

THE MINORCAN HORSE BREED: BLOOD GROUPS AND BIOCHEMICAL POLYMORPHISM

Rodríguez Gallardo, P.P.(1) and D.F.(2) of Andrés Cara

1 Service of Hemotypes. Jefatura de Cría Caballar. Apartado Oficial Suc.2.14071 Córdoba. España.
2 Departament of Genetics. CSIC-UCO. Facultad de Veterinaria. Av. Medina Azahara 9.14005 Córdoba. España.

ADDITIONAL KEY WORDS
Genetic distance. Genetic identity. Average heterozygosity. Incorrect paternity.

Summary

This present work represents a contribution to the study of the Minorcan horse breed by means of standard genetic blood scoring, blood groups and biochemical polymorphism.

176 specimens are studied, the majority of which are breeding stock of both sexes, the genetic frequencies for five loci of blood groups (A, C, D, P Y Q) and ten biochemical polymorphism systems (HB, ALB, TF, ES, A1B, PI, GC, PGM, 6PGD, GPI). From these results the average heterozygosity (H8) of the sample is estimated, the probability of an exclusion a priori (PE) of a falsely assigned paternity and the genetic Nei distances and identities with respect to Arab, English thoroughbred and Spanish thoroughbred breeds, whose past influence on the Minorcan breed is known.

Introduction

At the beginning of the 1980's, interest intensified on the island of Minorca (Spain) in an equine group called Minorcan that were extraordinarily linked to the popular equestrian "Jaleo" fiestas. Private initiatives and insular institutions promoted activities aimed at the official recognition of the said group as a breed and the establishment of a genealogical book for the breed. These objectives were achieved recently and backed up by the Sánchez Belda study (1987) and the acceptance of the founding register of the breed that was administered by the Consejo Insular de Menorca (the Minorcan Island Council).

The study of the genetic structure of the Minorcan horse breed by means of genetic scoring (blood groups and biochemical polymorphism) is tackled now each time as the racial breed is already officially established and its model or standard approved (anonymous), and there are sufficient samples of individuals standardized by genetic scoring. This line of work, already classic, can be seen currently and it is boosted by the protectionist spirit of the animal's genetic resources protected by the biodiversity conservation programmes set up in 1992 at the Río de Janeiro Conference in Brazil.

The Balearic insular reality as well as the location of the archipelago in the sea that meant contact with ancient civilizations, leads one to believe that the horse that is the object of this study was not unconnected to influences from diverse equine populations that settled in the islands over time and that helped form today's Minorcan breed. Based on this hypothesis, a comparative study of this breed with three others that arrived on the island in different periods is examined. The three other breeds are the Arab, the English Thoroughbred and the pure Spanish Breed.

MATERIAL AND METHODS

The sample studied was made up of 176 examples located on the island of Minorca (Spain), the majority of which were breeding horses of both sex, belonging to the Minorcan horse breed and checked by the corresponding genealogical book (Register of horses and mares of the Minorcan breed).

Each blood sample is made up of two 10ml. tubes, one with sodic EDTA as an anticoagulant for the analysis of the erythrocyte antigens and the biochemical polymorphisms of the Inter-erythrocyte proteins. The other tube is for the analysis of the biochemical polymorphism of the serum. The samples corresponding to those examples to be studied progressively arrived at the Blood Group Laboratory for Horse Breeding (Córdoba) between 1990 and 1996. From here a common minimum was fixed for all the individuals, made up of 25 antigen factors, distributed in 5 standard immune systems of hemagglutination and haemolysis (Podliachouk and Hesselholt, 1962; Stormont and Suzuki, 1964; Stormont et al., 1964).

Starting from the Ardí-weinberg balance assumption for the sample studied, the genic frequencies of the alleles of the blood groups will be calculated by the square root method in the systems in which it is possible (C and Q) and in the rest (A, D and P) by means of the Neiman-Sorensen (1956) iterative calculation.

Various standard electrophoresis methods, with a few modifications introduced since their conception, have been used to detect the variability of 10 genetic systems of proteins investigated and these are inhibitors of protease (PI), albumin (ALB), Gc protein tied to vitamin D (GC), esterase (ES), alb-glycoprotein (AIB) and transferrin (TF) (Juneja et al., 1978; Braend, 1973 and Trommershausen-Smith and Suzuki, 1978), from plasma and the intererythrocytes, glucophosphomutase (PGM), 6-phosphoglyconate dehydrogenase (6-PGD) and glucose phosphate isomerase (GPI) (Bengtsson and Sandberg, 1973) and haemoglobin (HB) by means of isoelectric focalization (Braend and Johansen, 1983).

To calculate the distance (D) and Nei genetic identities (I) between two populations (Nei,1972), as well as the average heterozygosity expected (He), the Dowling and Moore (1984) computer programme was used. Finally, for the exclusion probability calculation (PE) the computer programme developed by Huguet et al. (1988) was used, starting from the algorithm described by Ohno et al. (1982).

Tabla I. Frecuencias génicas de marcadores aloantigénicos eritrocitarios en el caballo de raza Minorquina.

SISTEMA D		SISTEMA Q		SISTEMA A	
Alelos	Frecuencias	Alelos	Frecuencias	Alelos	Frecuencias
cgmp	0,1413	b	0,1381	a	0,0045
dello	0,1534	c	0,0492	act	0,4562
bcn	0,3182	abc	0,0136	adg	0,0938
dvl	0,1364	ac	0,0712	b	0,0284
dghm	0,1193	(-)	0,7269	cle	0,0029
dn	0,0028			(-)/e	0,4162
dcgkm	0,0028	SISTEMA P			
cegkm	0,0573	ap/cd/d	0,3083	SISTEMA C	
ad	0,0425	b/s	0,0925	a	0,7868
cefgm	0,0145	(-)/d	0,5962	(-)	0,2132
cd	0,0114				

Click on the table to enlarge it

RESULTS AND DISCUSSION

The allele frequencies for the blood groups and biochemical polymorphism systems are presented in tables I and II, respectively. As far as blood groups are concerned, it is necessary to specify, in relation to the configuration of the alleles, that the Dcgm allele frequency will include that of the Dcgp as anti-DP reagent serum was not used in this study. The same occurs for the Del allele, that will include the Ddlo frequency. Similarly, in the P system, anti-Pc reagent serums are not used and thus there will be an accumulated genetic frequency for the Pa, Pacd and Pad alleles. Also, the void allele of the P system will include the Pd frequency. Finally, in the A system, the Ac genetic frequency will include that of the Ace allele, and that of the void allele will accumulate the Ae frequency as its corresponding reagent. Anti-serum was not used.

Tabla II. Frecuencias génicas de marcadores proteicos séricos y eritrocitarios en el caballo de raza Minorquina.

SISTEMA TF		SISTEMA PI		SISTEMA HB	
Alelos	Frecuencias	Alelos	Frecuencias	Alelos	Frecuencias
D	0,02	G	0,04	A1	0,02
F1	0,02	I	0,04	A2	0,11
F2	0,35	L	0,33	B1	0,53
H1	0,02	N	0,23	B2	0,24
H2	0,03	P	0,55		
J	0,02	S	0,18	SISTEMA GPI	
O	0,16	T	0,23	F	0,06
R	0,06	U	0,17	I	0,91
		W	0,21	S	0,01
		Orn	0,08		
SISTEMA ALB		SISTEMA PGD		SISTEMA AIB	
A	0,23	F	0,78	K	0,98
B	0,77	S	0,21	S	0,02
SISTEMA GC		SISTEMA PGM		SISTEMA ES	
F	0,96	F	0,11	F	0,56
S	0,04	S	0,89	G	0,27
				I	0,62
				S	0,23
				Orn	0,02

Click on the table to enlarge it

In the different blood group system one detects a genetic frequency distribution that differs from those obtained from the English Thoroughbred (Bowling and Clark, 1985 and Bouquet et al,1987), the Arab (Bowling and Clark, 1985) and Spanish Thoroughbred (Rodríguez-Gallardo et al.,1992), although there are some common elements. The most notable singular facts are the presence, though with a low genetic frequency (0.0028), of the Dcgm allele described in the Spanish thoroughbred horse by Aguilar et al. (1986) and considered typical of this breed. Similarly, the Dcefgm allele, absent in English and Arab thoroughbreds, is found in Spanish thoroughbreds in the Minorcan group. Finally, the DNL allele, that is absent in the Spanish and Arab Thoroughbred breed, is found in the Minorcan breed with a frequency of 0.0028.

Regarding biochemical polymorphism, the situation is similar. The presence of the TF F1 allele was detected and this is considered typical of the English Thoroughbred horse. This could indicate the influence of this breed on the Minorcan breed. Similarly, the TF J allele, that forms part of the Spanish Thoroughbred genetic heritage, is also present in the Minorcan breed, corroborating the influence of the Spanish horse on the Minorcan breed. In the HB system, the HB A1 and HB A2 alleles have been found that were not detected in the Arab Breed and the English Thoroughbred (Bowling and Clark, 1985) but were in the Spanish Thoroughbred (Rodríguez-Gallardo, 1992). Similarly, the presence of the GPI S allele was detected in the Minorcan breed, which is absent or very rare in the three breeds

that are being compared.

Tabla III. Medidas de identidad genética normalizada (I) y distancia genética estándar (D) de Nei entre la raza Minorquina (M) y las razas Árabe (A), Pura Raza Española (PRE) y Pura Sangre Inglés (PSI).

	M/A	M/PRE	M/PSI
I	0,952	0,990	0,910
D	0,049±0,020	0,073±0,027	0,094±0,040

Tabla IV. Probabilidad de exclusión, expresada en (%.100), de 15 sistemas genéticos en la raza caballo Minorquina.

Grupos sanguíneos		Polimerfismo Bioquímico	
A	32,74	ALB	14,57
C	13,36	TF	51,97
Q	64,66	GC	3,69
P	27,07	A1B	1,92
Q	15,49	ES	26,39
			6-PGD 13,83
	PE GS 88,89		PE PB 94,48
			PE Total 97,39

Click on the table to enlarge it

The Nei identity and genetic distance values between the Minorcan breed and the English Thoroughbred, Spanish Thoroughbred and Arab breeds are shown on table III and as can be observed, the least distance and greatest identity corresponds to the Arab breed, followed by the Spanish Thoroughbred. This result, that seems to contrast with the presence of scoring alleles of the Spanish Thoroughbred in the Minorcan breed, is due to the similar distribution of the frequencies of the alleles present in the Arab and Minorcan breeds. This would reflect the Arab base of the Minorcan horse to which one can add the influence of the Spanish Thoroughbred. The average heterozygosity (H_e) in the Minorcan breed, for the fifteen genetic systems analysed, was of 0.4390 ± 0.0649 , on average type value (Bowling and Clark, 1985), similar to that obtained for the Spanish Thoroughbred (0.4394 ± 0.0652) by the same systems and indicative of the wide genetic base of the breed (only in 1994 was its genealogical book closed).

Finally, we calculate the exclusion probability a priori to check the efficiency of the genetic systems studied in the relationship controls to develop for the genealogical book of the Minorcan breed, listing the results in table IV. The said results, for fifteen genetic systems (0.994), offer values greater than those obtained by Bowling and Clark (1985) in Arab (0.97) and English Thoroughbred (0.96) breeds for twenty genetic systems. However, the results are very similar, even superior in some systems, to those obtained by 17 genetic systems for the Spanish thoroughbred (0.99) by Rodríguez-Gallardo et al. (1992).