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GENETIC VARIABILITY AND POPULATION STRUCTURE IN LOCI RELATED TO MILK PRODUCTION TRAITS IN NATIVE ARGENTINE CREOLE AND COMMERCIAL ARGENTINE HOLSTEIN CATTLE

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ABSTRACT

Many cattle breeds have been subjected to high selection pressure for production traits. Consequently, population genetic structure and allelic distribution could differ in breeds under high selection pressure compared to unselected breeds. Analysis of κ-casein, α_{S1}-casein and prolactin gene frequencies was made for Argentine Creole (AC) and Argentine Holstein (AH) cattle herds. The calculated F_{ST} values measured the degree of genetic differentiation of subpopulations, depending on the variances of gene frequencies.The AC breed had considerably more variation among herds at the α_{S1}-casein and κ-casein loci. Conservation strategies should consider the entire AC population in order to maintain the genetic variability found in this native breed.

INTRODUCTION

Genetic markers for milk production traits

Genetic selection of cattle by selective breeding dates back to prehistoric times and has given rise to the diversity of current breeds. Primitive selection was based on milk yield (Boland *et al.*, 1992).

Genes involved in milk production have been studied in populations of several breeds in order to find differences between breeds or groups of breeds (Aschaffenburg and Thymann, 1965; Baker and Manwell, 1980; Poli and Antonini, 1992; Boland *et al.*, 1992; Velmala *et al.*, 1993; Medjugorac *et al.*, 1994). Classifications were established based on polymorphism. Correlation between allelic variants of milk proteins and milk production has been proposed by several authors (Ng-Kuai-Hang *et al.*, 1984; Lin *et al.*, 1986; Cowan *et al.*, 1990; van der Berg *et al.*, 1992; Lien and Rogne, 1993), but the results obtained have not always been consistent (Velmala *et al.*, 1995).

Many genes are involved in milk production. Among them, caseins are the major constituents of total milk proteins. In bovines, their genes are located within a 200-kb region in chromosome 6 (Ferretti *et al.*, 1990; Threadgill and Womack, 1990). Several DNA polymorphisms have been found for each casein gene, most of them based on previously described protein variants (Eigel *et al.*, 1984). In addition, the polypeptidic hormone prolactin is responsible not only for triggering lactation but also for mammary gland growth and lactogenesis (Tucker, 1981; Collier *et al.*, 1984). This feature suggests that this locus might be used as a genetic marker for milk production. Two allelic variants (*B* and *b*) have been distinguished at the DNA level, based on a *Rsa*I polymorphism in the third exon of the coding region. It has been suggested that prolactin alleles correlate with milk yield (Lewin *et al.*, 1992).

Creole cattle as a model of an unselected breed

Many cattle breeds have been selected for different production traits through high selection pressure. South American Creole breeds were adapted to different environments by natural selection after their introduction by European conquerors in the middle of the 15th century (Rabasa, 1993; Guglielmone *et al.*, 1991; Primo, 1992). Unselected *Bos taurus* Creole cattle are difficult to find at the present time since most of the Creole breeds frequently have been crossbred with *Bos indicus*. However, the introgression of *Bos indicus* in Argentine Creole cattle (AC) seems to be irrelevant since cytogenetic studies showed the absence of the typical acrocentric *Bos indicus* Y chromosome (De Luca *et al.*, 1997). Therefore, AC can be defined as pure descendants of animals brought over by Spanish conquerors.

The AC breed has passed through some bottlenecks in reaching the current equilibrium of about 300,000 animals. The population structure and behavior of AC is quite different from other breeds. First, it is composed of several subpopulations, with a low number of individuals per herd (around 200 or 300 each). Second, some of these herds are bred in subtropical dry forests, under rather wild conditions, where European breeds are poorly adapted (Rabasa, 1993).

Argentine Holstein: a breed selected for milk production

Argentine Holstein (AH) is the most important dairy breed in Argentina, with a population size of over three million. It was first introduced to Argentina from the

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Netherlands in 1883 (Inchausti and Tagle, 1967). During the last decades, animals, semen, and embryos have been imported mainly from Canada and USA by Argentine breeders (Poli and Antonini, 1992), in order to improve production traits.

AC and AH herds were compared through analysis of κ-casein, α_{S1}-casein and prolactin gene frequencies to determine if population genetic structure and gene frequencies are different in breeds under high selection pressure compared to unselected breeds.

MATERIAL AND METHODS

Blood samples

Blood samples (10 ml) of 113 AH (three herds) and 180 AC (six herds) were collected in ACD as anticoagulant (0.48 g citric acid, 1.32 g sodium citrate and 1.47 g dextrose, water to 100 ml), from which genomic DNA was isolated. DNA was extracted from leukocytes by proteinase K digestion and extraction with phenol:chloroform:isoamyl alcohol (25:24:1, v/v/v). DNA was precipitated with 1 M ammonium acetate and 100% ethanol. The DNA pellet was then washed with 70% ethanol and suspended in water.

Genotyping

Genotyping of κ-casein and prolactin genes was performed by PCR-RFLP, whereas α_{S1}-casein was typed by PCR-SSP (sequence specific primers). To analyze the κ-casein locus, a 586-bp fragment covering the sequence containing the mutation site was amplified according to the procedure proposed by Agrawala *et al.* (1992). The amplicon was digested with *Hin*fI restriction endonuclease to distinguish *A* and *B* alleles. A 156-bp fragment covering the sequence containing the polymorphism involving the *B* and *b* allelic types of prolactin gene was amplified by PCR using the primers and procedure reported previously by Lewin *et al*. (1992). After amplification, genotyping of prolactin allelic variants was carried out by digesting the amplified fragment with *Rsa*I restriction endonuclease. The α_{S1}-casein alleles were identified by PCR using sequence specific primers according to the procedure proposed by David and Deutch (1992). Results from amplification or digestion of the amplified fragments were analyzed by electrophoresis on 3% (w/v) agarose gels in 0.5 x TBE (100 v, 30 min), stained with ethidium bromide, and photographed under 320-nm UV light.

Statistical analysis

Gene frequencies for each herd and for the entire population were estimated by direct gene count method: $(n_{AB} + 2n_{BB})/2n$, where n_{AB} and n_{BB} were the numbers of AB and BB genotypes. Standard error of gene frequencies was calculated as $(p(1-p)/2n)^{1/2}$, where *n* is the sample size and *p* is the frequency of one allele. Deviation from Hardy-Weinberg expectations was tested by F_{IS} fixation index (Nei, 1987) using the formula $(h_e - h_o)/h_e$, where h_e and h_o are the expected and observed heterozygosity (fraction of heterozygotes) within each population. Differences between the distributions of genotypic frequencies were tested using χ^2 analysis according to Nei (1987).

Standardized variance of gene frequencies within each breed was calculated using the F_{ST} statistic as described by Nei (1987), using $(h_S - h_t)/h_S$, where h_S and h_t are the heterozygosities in a subpopulation and in the total population. The F_{ST} values were tested by χ^2 analysis according to Chesser (1983). For this analysis each breed was considered as a population, whereas each herd was considered as a subpopulation.

RESULTS

Three genotypes were identified for κ-casein (*AA*, *AB*, and *BB*) and α_{S1}-casein (*BB*, *BC*, and *CC*) in both breeds (Table I). Prolactin allele *b* seems to be almost fixed in AC, since the *B* allele was detected only in heterozygote form.

Nonsignificant differences between breeds were found for the κ-casein locus (Figure 1). On the other hand, comparison of prolactin and α_{S1}-casein genotypic frequencies showed significant differences by χ^2 analysis. A clearly significant difference in the gene frequencies for α_{S1}-casein loci was observed. This could be related to the tight correlation between alleles and milk yield. In fact, most dairy breeds have gene frequencies higher than 90% for α_{S1}-casein B allele (Ng-Kuai-Hang *et al.*, 1984; Lin *et al.*, 1986).

Results from F_{ST} analysis indicated major differences in the genetic architecture of the two breeds. The different AH herds exhibited similar gene and genotypic frequencies of the loci analyzed (Table II). The AC cattle

Table I - Gene frequencies, standard errors of gene frequencies, F_{IS} statistic and Hardy-Weinberg equilibrium for κ-casein, α_{S1}-casein and prolactin genes in Argentine Holstein (AH) and Argentine Creole (AC) cattle .

Locus	*Breed*	*Allelic frequencies*		*SE*	F_{IS}	χ^2
		A	**B**			
κ-casein	AH	0.656	0.344	0.032	-0.025	0.097 ns
	AC	0.647	0.353	0.025	-0.103	0.000 ns
		B	**C**			
α_{S1}-casein	AH	0.923	0.077	0.027	0.097	0.737 ns
	AC	0.747	0.253	0.023	0.049	0.433 ns
		B	**b**			
Prolactin	AH	0.129	0.871	0.022	-0.027	0.000 ns
	AC	0.044	0.956	0.011	-0.081	0.000 ns

ns: Nonsignificant differences; SE: Standard error of the gene frequencies; F_{IS} and χ^2: F_{IS} index and deviations from the expected values, as described in Material and Methods.

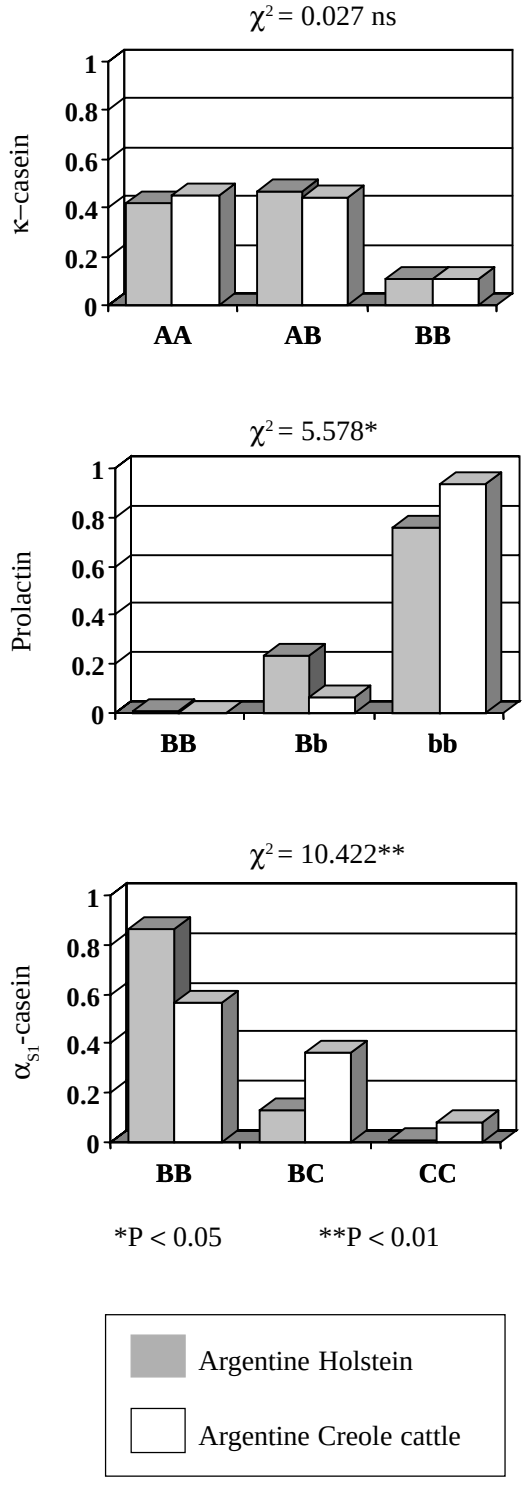

Figure 1 - Comparisons of genotypic frequencies for κ-casein, α_{S1}-casein and prolactin loci between AH and AC breeds.

Table II - F_{ST} values and chi-square significance tests for the three loci analyzed in Argentine Hosltein (AH) and Argentine Creole (AC) breeds.

Breed	AH		AC	
	F_{ST}	χ^2	F_{ST}	χ^2
κ-casein	0.014	3.223 ns	0.066	25.151***
α_{S1}-casein	0.039	8.891 ns	0.09	34.361***
Prolactin	0.006	0.958 ns	0.036	13.730 ns

***P < 0.001.

showed a quite different genetic status, with strong differences between herds. In this breed, the values obtained for the F_{ST} statistic per locus always were highly significant, except for the prolactin locus, due to fixation of one allele (Table II).

DISCUSSION

Studies on protein variation using electrophoretic and molecular techniques have permitted the estimation of two parameters of preeminent importance in evolutionary theory: a) the degree of genetic variability within populations, and b) the degree of genic differentiation between populations (Avise *et al.*, 1975). A variety of statistics have been devised to measure genetic differentiation (or its opposite, genetic similarity) between populations. Genetic divergence among populations of the same or different breeds usually is quantified by fixation indices or *F* statistics (Wright, 1955).

Assuming that both breeds (AH and AC) have similar limitations, Hardy Weinberg equilibrium could be compared. The results showed that both breeds are in equilibrium for the loci analyzed. This fact could be considered an indicator that artificial selection, at present, is not disturbing the equilibrium of gene frequencies in these milk production-related loci. No evidence of inbreeding was found in AH, which is a highly selected breed, according to the results obtained from analysis of averaged F_{IS} across subpopulations (Table I). F_{IS} does not directly detect inbreeding. However, an increase in the fraction of homozygotes (with a positive F_{IS}) would be expected. The high level of gene flow supported by sire exchange or artificial insemination could be responsible for the low level of inbreeding found in AH breed. In fact, a great similarity between different AH herds was shown by F_{ST} results. Gene flow seems to be increasing the effective population size of this breed in a way that all herds behave as replicates of a master population widespread all over the country.

Different from AH genetic architecture, the AC breed showed considerable difference between herds, as revealed by F_{ST} values of α_{S1}-casein and κ-casein loci (Table II). Isolation and low levels of gene flow could be the main causes of maintenance of this subdivided status. Given the small size of each AC herd, genetic drift could be the

mechanism originating genetic differentiation between AC populations. Differences in prolactin were also observed between breeds (Figure 1). This locus did not reveal subdivision in AC, since *B* allele seems to be almost fixed in all the herds (Table I). This fact could be explained by two hypotheses: 1) the same status for this locus in the founding population, and 2) selection against the *B* allele in AC during development of the original population.

Preservation of gene diversity in natural and farm animal populations is crucial for their long-term survival (Avise, 1994). As the population structures of AH and AC differ, the scheme for conservation of genetic variability would necessarily be different for each breed. AH genetic structure seemed to be maintained by a high level of gene flow, at least in the studied loci, which are related with milk production. In contrast, the gene pool (averaged gene frequencies) in the AC breed is the result of the individual contribution of each heard. Conservation strategy would have to consider the whole population in order to mantain the genetic variability found in this locally adapted breed.

RESUMO

Muitas raças de gado foram submetidas a alta pressão de seleção para caracteres de produção. Conseqüentemente, a estrutura genética e a distribuição alélica da população poderiam diferir em raças sob alta pressão de seleção, quando comparadas a raças não selecionadas. Foi feita a análise das freqüências dos genes de κ-caseína, α_{S1}-caseína e prolactina em rebanhos de gado Creole argentino (AC) e Hosltein argentino (AH). Os valores de F_{ST} calculados mediram o grau de diferenciação genética de subpopulações, dependendo de variações na freqüência dos genes. A raça AC apresentou variação consideravelmente maior entre os rebanhos nos loci de α_{S1}-caseína e κ-caseína. Estratégias de conservação devem considerar a população inteira de AC de forma a manter a variabilidade genética encontrada nesta raça nativa.

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