Greater genetic variability in Argentine Creole than in Thoroughbred horses based on serum protein polymorphisms

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ABSTRACT. Genetic polymorphism was analyzed for five blood proteins: albumin - Al, esterase - Es, α_1B-glycoprotein - Xk, transferrin - Tf and hemoglobin - Hb in 200 Thoroughbred (TB) and 124 Argentine Creole (AC) horses. Of the five systems examined, Tf and Hb were not in Hardy-Weinberg equilibrium in either breed and Es was not in equilibrium in the Creole breed. Genetic variability, estimated as average heterozygosity, was higher in AC (H = 0.585 ± 0.131) than in TB (H = 0.353 ± 0.065). The genetic differentiation between these two populations (F_{ST}) was 0.109. Thus, of the total genetic differences between breeds, the proportion of genetic variation attributable to breed differences was about 10%; the remaining 90% was due to individual variation within breeds. The high degree of genetic variability seen in Argentine Creole horses could be a consequence of natural selection. Selection of TB through the centuries has most likely modified the gene pool of the ancestral population, with a consequent reduction in variability at certain loci. Probably, different mechanisms exist for maintaining polymorphism at these loci in TB and in AC horses. Heterozygosity may have played a fundamental role in adaptation.

Key words: Horses, Argentine Creole, Thoroughbred, Heterozygosity, Genetic variability, Serum protein polymorphysm

INTRODUCTION

Thoroughbred horses originated from a small group of North African and Middle Eastern horses were taken to England and bred for their racing ability. Arabian, Barb and Persian stallions were crossed with native mares, and made a definite contribution to Thoroughbred development. Genetic studies based on genealogical data have shown that, if all ancestors of contemporary horses with a contribution of ≥1% are considered, only 21 individuals are responsible for 80% of the genes present in the modern Thoroughbred population (Cunningham, 1991).

The Argentine Creole horse breed is descended from the horses brought to the Americas by the Spanish conquerors. The descendants of these horses spread throughout America, and became wild horses called *baguales* or *cimarrones* in Argentine. At the end of the 19th century and the beginning of the 20th century, there was a renewed interest in recovering this breed, which had nearly disappeared through miscegenation with other breeds. This movement strived to restore the racial integrity of this breed by choosing sires with characteristic morphological features. The initial selective stage began with the establishment of the Selective and Genealogical Record, in 1939. Two important characteristics of the Creole breed are its hardiness, as shown by the ability to consume and assimilate any type of forage, and its resistance to and rapid recovery from fatigue. This breed also shows elevated fertility and longevity, and the animals are particularly strong (Dowdal, 1982). This breed also has phenotypic variability, but few studies (Peral García, 1994, 1996) have been done to determine the degree of genetic variability.

Biochemical polymorphisms are useful tools for evaluating genetic variability. The number and frequencies of alleles at different loci can be used to determine the genetic profile of a breed and to distinguish between individuals, populations and breeds. Genetic divergence among populations or breeds is usually measured by analysis of genetic distance and by fixation indexes (Wright, 1965). Nei (1977) and Wright (1978) concluded that all the fixation indices can be defined using the observed and expected heterozygosities at the equilibria of each population, when studying differentiation among populations of the same or different breeds.

We compared the genetic variability of Thoroughbred and Argentine Creole horses based on serum protein polymorphisms.

MATERIAL AND METHODS

Blood samples were obtained from 200 Thoroughbred and 124 Argentine Creole horses in the province of Buenos Aires. The individuals were chosen randomly from those registered by their respective official recording organization (Argentine Stud Book and Selective and Genealogical Record of Creole Horses). Electrophoresis techniques (Braend, 1967; Gahne et al., 1977; Juneja et al., 1978) were used to study the loci that codify the proteins: albumin - Al, esterase - Es, α_1B-glycoprotein - Xk, transferrin - Tf and hemoglobin - Hb.

Measures of genetic variability such as the heterozygosity per locus (h) and average heterozygosity for joint loci (H) were determined by means of $h = 1 - \sum x_i^2$ and $H = \sum h/r$, respectively, where r is the number of loci and x_i^2 is the square of the frequency of each allele for the i loci. The variance of the average heterozygosity was estimated by $V(H) = \sum (hj - H)^2/(r - 1)/r$, where $\sum (hj - H)^2/(r - 1)$ corresponds to $V(h)$ or the heterozygosity per locus variance, as

described by Crow and Kimura (1970) and Nei (1987).

The genetic differentiation between the horse breeds was analyzed by using fixation indices (Wright, 1965), modified by Nei (1977) and Wright (1978). The F_{IS} and F_{IT} statistics measure gene frequency differences by determining the excess or deficit of heterozygote phenotypes in each population and in the whole population, respectively. These statistics can have negative or positive values. The F_{ST} statistic measures the degree of differentiation among populations. Its value lies between zero and 1. The analyses were performed for each locus and for the mean of joint loci (Nei, 1987).

The deviations of F_{IS} and F_{IT} from zero were tested by the chi-square test $\chi^2 = NF^2$ (Hedrick, 1985). The significance of the genetic differences between populations (F_{ST}) was evaluated by a chi-square analysis [$\chi^2 = 2N F_{ST} (k - 1)$] with $(k - 1)(s - 1)$ degrees of freedom, where N is the total sample size, k is the number of alleles and s is the number of populations (Workman and Niswander, 1970; Chesser, 1983). The calculations were made using the statistical package BIOSYS (Version 1.7; Swofford and Selander, 1989).

RESULTS AND DISCUSSION

Of the five systems examined, Tf and Hb were not in Hardy-Weinberg equilibrium in either breed and Es was not in equilibrium in the Creole breed (Table 1). The observed fraction of heterozygotes for the five loci (H), was high in both breeds. In the Creole horses, the genetic

Table 1. Allele frequencies and chi-square test for deviation from Hardy-Weinberg equilibrium results for the albumin (Al), esterase (Es), α_1B-glycoprotein (Xk), transferrin (Tf) and hemoglobin (Hb) systems.

Systems	Alleles	Allelic frequencies			
		Thoroughbred	χ^2	Argentine Creole	χ^2
Albumin (Al)	*Al-A*	0.153		0.496	
	Al-B	0.847	n.s.	0.504	n.s.
Esterase (Es)	*Es-F*	0.047		0.153	
	Es-G	0		0.242	
	Es-H	0		0.121	
	Es-I	0.880	n.s.	0.464	P<0.001
	Es-O	0		0.020	
	Es-S	0.073		0	
α_1B-glycoprotein (Xk)	*Xk-F*	0		0.020	
	Xk-K	0.995	n.s.	0.479	n.s.
	Xk-S	0.005		0.500	
Transferrin (Tf)	*Tf-D*	0.290		0.359	
	Tf-F₁	0.295		0	
	Tf-F₂	0.195		0.065	
	Tf-F₃	0		0.224	
	Tf-H	0.073	P<0.001	0.117	P<0.001
	Tf-J	0		0.044	
	Tf-M	0		0.012	
	Tf-O	0.083		0.101	
	Tf-R	0.065		0.060	
Hemoglobin (Hb)	*Hb-A*	0.500		0.310	
	Hb-B	0.500	P<0.001	0.690	P<0.05

n.s - nonsignificant.

variability was H = 0.585 (Table 2). Heterozygosity was greatest for the transferrin locus in both breeds. In Creole horses, about 70% of the individuals were heterozygotes for Tf and Es genotypes, with values of 0.4-0.6 at the other loci. In Thoroughbreds, the heterozygosity of the other systems was very diversified, with Xk showing a diversity of h = 0.01 (Table 2). The average heterozygosity is not representative of the actual situation at each locus, since important discontinuities were found among the diversity values of Thoroughbreds. The discontinuities indicate that the Thoroughbred population has a greater than expected variability.

The prevalence of heterozygote phenotypes in Creole horses (Table 2) confirms the

Table 2. Average heterozygosity for a locus (h) and average heterozygosity for the joint loci (H*)* in Thoroughbred and Argentine Creole horses, and estimates of F_{IS} and F_{IT} excess or deficit of heterozygotes and F_{ST} degree of genetic differentiation for each locus and for the average of loci.

Locus	Thoroughbred		Argentine Creole		F_{IS}	F_{IT}	F_{ST}
	h	SE	h	SE			
Albumin (Al)	0.259	(0.047)	0.500	(0.042)	0.100n.s.	0.221*	0.131*
Esterase (Es)	0.218	(0.068)	0.688	(0.050)	-0.004n.s.	0.123n.s.	0.124***
α_1B-glycoprotein (Xk)	0.010	(0.171)	0.519	(0.033)	0.056n.s.	0.361***	0.323***
Transferrin (Tf)	0.775	(0.210)	0.779	(0.097)	-0.153n.s.	-0.117n.s.	0.029***
Hemoglobin (Hb)	0.500	(0.073)	0.428	(0.079)	-0.639***	-0.581***	0.033*
Average (H)	0.353	(0.131)	0.585	(0.065)	-0.156	-0.026	0.109

V(h) = per locus variance
H_0: F_{ST} = 0; H_1: F_{ST} >0; n.s. = nonsignificant; *P≤0.05; ***P≤0.001.

high degree of genetic diversity in this breed. Although there was extensive heterozygosity at each locus, the differences among the values of heterozygosity between loci were minimal. Although there is an important degree of genetic diversity among Creole horses, the average heterozygosity of a horse for those loci was similar for all individuals of this breed. Apparently there is some sort of mechanism for maintaining numerous allelic variants at these loci.

The F_{IT} statistic (Table 2), calculated for the population as a whole (N = 324), indicated an overall increase of 2.6% in heterozygote phenotypes (F_{IT} = -0.026) compared to homozygotes. The average excess of heterozygotes for each breed was 15.6% (F_{IS} = -0.156). At each locus, in Tf, Es and Hb the fraction of heterozygotes exceeded the fraction of homozygotes, with significant values only for Hb (F_{IS} = -0.639). For Al and Xk, the predominant phenotypes were homozygotes.

The degree of differentiation between breeds was highly significant for Xk (F_{ST} = 0.131), Es and Tf, and significant for Al and Hb (Table 2). The average genetic differentiation between breeds was 10.9% (F_{ST} = 0.109). This average estimate was similar to those determined for certain human races (F_{ST} = 0.088) (Nei and Roychoudhury, 1982), as well as breeds of Spanish dogs (F_{ST} = 0.099) (Jordana et al., 1992). The principal genetic differences at the population level (breed differences) were also about 10% (F_{ST} = 0.109) in our study. The remaining 90% of the total genetic variability was attributable to individual differences.

The heterozygosity of the hemoglobin loci in the two breeds and of the esterase and transferrin loci in Creole horses might have conferred some selective advantage on individuals bearing two different alleles. The genetic characteristics of these breeds reflect responses to environmental changes and to the type and magnitude of genetic variability originally present.

The response to selection pressures apparently has favored maintenance of some multiallelic systems in Argentine Creole horses. Heterozygosity may have played a fundamental role in adaptation to new situations.

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