es^F in Andalusian breed is lower than that of Thoroughbreds and just a little higher than that of Arabs. The same question as previously for Tf^{F_1} arises, how the level of Es^F in Thoroughbreds has been reached?

The problem is exactly the opposite for Es^G, the Andalusian breed being rich in this variant; its frequency is the highest among the compared breeds, globally 0.230. Among Arab horses different strains contain various amounts of Es^G, and there is evidence that around the eighteenth century horses from Arabian peninsula did contain it (Kaminski and Tomaszewska-Guszkiewicz, 1978). Both Andalusian and Arab horses contributed to the creation of Thoroughbreds which are deprived of the variant Es^G. Is it due to a "negative" founder effect or is it a result of the selection? It seems that genetic drift by itself would not be enough to account for elimination of a rather frequent gene.

For Es^S there is again the question of how it came to Thoroughbreds, since the global frequency in Andalusian horses is very low (0.006) and that of Arabs a bit higher, but still lower than in Thoroughbreds.

The occurrence of Es^H in Andalusian horses, even though at low frequency, is one of differences between them and Arab horses, lacking of this variant. The relatively high level of Es^H in Tarpans, and its presence among the mixed-breed group could be taken as an indication of a common ancestry with Andalusian breed. Such an idea appears unlikely however since no intermediate breeds are known between these south-western and eastern horse types.

Concerning the contribution of Andalusian and Arab horses to the genetic pool of Thoroughbreds with respect of locus Xk, Xk^S was not observed among the latter while both former breeds carry notable frequencies of this variant. Here again the "negative" founder effect seems an appropriate explanation.

Finally the "profile" of various allelic components of Pr system yields a meaningful distinction between Andalusian and Arab horses, both different from their "offspring" Thoroughbreds.

In conclusion, the Andalusian breed displays a real individuality with respect to various electrophoretic genetic markers at many loci. It appears different from Arab horses on the one hand and from the breeds known historically as derived from it, such as Thoroughbreds or Connemara on the other hand. The Andalusian horses disclose an unpredicted although limited similarity with Tarpans. Their sharing of Es^S and Pr^M could lead to postulate a common ancestry; however, the exclusive occurrence of Tf^J and of Xk^S in Andalusian breed pleads against. The observed similarity is rather a convergence or else it is due to an unknown migration with partial interbreeding.

Concerning the two populations of Andalusian breed, some differences have been noted. They appear to be due to different population structures, Spanish horses being probably a quite mixed-up population, as often is the case in National Studs. On the contrary, the small groups bred in France are often isolated from one another in spite of common ancestors. The inbreeding within these groups leads to production of the progeny carrying indeed the genes from the stallions and mares available (Tables 7 and 8). Some isolated horses carried variants not encountered in the breeding groups.

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