BLOOD GROUP AND PROTEIN POLYMORPHISM GENE FREQUENCIES FOR THE ANDALUSIAN HORSE BREED. A COMPARISON WITH FOUR AMERICAN HORSE BREEDS

FRECUENCIAS GENICAS DE GRUPOS SANGUINEOS Y POLIMORFISMOS PROTEICOS EN EL CABALLO DE RAZA ANDALUZA. COMPARACION CON CUATRO RAZAS DE CABALLO AMERICANAS

Rodríguez-Gallardo, P.P., P.Aguilar Sánchez, J.L.Vega Plá and D.F. de Andrés Cara

Laboratorio de Grupos Sanguíneos Cría Caballar-C.S.I.C.-U.CO. Instituto de Zootecnia. 14005 Córdoba. España.

Additional Keywords

Andalusian horse. Average heterozygosity. Genetic distance. Genetic identity. Incorrect paternity.

Palabras clave adicionales

Caballo andaluz. Heterocigosidad media. Distancia genética. Identidad genética. Paternidad incorrecta.

SUMMARY

Gene frecuencies at seventeen blood group and protein polymorphism *loci* for the andalusian horse breed are given.

Standard methods of starch and polyacrylamide gel electrophoresis were used to identify inherited variants at the following enzyme and other protein *loci*: albumin (Al), transferrin (Tf), carboxylesterase (Es), A1B glycoprotein (Xk), vitamin D binding protein (Gc), protease inhibitor (Pi), 6-phosphogluconate dehydrogenase (PGD), phosphoglucomutase (PGM) and glucose-phosphate isomerase (GPI). Polyacrylamide gel isoelectric focusing was used for haemoglobin (Hb).

Standard hemaglutination and complement mediated hemolysis were used to detect red cell alloantigens at the following *loci*: A, C, D, K, P, Q and U.

These data are used to calculate the effectiveness of the battery of tests for recognizing incorrect paternity (Pex) in this breed and to calculate Nei's measures of normalized genetic identity (I) and standard genetic distance (D) between two populations (Nei, 1972), applied to studies of breed relationships between Andalusian and Paso Fino, Paso Peruano, Quarter and Morgan horses (Blood group and protein polymorphism gene frequencies for Paso Fino, Paso Peruano, Morgan and Quarter horses are from Trommershausen-Bowling & Clark, 1985).

RESUMEN

Se presentan las frecuencias génicas de diecisiete *loci* de grupos sanguíneos y polimorfismos bioquímicos en el caballo Andaluz.

Se emplearon métodos estándar de electroforesis en geles de almidón y poliacrilamida para identificar las variantes hereditarias de los siguientes *loci* proteicos: albúmina (Al), transferrina (Tf), carboxilesterasa (Es), A1B

glucoproteína (Xk), proteína de unión a la vitamina D (Gc), inhibidor de proteasas (Pi), 6-fosfogluconato deshidrogenasa (PGD), fosfoglucomutasa (PGM), glucosa-fosfato isomerasa (GPI). Para la hemoglobina (Hb) se utilizó isoelectroenfoque en gel de poliacrilamida.

Se emplearon hemólisis mediada por el complemento y hemaglutinación estándar para detectar aloantígenos de grupos sanguíneos de los siguientes *loci*: A, C, D, K, P, Q y U.

Estos datos se utilizan para calcular la eficacia de la batería de tests para reconocer paternidades incorrectas (Pex) en esta raza y para calcular la medidas de identidad genética normalizada (I) y distancia genética estándar de Nei entre dos poblaciones (Nei, 1972), aplicadas a estudios de parentesco entre las razas de caballo Andaluz y Paso Fino, Paso Peruano, Morgan y Cuarto de milla (los valores de frecuencias génicas de grupos sanguíneos y polimorfismos bioquímicos de las razas Paso Fino, Paso Peruano, Morgan y Cuarto de milla proceden de Trommershausen-Bowling y Clark, 1985).

INTRODUCTION

Since the times of domestication human needs have modified the genetic pools of ancestral populations, first by breeding habits, then through migrations and invasions, and more recently by means of importations of horses bearing desired particular characteristics, and arbitrary crosses. This admixture of ancient local populations with imported lines, followed by selection practises in a more or less definite direction and the lack of cross-breeding with other populations has created the present day horse breeds.

Concerning the American horse breeds, there are many interrelations among their ancestors, which trace up to horses brought to the New World by the spanish explorers, particularly andalusian horses.

This paper gives gene frequencies at seventeen blood group and protein polymorphism loci for the Andalusian horse breed, that are used to calculate Nei's measures of normalized genetic identity (I) and standard genetic distance (D) between two populations (Nei, 1972), applied to studies of breed relationships between Andalusian and Paso Fino, Paso Peruano, Quarter and Morgan horses. Values of average heterozygosity (Jx) and of the effectiveness of the battery of tests for recognizing incorrect paternity (SPex) in this breed, are also given.

MATERIALS AND METHODS

Breed information has been obtained from Van Vleck (1979), Edwards (1980) and from the Spanish Pure Breed horse stud-book.

The Andalusian as well as the Barb horses, are believed to descend from the horses brought by a population coming from Egypt, related to the Hittites, whose horses would be of Przewalski origin. These people settled in north Africa then passed to the Iberic peninsula sometime around the thirteenth century before our era. Among the aptitudes of Andalusian horses were speed and a unique behaviour developed during fighting being used for military or festive purposes, and to improve other breeds. The Andalusian horse today (officially named Spanish Pure Breed horse) is

primarily raised for pleasure.

The Spanish Pure Breed horse stud-book (closed in 1929) requires that all breeding stallions and mares be blood-typed before their foals can be registered. Foal blood-typing and parentage verifying are required before definitive registration. The effectives of the breed are about 12000 horses and annual foal registration is approaching 4000.

Paso horses are naturally-gaited riding breeds, developed in Perú (Paso Peruano, PP) and in Puerto Rico, Cuba, Colombia (Paso Fino, PF), that descend from horses (Andalusians, Barbs, Jennets) carried to the New World by the spanish explorers and colonists. According to its history, the Paso Peruano breed has remained genetically isolated for several centuries. Paso horses have been selectively bred for a natural lateral gait (a characteristic of most riding horses prior to the 17th century) in contrast with the square trotting gait seen in most horses today. This gait is extremely comfortable for the rider and is relatively non-tiring for horses to perform. Paso Peruano horses have also been selected for their characteristic foreleg movement (similar to the arm motions of a crawl swimmer). These horses are primarily used for pleasure riding and showing.

Morgan horses all trace to one stallion, Justin Morgan (formerly Figura), which breeding origins are not reliably documented. However, the gene pool of the breed is expected to be quite diverse because of the variety of mare lines used in the beginnings of the breed, the subsequent use of cross-breeding, and selection practises based on low heritability characters. At the present time, the breed registry is closed. Morgan horses were originally used for weight-pulling and saddle and harness racing; they are nowadays very popular for saddle riding and showing.

Quarter horses have their origins in Thoroughbreds and other imports combined with horses descending from those brought to America by the spanish explorers. Quarter horses were bred for the ability to gallop very fast over short distances and for cattle work. Today, the breed is primarily raised for pleasure. The stud-book has remained open until recently and cross-breeding with Thoroughbreds is still allowed, so that the amount of genetic diversity is expected to be quite large.

Blood group and protein polymorphism gene frequencies for Paso Fino, Paso Peruano, Quarter and Morgan horses are from Trommershausen-Bowling and Clark (1985).

Blood samples of Andalusian horses (N= 1099) were routinary tested in the period 1988 to 1991. Not every sample was tested for all the specificities.

Standard methods of starch and polyacrylamide gel electrophoresis were used to identify inherited variants at the following enzyme and other protein *loci*: albumin (Al), transferrin (Tf), esterase (Es), A1B glycoprotein (Xk), Vitamin D binding protein (Gc), protease inhibitor (Pi), 6-phosphogluconate dehydrogenase (PGD), phosphoglucomutase (PGM) and glucose-phosphate isomerase (GPI). Polyacrylamide gel isoelectric

focusing was used for haemoglobin (Hb). Computation of allelic frequencies was done by direct counting from phenotypes.

Standard hemagglutination and complement mediated hemolysis were used to detect red cell alloantigens. Blood group frequencies were calculated as follows: for C, K and U by the square-root method; for P, A and Q, with the square-root method to obtain frequencies of the null alleles, followed by calculation and adjustement of the other allele frequencies to obtain the best fit to the data under the assumption of Hardy-Weinberg equilibrium (Neimann-Sfrensen, 1956); for D, by direct counting assuming no ambiguous phenotypes, then by iterative approximations under Hardy-Weinberg equilibrium (Neimann-Sorensen, 1956).

Nei's measures of normalized genetic identity (I) and standard genetic distance (D) between two populations, and average heterozygosity (Jx) (Nei, 1972) were calculated using the computer programme of Dowling and Moore (1984).

Probabilities of exclusion (Pex) for blood group and protein polymorphism loci were estimated according to Carracedo and coworkers using their computer programme (Carracedo *et al*., 1988).

RESULTS AND DISCUSSION

Electrophoretic serum and red cell marker frequencies are presented on **table I** and alloantigenic red cell marker frequencies on **table II**, for Andalusian horses.

On **tables III** and **IV** we present blood marker gene frequencies for Paso Fino, Paso Peruano, Quarter and Morgan horses taken from Trommershausen-Bowling and Clark (1985) and for Andalusian horses arranged in order to permit comparisons (Data on these tables have been used for breed relationship calculations).

EFFICACY OF BLOOD-TYPING TESTS. The calculated effectiveness of the tests for detecting incorrect paternity when blood samples are tested from sire, dam and offspring was at least 99% using 17 internationally defined *loci* (**table V**). In actual practice, the effectiveness could be slightly lower because when mares are bred to two stallions, they usually are not selected at random from the breed.

The efficacy of a *locus* depends on the number of alleles and their frequencies, the most effective *loci* in this study being D, A, P, Q, Tf, Pi and Es (estimated individual Pex greater than 20%), each of them having five or more alleles with appreciable frequencies. Taken together, these seven *loci* have a theoretical Pex greater than 98%

BREED RELATIONSHIPS. The broad genetic base of the Andalusian horse breed, with only sixty years of a closed stud-book, combined to breeding programmes in which no one trait has been highly selected, is reflected in the rather high number of blood type variants found in the breed (78 out of 90 assayed) and the

mid-range average heterozygosity value (0.457±0.070), compared to the values of the same parameters (51 out of 76, 0.378±0.069, respectively) in Thoroughbreds (Trommershausen-Bowling and Clark, 1985), intensively selected for a single trait of rather high heritability and whose stud-

Table I. *Gene frequencies of serum and red cell protein markers for Andalusian horses (N= 1099).* (Frecuencias génicas de marcadores proteicos séricos y eritrocitarios para caballos andaluces).

Locus	Marker		*Locus*	Marker	
Al	A	0.517	Xk	F	0.031
	B	0.483		K	0.919
				S	0.050
Tf	D	0.375			
	F1	0.003	Es	F	0.027
	F2	0.316		G	0.267
	H1	0.185		H	0.021
	H2	0.000		I	0.675
	J	0.017		S	0.009
	M	0.000			
	O	0.051	Gc	F	0.993
	R	0.053		S	0.007
Pi	F	0.007	PGD	D	0.001
	G	0.013		F	0.884
	H	0.000		S	0.116
	I	0.014			
	K	0.002	PGM	F	0.080
	L+L2	0.171		S	0.920
	N	0.059		V	0.000
	O	0.000			
	P	0.043	GPI	F	0.084
	Q	0.003		I	0.916
	R	0.015		S	0.000
	S+T	0.493			
	U	0.057	Hb	A	0.005
	V	0.003		AII	0.057
	W	0.094		BI	0.773
	Z	0.030		BII	0.165

Table II. *Gene frequencies of alloantigenic red cell markers for Andalusian horses.* (Frecuencias génicas de marcadores alloantigénicos, eritrocitarios para caballos andaluces).

Locus (N)	Allele		*Locus* (N)	Allele	
A	a	0.000	K	a	0.002
(932)	adf	0.126	(419)	-	0.998
	adg	0.208			
	b	0.128			
	bc	0.007			
	c	0.039	P	a	0.018
	cd	0.000	(384)	ac	0.037
	-	0.492		acd	0.017
				ad	0.421
				b	0.020
C	a	0.433		bd	0.000
(1024)	-	0.567		d	0.002
				-	0.486
D	ad	0.038			
(1028)	adn	0.000			
	bc	0.054	Q	abc	0.112
	cefg	0.046	(988)	ac	0.007
	cegin	0.007		b	0.104
	cf	0.000		c	0.258
	cg	0.074		-	0.519
	cfgk	0.053			
	de	0.296			
	dfk	0.252			
	dgh	0.044	U	a	0.441
	dk	0.137	(573)	-	0.559
	dn	0.000			
	-	0.000			

book was closed two hundred years ago.

The closest relationship found, as shown by data on **table VI**, was between Andalusian and Paso Fino horses, as expected from Paso Fino breed history. Moreover, variant Tf J, considered as a blood genetic marker of the Andalusian horse breed (Kaminski and de Andrés, 1986), was

Table III. *Gene frequencies of serum and red cell protein markers.* (Frecuencias génicas de marcadores protéicos séricos y eritrocitarios).

Locus	Marker	Andalusian (N=1099)	PF* (N=109)	PP* (N=100)	MH* (N=82)	QH* (N=100)
Al	A	0.517	0.402	0.275	0.445	0.355
	B	0.483	0.598	0.725	0.555	0.645
Tf	D	0.375	0.262	0.295	0.293	0.225
	F1	0.003	0.000	0.000	0.000	0.185
	F2	0.316	0.178	0.225	0.543	0.335
	H1	0.185	0.145	0.130	0.067	0.050
	H2	0.000	0.065	0.025	0.000	0.005
	J	0.017	0.011	0.000	0.000	0.000
	M	0.000	0.000	0.000	0.043	0.000
	O	0.051	0.154	0.195	0.018	0.070
	R	0.053	0.112	0.110	0.024	0.115
	other	0.000	0.073	0.020	0.012	0.015
Pi	F	0.007	0.000	0.000	0.019	0.005
	G	0.013	0.066	0.000	0.037	0.040
	I	0.014	0.014	0.000	0.049	0.040
	L+L2	0.171	0.448	0.455	0.303	0.379
	N	0.059	0.042	0.025	0.179	0.126
	S+T	0.493	0.236	0.140	0.079	0.218
	U	0.057	0.099	0.145	0.291	0.177
	other	0.186	0.094	0.235	0.043	0.015
Xk	F	0.031	0.017	0.005	0.035	0.010
	K	0.919	0.896	0.780	0.910	0.940
	S	0.050	0.087	0.215	0.055	0.050
Es	F	0.027	0.056	0.125	0.177	0.045
	G	0.267	0.178	0.240	0.085	0.061
	H	0.021	0.023	0.000	0.006	0.020
	I	0.675	0.701	0.465	0.689	0.834
	S	0.009	0.037	0.170	0.037	0.040
	other	0.000	0.005	0.000	0.006	0.000
Gc	F	0.993	0.982	0.983	0.877	0.878
	S	0.007	0.018	0.017	0.123	0.122
PGD	D	0.001	0.006	0.000	0.000	0.005
	F	0.884	0.867	0.725	0.835	0.677
	S	0.116	0.127	0.275	0.165	0.318
PGM	F	0.080	0.133	0.136	0.148	0.027
	S	0.920	0.867	0.864	0.852	0.973
	V	0.000	0.000	0.000	0.000	0.000

*Trommershausen-Bowling & Clark, 1985.

Table IV. *Gene frequencies of alloantigenic red cell markers.* (Frecuencias génicas de marcadores aloantigénicos eritrocitarios).

Locus	Allele	Andalusian (N/ locus)	Breed PF* (N=108)	PP* (N=100)	MH* (N=84)	QH* (N=100)
A	a	(932) 0.000	0.000	0.000	0.000	0.000
	adf	0.126	0.263	0.321	0.529	0.440
	adg	0.208	0.233	0.214	0.040	0.050
	b	0.128	0.144	0.065	0.201	0.150
	bc	0.007	0.014	0.000	0.006	0.010
	c	0.039	0.042	0.004	0.047	0.010
	-	0.492	0.304	0.396	0.177	0.340
C	a	(1024) 0.433	0.369	0.735	0.688	0.755
	-	0.567	0.631	0.265	0.312	0.245
D	ad	(1028) 0.038	0.079	0.100	0.135	0.050
	bc	0.054	0.218	0.170	0.065	0.185
	cefg	0.046	0.037	0.100	0.040	0.005
	cegi	0.007	0.005	0.010	0.005	0.050
	cg	0.074	0.144	0.100	0.300	0.110
	cfgk	0.053	0.000	0.000	0.000	0.000
	de	0.296	0.153	0.145	0.100	0.155
	dfk	0.252	0.065	0.015	0.005	0.035
	dgh	0.044	0.060	0.006	0.085	0.075
	dk	0.137	0.213	0.350	0.260	0.290
	d	0.000	0.027	0.004	0.005	0.045
K	a	(419) 0.002	0.001	0.025	0.006	0.041
	-	0.998	0.999	0.975	0.994	0.959
P	a	(384) 0.492	0.307	0.200	0.220	0.260
	b	0.020	0.069	0.020	0.073	0.020
	-	0.488	0.624	0.780	0.707	0.721
Q	abc	(988) 0.112	0.076	0.020	0.006	0.175
	ac	0.007	0.000	0.000	0.000	0.000
	b	0.104	0.082	0.219	0.037	0.099
	c	0.258	0.210	0.250	0.296	0.206
	-	0.519	0.632	0.510	0.661	0.520
U	a	(573) 0.441	0.347	0.329	0.267	0.252
	-	0.559	0.653	0.671	0.733	0.748

*Trommershausen-Bowling & Clark, 1985.

Table V. *Probability of exclusion (Pex) at seventeen loci in the Andalusian horse breed.* (Probabilidad de exclusión de 17 *loci* en la raza de caballo andaluz).

Blood group		Biochemical polymorphisms	
Locus	Pex	Locus	Pex
A	0.45	Al	0.19
C	0.19	Tf	0.48
D	0.64	Pi	0.52
K	<0.01	Xk	0.08
P	0.30	Es	0.23
Q	0.39	Gc	<0.01
U	0.19	PGD	0.09
		PGM	0.07
$\Sigma Pex_{\text{blood group loci}}$	= 0.95	GPI	0.07
		Hb	0.19
		$\Sigma Pex_{\text{biochem.polym.loci}}$	= 0.91
Total Pex for 17 *loci*	>0.99		

only present in the sample of Paso Fino horses. The level of relationship found between Andalusian and Paso Peruano horses was lower, and similar to that found between Andalusian and Morgan and Quarter horses, although the kinship of Morgan and Quarter horses with the Andalusian breed might be expected to be lesser according to their respective origins.

Divergences between Paso Peruano and Andalusian horses, might be explained from the isolation of the Paso Peruano breed for several centuries, plus its selective breeding for specific traits no longer present in the Andalusian breed.

Table VI. *Nei's measures of normalized genetic identity (I) and standard genetic distance (D).* (Medidas de identidad genética normalizada y distancia genética estándar de Nei).

	And/PF	And/PP	And/MH	And/QH
I	0.954	0.913	0.919	0.927
D	0.047±0.030	0.091±0.036	0.084±0.35	0.076±0.024

REFERENCES

Carracedo, A., E. Huguet y F. Barros. 1988. Parámetros estadísticos en la investigación biológica de la paternidad. Exclusión y prueba positiva de paternidad. En "Introducción a la Investigación Biológica de la Paternidad" (E. Huguet, A. Carracedo y M. Gené, Eds.). PPU, Barcelona. pp. 171-188.

Dowling, T.E. and W.S. Moore. 1984. A program for estimating genetic variability within and between populations. *Journal of Heredity* 75:416.

Edwards, E.H. 1980. A standard guide of horse and pony breeds. McGraw-Hill, New York.

Kaminski, M. and D.F. de Andrés Cara. 1986. Electrophoretic markers of Andalusian Horses: comparison of Spanish and Lusitanian lineages. *Comparative Biochemistry and Physiology* 83B(3):575-588.

Nei, M. 1972. Genetic distance between populations. *American Naturalist* 106:283-292.

Neimann-Sfrensen, A. 1956. Blood groups and breed structure as exemplified by three Danish breeds. *Acta Agriculturae Scandinavica* VI:115-137.

Trommershausen-Bowling, A. and R.S. Clark. 1985. Blood group and protein polymorphism gene frequencies for seven breeds of horses in the United States. *Animal Blood Groups and Biochemical Genetics* 16:93-108.

Van Vleck, L.D. 1979. Razas en Estados Unidos. En "El Caballo" (J.Warren Evans, A.Borton, H.F.Hintz y L.Dale Van Vleck, Eds.). Acribia, Zaragoza. pp. 18-109.