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κ-Casein gene frequencies support subdivision and historical origin of Argentine Creole cattle

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ABSTRACT

Gene frequencies at the κ-casein locus were estimated in six different herds (N = 180) of Argentinean Creole cattle. The results showed a strong influence of subdivision and independent evolution on the divergence of the observed gene frequencies. These results suggest that the population structure of these herds favor the maintenance of polymorphism, which is of crucial importance for the long-time survival of populations.

INTRODUCTION

Anthropological and paleontological evidence show that *Bos taurus* members of the bovidae family were first introduced into America by Spanish conquerors. When Cristobal Colón landed on La Española Island (Haiti and Dominican Republic) for the second time, in 1493, he brought the first cattle to the new world.

The founding population consisted of almost 300 animals belonging to the Iberian breeds Retinta, Berrenda, Cacere and Black Andaluza (Primo, 1992). Only a few years later, these cattle were taken to Central and South America, and the southwest and southeast of what is now the United States, spreading over the continent. Since the introduction of these animals into the new world, no crosses with other breeds have been registered (Rabasa, 1993).

The Argentinean Creole cattle originated from three different introductions. In 1549, Juan Nuñez de Prado introduced the first bovines from Potosí (Bolivia). Later, Francisco de Aguirre brought his herds from Chile through the Andes mountains and Juan de Salazar, landing in Brasil, introduced more animals to the Argentine Pampas, from the north (Paraguay) (Gilberti, 1961; Montoya, 1984).

After many years of natural selection, the Creole cattle became wild, acquiring resistance to a wide range of environmental conditions. This breed shows a high level of phenotypic diversity and exhibits a high degree of longevity, fertility and resistance to many subtropical diseases (Guglielmone *et al.*, 1991; Rabasa, 1993; Hansen, 1994).

The actual Argentinean Creole cattle population is divided into small subpopulations, with a low degree of gene flow among them. Some are located in isolated places, where human management is almost excluded. This suggests that this breed could be considered as a genetic reservoir for a species under high selective pressure elsewhere.

κ-casein is one of the four casein proteins found in milk (Jenness *et al.*, 1956; Whitney *et al.*, 1976; Eigel *et al.*, 1984; Boland *et al.*, 1992). Gene and genotype frequencies of the κ-casein locus have been studied by polymerase chain reaction and restriction fragment length polymorphisms (PCR-RFLP) methods (Rogne *et al.*, 1989; Damiani *et al.*, 1990; Denicourt *et al.*, 1990; Pinder *et al.*, 1991; Agrawala *et al.*, 1992). Only two milk protein variants, A and B, have been identified and they occur in every breed, suggesting that this is a very ancient polymorphism. A comparative study between

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different bovine breeds has shown that certain breed groups could be readily differentiated by means of κ-casein gene frequencies (Baker and Manwell, 1980).

MATERIAL AND METHODS

Peripheral blood was sampled from 30 Argentinean Creole cattle from each of six herds (Table I). These herds are situated in quite different geographical locations. In particular, two of these herds (Chasquivil and Arroyo del Medio) have been isolated by geographical barriers for a long period (Table I).

Genomic DNA was isolated from white blood cells according to the method of Sambrook *et al.* (1989). Animals were chosen at random from each herd, and DNA samples were typed for the κ-casein (κ-Cn) locus, using the PCR-RFLP method, described by Agrawala *et al.* (1992).

Table I - Historical origin of each Argentinean Creole cattle population.

Subpopulation	Historical origin
Balcarce	Derived from a population located in the center-north (INTA Leales, Tucumán)
Las Acacias	Derived from two different populations (INTA Leales and Buenos Aires Province)
Cruz de Guerra	Multiple origins, including INTA Leales
Concordia	Derived from a population located in the northeast (Romero herd)
Chasquivil	Population under geographical isolation in a high altitude grassland for more than 25 generations
Arroyo del Medio	Population located in the northwest (Jujuy and Salta Provinces)

Reaction conditions

The PCR amplification was carried out in 50 µl, in a Perkin-Elmer thermal cycler, according to the following reaction conditions: 10 mM Tris-HCl, pH = 8, 50 mM KCl, 1.5 mM $MgCl_2$ and 0.1% Triton X100, 100 µM of each dNTP, 1.25 U of Taq polymerase, 0.5 µM of each primer (*5′-GTGACAAAATAGCCAAATAT-3′* and *5′-ACAGATTTAATTTATCCATCT-3′*) and 100 ng of genomic DNA. A 4-min denaturation step at 94°C was followed by 30 cycles with denaturation at 94°C (1 min), annealing at 55°C (1 min 30 sec) and extension at 72°C (2 min). The last polymerization step was extended to 10 min at 72°C. Samples of PCR products (5 µl) were digested with Hae III according to the manufacturer's recommendations.

The restriction fragments were analyzed by electrophoresis (100 V, 30 min) in TBE buffer with ethidium bromide stained 3% agarose gels.

Genetic structure analysis

The Wright's fixation index F_{isk} (Wright, 1931; Nei, 1987) was used to estimate the deviation from Hardy-Weinberg equilibrium in each subpopulation and in the whole population.

Heterogeneity was tested by a χ-square contingency table. An angular transformation of each sample gene frequency was used to detect specifically which gene frequencies differed from each other, according to Zar (1984).

RESULTS

The observed genotypic frequencies did not deviate from the predicted Hardy-Weinberg equilibrium values in each herd (Table II). However, the observed equilibriums for each herd were different, suggesting that the different subpopulations reached distinct adaptive peaks. The F_{isk} statistic showed negative values in all the herds except Cruz de Guerra, suggesting a tendency towards the heterozygotic form (Table II).

The population gene frequencies were obtained by pooling the observed gene frequencies from each subpopulation. These "artificial" population frequencies did not deviate from Hardy-Weinberg equilibrium expectations. However, the F_{ST} statistic was quite high, with a highly significant value (F_{ST} = 0.071; $P < 0.01$); suggesting a high degree of heterogeneity.

Table II - Gene and genotype frequencies of κ-Cn locus, values of F_{isk} and χ^2 test of Hardy-Weinberg equilibrium for each subpopulation and the whole population.

Subpopulation	A	B	AA	AB	BB	F_{isk}	χ^2
Balcarce	0.821	0.179	0.641	0.359	0.000	-0.210	1.874
Cruz de Guerra	0.725	0.275	0.550	0.350	0.100	0.122	0.298
Las Acacias	0.806	0.194	0.610	0.380	0.000	-0.240	1.050
Concordia	0.595	0.405	0.333	0.524	0.143	-0.087	0.159
Chasquivil	0.565	0.435	0.291	0.548	0.161	-0.110	0.410
Arroyo del Medio	0.516	0.484	0.281	0.469	0.250	0.062	0.121
Pooled Data	0.647	0.353	0.466	0.483	0.111	-0.058	0.615

In order to detect which gene frequencies differed from each other, an angular transformation of each gene frequency was used (Table III). The comparison revealed that the herds could be classified into two groups. One of these groups (Balcarce, Cruz de Guerra and Las Acacias) showed κ-Cn A gene frequencies higher than 0.7, whereas in the other group (Concordia, Chasquivil and Arroyo del Medio) this value was lower than 0.6. However, the gene frequencies estimated for Cruz de Guerra, which was classified in the first group, showed no significant differences from the two subpopulations belonging to the other group (Chasquivil and Concordia) (Table III).

Table III - Multiple comparison of gene frequencies using arcsin transformation to determine which frequencies were different from each other.

Subpopulation	B	C	Ac	Ch	ER	AM
B	-					
C	**2.936**	-				
Ac	**0.619**	**1.982**	-			
Ch	7.553***	***3.716***	5.788***	-		
ER	5.828***	***2.672***	4.560*	**0.744**	-	
AM	8.694***	4.748*	6.793***	**1.134**	**1.763**	-

B = Balcarce, C = Cruz de Guerra, Ac = Las Acacias, Ch = Chasquivil, ER = Concordia, AM = Arroyo del Medio.
* Critical value is $q_{0.05, \infty, 6} = 4.030$
***Critical value is $q_{0.001, \infty, 6} = 5.619$
Bold type indicates the defined groups.

The analysis of data from this system suggests subdivision in the gene frequencies of the small wild cattle populations and correlation with the origin of each subpopulation. On the other hand, pooled data from the six herds correspond with the gene frequencies reported for Iberian breeds (Baker and Manwell, 1980).

DISCUSSION

Farm animals are not usually Mendelian populations. Several factors such as inbreeding, selection, breeding structure, etc. can disturb Hardy-Weinberg equilibrium. In spite of these limitations κ-casein frequencies were in equilibrium in each herd and the whole population of Argentine Creole cattle. By contrast, other breeds present significant differences in genotypic frequencies compared with the expected frequencies (Poli and Antonini, 1991). In Argentine Creole cattle the F_{isk} analysis suggested a trend toward an increment of heterozygosity, according to the theory of finite population dynamics (Crow and Kimura, 1970), since the effective number in each herd was quite small.

From the time the first animals arrived in the Argentina until about a hundred years ago, Creole cattle formed one large population, with a high degree of natural and artificial vagility, spreading all over the country. Near the end of the last century, European immigrants brought other European cattle breeds. Afterwards, Creole cattle populations were restricted to peripheral areas and split into finite populations. The introduction of *Bos indicus* (1930) displaced Creole cattle populations, increasing their subdivision. The actual population size is now limited to approximately 300,000 head.

Our analysis of the κ-Cn locus showed that the Argentinean Creole cattle population has a high degree of heterogeneity between subpopulations, evidenced by F_{ST} statistic analysis. Such genetic heterogeneity among subpopulations could be explained by several factors, such as mating structure, finite population size, genetic drift or selection. Thus, the population structure of Argentinean Creole cattle could be relevant in the maintenance of polymorphism, which is thought to be crucial for the long-time survival of populations. In this sense, our results suggest that Creole cattle could be considered as a genetic reservoir.

The gene frequencies of A and B alleles could be related to the original ancestors: the herds from Balcarce, Cruz de Guerra and Las Acacias, belonging to the same group, have been reported to be descendents of a population found in northern Argentina (Table I). Herds classified in the other group had three different origins, Concordia originated in a population located in the northeastern part of the country, the herd from Chasquivil has been isolated in the high grasslands for more than 25 generations, and the population from Arroyo del Medio has been located for a long time in an inaccessible site in the northwestern part of the country (Table I). The multiple historical origin of the Cruz de Guerra subpopulation could account for the gene frequencies observed in this herd, comparable to those from Concordia and Chasquivil (Table III).

Analysis of data from a biallelic system, the κ-casein locus, made evident interesting features concerning independent evolution of the creole cattle herds studied. Moreover, the observed gene frequencies reflected the ancestral trunk of each subpopulation studied.

The subdivision observed in Argentine Creole cattle population could be taken as evidence of gene frequency differentiation, a feature which is considered relevant in conservation programs (Crow and Kimura, 1970)

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RESUMO

As freqüências genéticas no locus da κ-caseína foram estimadas em seis diferentes rebanhos (N = 180) de gado Creole argentino. Os resultados mostraram uma forte influência de subdivisão e evolução independente na divergência de freqüências gênicas observadas. Estes resultados sugerem que a estrutura populacional dos rebanhos estudados favorece a manutenção do polimorfismo, o qual é de crucial importância para a sobrevivência a longo prazo das populações.

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