Rev. Brasil. Genet. 12, 1, 27-37 (1989)
(Brazil. J. Genetics)

A STUDY OF BIOCHEMICAL POLYMORPHISMS IN EUROPEAN/ZEBU DAIRY CROSSBRED CATTLE AND OF THEIR RELATIONSHIP WITH EUROPEAN AND ZEBU CATTLE

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ABSTRACT

The objective of the present investigation was to study the genetic structure of a dairy crossbred herd. Hemoglobin, amylase, albumin, transferrin, carbonic anhydrase and purine nucleoside phosphorylase were studied in 505 females divided into five genetic groups (3/8, 1/2, 5/8, 3/4 and 7/8 crosses between European and Zebu cattle). Highly significant ($P < 0.01$) variation was detected in gene frequency in all systems analyzed, depending on the "type of blood" contributing to each cross (European or Zebu).

INTRODUCTION

Characterization of the genetic structure of different breeds through biochemical polymorphisms is extremely important and has been used to study evolutionary relationships among cattle breeds (Baker and Manwell, 1980; Manwell and Baker, 1980; Singh and Nair, 1981; Baker and Manwell, 1983; Fernandez, 1985).

Marked differences have been found for gene frequencies between *Bos taurus* and *Bos indicus*. Thus, the bovine hemoglobin types are controlled by two alleles, Hb-A and Hb-B (Cabannes and Serain, 1955). Hb-A occurs in all cattle breeds studied so far. The less common Hb-B has been found in Asian and African Zebu cattle (frequency 0.5), in the Channel Island breeds, especially Jersey and the Upland breeds, and in other European breeds where the average gene frequencies range from 0.1 to 0.3 (Braend, 1972; Baker and Manwell, 1980).

The serum amylase I locus has been widely studied and has been reported to

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be encoded by three codominant alleles, Am I^A, Am I^B and Am I^C (Ashton, 1965; Gasparski and Stevens, 1968; Gebicke-Haerter and Geldermann, 1977; Archibald and Spooner, 1978). Data on amylase I^A, which was considered by Ashton (1965) to be a *Bos indicus* marker, indicate that it has a frequency of 0.41 in Jersey cattle and of 0.07 to 0.20 in other genetic groups. The frequency of amylase I^B ranges from 0.20 to 0.53 in European cattle breeds (Archibald and Spooner, 1978). Special attention is needed to distinguish amylase I^A from amylase I^B. Amylase I^C frequencies range from 0.26 to 0.59 in European cattle breeds (Archibald and Spooner, 1978) and are very low in Zebu cattle (Baker and Manwell, 1980).

Serum albumin (Alb) presents two codominant alleles, Alb-F and Alb-S. The frequency of allele Alb-S is above 0.9 in most Zebu breeds and virtually zero in North European and Pied Lowland breed groups (Khanna and Singh, 1972; Baker and Manwell, 1980).

The transferrin locus has been shown to be usually encoded by four alleles, Tf-A, Tf-D_1, Tf-D_2 and Tf-E (Smithies, 1957; Ashton, 1959; Kristjansson, 1960; Spooner *et al.*, 1970). For Asian and African cattle the frequencies of two additional transferrin variants, B and F, have been reported by Ashton (1959). Transferrin A, D_1 and D_2 do not seem to be specific for European or Zebu type cattle. Allele E is most frequent in Celtic and Scandinavian *Bos taurus* breeds, in *Bos indicus* and African humped (Sanga) cattle (Ashton, 1959; Osterhoff and Van Heerden, 1965; Manwell and Baker, 1980).

Carbonic anhydrase (CA) polymorphism was first described by Sartore *et al.* (1969); usually, CA presents two alleles, Ca-S and Ca-F. A new allele detected by the hemoglobin precipitation technique, Ca-Z, has been recently reported to occur at a frequency of 0.24 in the Nellore breed (Penedo *et al.*, 1982; Panepucci, 1988a). Carbonic anhydrase S (Ca-S) is the predominant allele in all breeds, at a frequency of 0.59 to 1.00 (Sartore *et al.*, 1969; Soos, 1972; Han and Suzuki, 1976; Efremov *et al.*, 1980; Shanker *et al.*, 1983). According to Baker and Manwell (1980) no statistically significant differences in carbonic anhydrase gene frequencies were detected among the different breed groups.

Purine nucleoside phosphorylase presents three phenotypes (Ansay and Hanset, 1972; Ansay, 1975). The common phenotype, NP-L, is represented by a thin anodal band; the second phenotype, NP-H also anodal, is a highly active large band and the third phenotype is also highly active and the band has slightly more anodal mobility. Mating analysis indicated that the NP-L phenotype is controlled by a recessive gene. NP-L frequencies are high in the European breeds studied, ranging from 0.52 to 0.94 (Ansay and Hanset, 1972; Gonzalez *et al.*, 1987). In Brazilian Zebu type Nellore cattle, the frequency of NP-H is a high 0.77 (Panepucci, 1988b).

This investigation shows the genetic structure of different crosses between *Bos taurus* and *Bos indicus* dairy cattle herds and their variation in gene frequencies in relation to the original frequencies of the two major cattle breed groups.

MATERIALS AND METHODS

Experimental animals

Blood samples were collected from 505 dairy crossbred females of different types of crosses between European and Zebu cattle. The crossbred individuals were divided into five genetic groups (3/8, 1/2, 5/8, 3/4 and 7/8 European) from farms in the states of Minas Gerais and São Paulo, Brazil. The European breeds involved in the crosses were: Brown Swiss, Holstein-Friesian, Red and White Holstein, Simmenthal, Danish and Jersey. The Zebu breeds involved were: Guzerat, Gyr and Indu-Brazil (which is a mixture of different *Bos indicus* breeds). These females are daughters of crossbred bulls (5/8, 3/4 and 7/8 European) involved in a progeny test.

Preparation of extracts and electrophoresis

Blood was taken from the jugular vein using heparine as anticoagulant and kept refrigerated until arrival to the laboratory. Samples were centrifuged for 15 minutes at 3000 rpm at room temperature. Plasma was collected and erythrocytes were washed three times in 0.9% NaCl. Six genetic systems were analyzed, including three red cell systems: hemoglobin (Hb), carbonic anhydrase (CA) and purine nucleoside phosphorylase (NP); and three plasma systems: amylase (Am), albumin (Alb) and transferrin (Tf). Genetic markers were identified by horizontal starch gel electrophoresis using the specific technique for each: hemoglobin (Braend, 1971), carbonic anhydrase by the hemoglobin precipitation technique (Shanker *et al.*, 1983), purine nucleoside phosphorylase by the method of Edwards *et al.* (1971), amylase by the method of Gebicke-Haerter and Geldermann (1977), transferrin by the method of Geldermann (1970) and albumin by the method of Kristjansson (1963). Data for discrimination between Tf D_1 and D_2 were not used because of technical problems at the beginning of the study that prevented this subtyping in 100 animals. Gene frequencies were determined by direct counting. The sample size for each genetic group in every protein system considered was over 30 individuals as recomended by Baker and Manwell (1980). Chi-square tests were performed to verify association between gene frequency and genetic groups, *i.e.* whether genetic groups show different gene frequencies.

RESULTS

The gene frequencies of the systems analyzed are given in Table I and Figure 1. Zebu "blood" decreases from left to right (Table I and Figure 1), while European "blood" increases in the same direction. Hemoglobin B, albumin S transferrin E and

Table I - Estimated gene frequencies (a) of the different systems analyzed in the different genetic groups and in comparison with foundation breeds.

System		Genetic groups						
		Zebu*	3/8	1/2	5/8	3/4	7/8	European*
Hb	A	–	0.69	0.74	0.79	0.81	0.85	–
	B	0.40	0.31	0.26	0.21	0.19	0.15	0.10-0.20
Am	B	–	0.88	0.78	0.67	0.64	0.68	–
	C	0.05	0.12	0.22	0.33	0.36	0.32	0.20-0.50
Alb	F	–	0.42	0.60	0.67	0.79	0.85	–
	S	0.90	0.58	0.40	0.33	0.21	0.15	0.10
Tf	A	0.15	0.32	0.30	0.33	0.42	0.44	0.30-0.60
	D	–	0.38	0.42	0.47	0.39	0.44	–
	E	0.30	0.30	0.28	0.20	0.19	0.12	0.10
Ca	S	–	0.91	0.84	0.90	0.79	0.87	–
	F	0.00	0.07	0.10	0.08	0.13	0.10	0.10-0.25
	Z	0.24[1]	0.02	0.06	0.02	0.08	0.03	–
NP	H	0.77[2]	0.54	0.52	0.39	0.26	–	0.25[3]
	L	0.23[2]	0.46	0.48	0.61	0.74	–	0.80[3]

(a) Gene frequencies were calculated from observed number of individuals shown in Table II.

*Average frequencies (Baker and Manwell, 1980).

[1] Average frequencies (Panepucci, 1988a).

[2] Average frequencies (Panepucci, 1988b).

[3] Average frequency (Ansay and Hanset, 1972).

purine nucleoside phosphorylase H gene frequencies show decreasing values from 3/8 to 7/8 genetic groups while amylase C shows an increasing value in the same direction. Carbonic anhydrase does not show a consistent trend with genetic groups.

The values observed for the different phenotypes detected in the various genetic groups are shown in Table II. The number of individuals tested was not the same for each system, having small variations except for nucleoside phosphorylase due to economical reasons. The chi-square values obtained when testing for Hardy-Weinberg

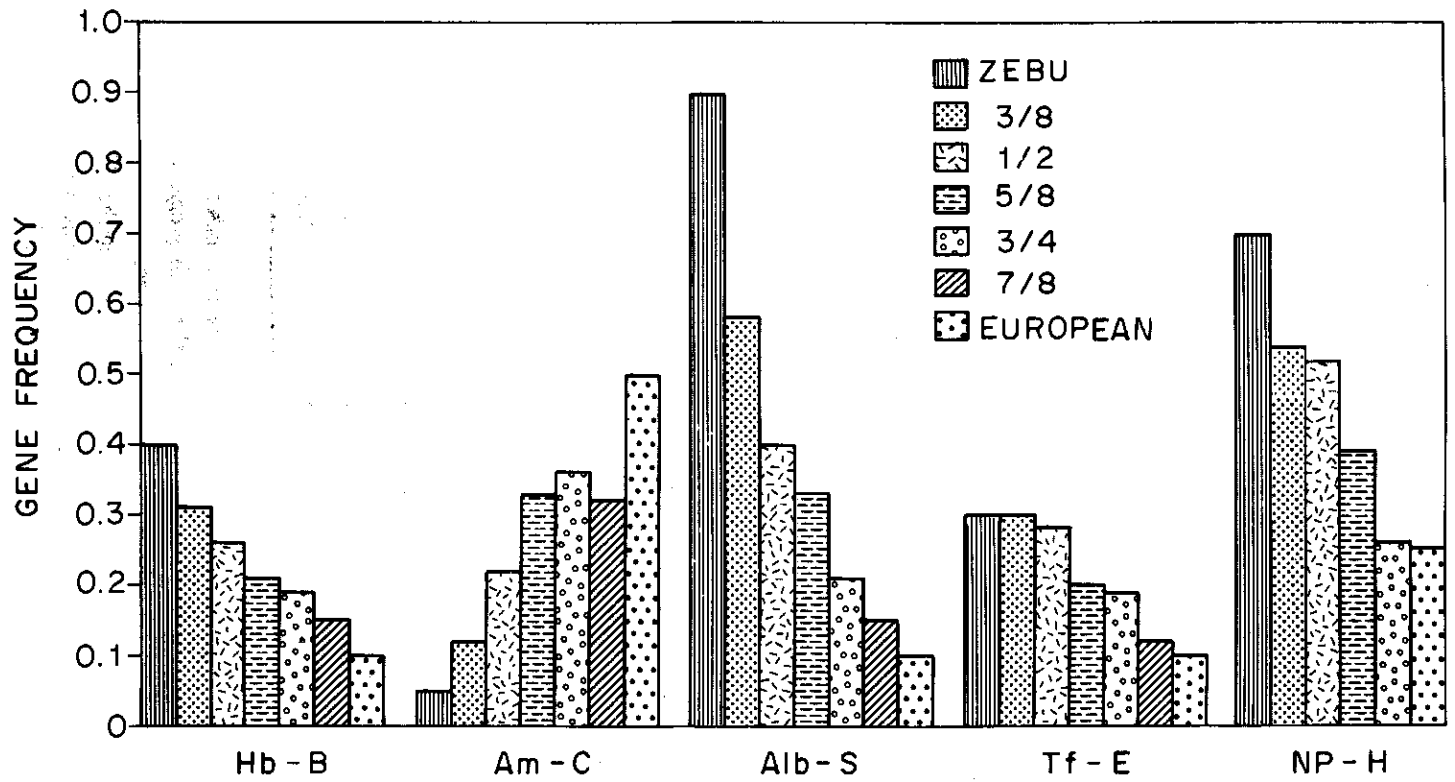

Figure 1 - Representation of the gene frequency of the different alleles according to genetic groups.

Table II - Observed numbers of the different phenotypes detected in the different genetic groups.

System		Genetic groups				
		3/8	1/2	5/8	3/4	7/8
Hb	AA	29	57	108	79	31
	AB	32	44	59	32	11
	BB	4	6	6	6	1
Am	BB	49	51	63	48	16
	BC	16	23	45	45	14
	CC	–	6	19	18	4
Alb	FF	6	25	54	70	27
	FS	43	51	63	43	12
	SS	16	8	11	3	–
Tf	AA	7	12	21	29	10
	DD	15	34	44	34	12
	EE	10	13	12	6	1
	AD	14	14	24	17	10
	AE	13	26	19	25	6
	DE	6	8	8	9	2
CA	SS	53	73	153	65	28
	FF	–	–	1	1	–
	ZZ	–	–	–	–	–
	SF	9	19	23	23	8
	SZ	3	9	5	13	2
	FZ	–	3	2	3	–
NP	H	70	90	31	30	–
	L	21	21	18	36	–

equilibrium are shown in Table III. Chi-square values for transferrins were highly significant in all groups tested, except for the 7/8 group. There were also significant chi-square values for albumin in 3/8 and 1/2 crosses.

Dependency tests of gene frequency between the different genetic groups are shown in Table IV. All genetic systems showed highly significant values. Only two animals with the Am-AC phenotype were found in the 3/4 genetic group.

Table III - Chi-square values for Hardy-Weinberg equilibrium obtained for the different phenotypes detected in the different genetic groups.

System	Genetic groups				
	3/8	1/2	5/8	3/4	7/8
Hb	1.56	0.32	0.39	1.26	0.00
Am	1.31	2.00	4.78*	1.79	0.13
Alb	8.24**	5.93*	1.55	1.48	1.37
Tf	11.80*	36.75**	38.77**	30.27**	6.97
CA	0.77	4.22	4.27	1.42	–

*P $<$0.05; **P $<$0.01.

DISCUSSION

Many authors have reported blood protein polymorphisms in breeds and crossbred populations of cattle. The present study shows varying gene frequencies in crossbred populations and their relationship to original breeds.

These results clearly indicate the presence of increasing and decreasing allele frequencies, depending on the system analyzed and according to the proportion of *taurus* "blood" that participates in the crosses. Hemoglobin has been studied in crossbred populations (Singh and Bhat, 1980; Kumaran *et al.*, 1982). As shown by several authors, hemoglobin B has a relatively high frequency in Zebu cattle and shows decreasing frequency from 3/8 to 7/8 European-Zebu crosses. The values obtained for the 1/2 cross were quite similar to those obtained by Kumaran *et al.* (1982) in 1/2 Holstein-Friesian x Hariana.

As stated before, amylase C has a very low frequency in Zebu cattle (Baker and Manwell, 1980). This study shows an increasing frequency of this allele with increasing proportion of European blood.

Albumin allele frequencies were found to be very specific for cattle breeds. The Alb-S frequency of Charolais cattle was found to be extremely similar in populations from Sweden, England and South Africa, ranging from 0.16 to 0.18 (Osterhoff, 1967; Spooner and Oliver, 1969; Gahne *et al.*, 1977). The Alb-S frequency in most Indian breeds is over 0.90 (Baker and Manwell, 1980). The variation found in the present study strongly confirms data in the literature and shows that albumin can be an excellent gene marker for breed relationship studies.

Transferrin E showed a clear variation, with higher frequencies in the 3/8 group and lower frequencies in the 7/8 group. This confirms other findings of higher

Table IV - Dependency test of gene frequency and different genetic groups.

Alleles		Genetic groups					χ^2
		3/8	1/2	5/8	3/4	7/8	
Hb	A	90	158	275	190	73	12.11*
	B	40	56	71	44	13	
Am	B	114	125	171	141	46	29.83**
	C	16	35	83	81	22	
Alb	F	55	101	171	183	66	64.84**
	S	75	67	85	49	12	
Tf	A	41	64	85	100	36	21.45**
	D	50	90	120	94	36	
	E	39	60	51	46	10	
Ca	S	118	174	298	166	66	19.19**
	F	9	22	27	28	8	
	Z	3	12	7	16	2	
NP[1]	H	91	125	38	35	–	32.90**
	L	91	97	60	97	–	

[1] Values assuming Hardy-Weinberg equilibrium.

*P $<$0.05; **P $<$0.01.

frequencies of this allele in Zebu cattle (Braend, 1972). As expected, transferrin A and D did not show marked variation.

Purine nucleoside phosphorylase also showed a clear variation in gene frequency which confirmed a high NP-H value in crosses with high Zebu type blood and declining in 7/8 European-Zebu crosses (Ansay and Hanset, 1972; Gonzalez *et al.*, 1987; Panepucci, 1988b).

The chi-square values obtained for the different systems analyzed within each genetic group showed deviations from Hardy-Weinberg equilibrium in several cases (Table III). Since all animals are daughters of bulls which are in a progeny test, they are not the product of random mating, a fact that may explain the deviations from expectation. Dependency tests between number of alleles and different genetic groups showed, as expected, significant values for all systems analyzed (Table IV).

Biochemical characterization of crossbred cattle is important for future studies and for possible correlation with disease resistance, tick resistance and production traits, especially when working with a dairy herd. The knowledge of gene markers can be extremely useful for the study of germplasm resources in domestic cattle, and also to monitor the gene diffusion rate among crossbred populations. It could also be used to grossly estimate the "blood" proportion of unknown crossbred herds that need to be analyzed.

ACKNOWLEDGMENTS

The author is grateful to Dr. Mauricio Mello de Alencar for valuable discussion and suggestions. Publication supported by FAPESP.

RESUMO

O objetivo do presente trabalho foi determinar a estrutura genética de um rebanho mestiço leiteiro. Hemoglobina, amilase, albumina, transferrina, anidrase carbônica e purina nucleosídeo fosforilase foram estudadas em 505 fêmeas pertencentes a cinco grupos genéticos (3/8, 1/2, 5/8, 3/4 e 7/8 cruzamentos Europeu-Zebu).

Foi detectada variação significativa ($P < 0.01$), na frequência gênica, para todos os sistemas protéicos analisados. Essa variação foi dependente do "tipo de sangue" que contribui para cada grupo genético (Europeu-Zebu).

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(Received July 11, 1988)