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Research Article

Genetic characterization of Aberdeen Angus cattle using molecular markers

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Abstract

Aberdeen Angus beef cattle from the Brazilian herd were studied genetically using restriction fragment length polymorphism (RFLP) of the κ-casein - *Hin*fI (*CSN*3 - *Hin*fI), β-lactoglobulin - *Hae*III (*LGB* - *Hae*III) and growth hormone *Alu*I (*GH- Alu*I) genes, as well as four microsatellites (TEXAN15, CSFM50, BM1224 and BM7160). The RFLP genotypes were determined using the polymerase chain reaction (PCR) followed by digestion with restriction endonucleases and electrophoresis in agarose gels. With the exception of the microsatellite BM7160, which was analyzed in an automatic sequencer, the PCR products were genotyped by silver staining. The allele and genotype frequencies, heterozygosities and gene diversity were estimated. The values for these parameters of variability were comparable to other cattle breeds. The genetic relationship of the Aberdeen Angus to other breeds (Caracu, Canchim, Charolais, Guzerath, Gyr, Nelore, Santa Gertrudis and Simmental) was investigated using Nei's genetic distance. Cluster analysis placed the Aberdeen Angus in an isolated group in the *Bos taurus* breeds branch. This fact is in agreement with the geographic origin of this breed.

Key words: Aberdeen Angus, bovine, DNA, characterization, RFLP, microsatellite.

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Introduction

Various aspects of a population are useful in its characterization, including phenotypic traits (monogenic and polygenic), reproduction, geographic distribution, origin and habitat. The genetic characterization of populations, breeds and species allows the assessment of genetic variability, a crucial element in determining breeding strategies and genetic conservation programs.

The assessment of genetic variability is especially important in highly specialized livestock breeds since the use of assisted reproduction techniques, such as artificial insemination and embryo transfer, to maintain these breeds can rapidly reduce the genetic variability of the population. Molecular markers have been widely used to access this variability since they provide information on every region of the genome, regardless of the level of gene expression. RFLP and microsatellites (highly polymorphic simple sequence repeats) are currently the most widely used molecular markers, mainly because of the possibility of combining their analysis with the polymerase chain reaction (PCR). These markers have been used to explain bovine domestication and migration patterns (Loftus *et al*., 1994; Bradley *et al*., 1994) and to characterize cattle populations (MacHugh *et al*., 1994; Kemenes *et al*., 1999; Tambasco *et al*., 2000). Another application of molecular markers is in uncovering parentage mistakes. This is especially important for guaranteeing the accuracy of breeding programs in which the relationship between individuals is used to estimate breeding value. For this application, the frequencies of each marker in the population must be known.

Aberdeen Angus cattle have been bred in Scotland for more than 400 years, although the presence of polled cattle in the counties of Aberdeen and Angus dates from prehistoric times, as suggested by prehistoric carvings found in these regions (http://www.ansi.okstate.edu/breeds/cattle/). This polled black breed of cattle is considered to have a long life span, high fertility, precocity and easy calving (Hermsdorf, 1941). The latter attribute makes Aberdeen

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Angus sires a good choice for first parity females. The polled phenotype is another attractive attribute since it reduces losses from injury to cohorts and allows for denser occupation. Aberdeen Angus cattle were introduced to Brazil in 1906 by Leonardo Colares Sobrinho in Bagé, in the state of Rio Grande do Sul. In southern Brazil, the breed is used in purebred schemes, whereas in southeast and midwest Brazil it is used in crosses with Nelore cattle.

The aim of the present work was to evaluate the genetic variability of the Brazilian herd of Aberdeen Angus cattle and it's genetic relationship to other beef cattle breeds used in Brazil, in order to provide information for beef cattle breeding programs.

Material and Methods

Animals

Fifty-two unrelated Aberdeen Angus animals were sampled from the Bela Vista farm at Pardinho, São Paulo state (N = 25), from artificial insemination centers (N = 4), and from two farms in Paraná state (N = 23). These animals were chosen to represent all of the sire lines used in the Brazilian Aberdeen Angus herd.

Markers

Four microsatellites (TEXAN15, CSFM50, BM1224 and BM7160) and three RFLPs (*CSN3* - *Hinf*I, *LGB* - *Hae*III and *GH* - *Alu*I) were analyzed by PCR. The corresponding chromosomal location and primer sequences of these markers are given in Table 1.

DNA extraction

DNA was extracted from blood or semen samples using the salting out procedures described by Regitano (2001).

Genotyping

PCR reactions were done in a GeneAmp 2400 thermocycler with 200 ng of DNA in PCR solution (20 mM Tris-HCl, pH 8.4, 50 mM KCl) plus 1.5 mM $MgCl_2$, 0.2 μM of each dNTP, 0.4 μM of each primer and 0.5 U of *Taq* DNA polymerase in a final volume of 25 μL. For the microsatellite BM7160, the forward primer was labeled with fluorescein at the 5' end. The RFLP cycling conditions were those described in the references listed in Table 1. After digestion with the appropriate restriction enzyme, fragments were analyzed in 3% agarose gels in 1X TBE buffer, stained with ethidium bromide and photographed using an EDAS (Kodak ^{TM}) digital system. For microsatellite loci the cycles were the same as described by Bishop *et al*. (1994) and the PCR products were analyzed in 8% polyacrylamide gels in a manual sequencing apparatus, followed by silver staining (Comincini *et al*., 1995). Genotypes for the locus BM7160 were determined with an automatic laser fluorescence DNA sequencer (Pharmacia Biotech).

Statistical analysis

Gene and genotype frequencies were estimated according to Weir (1996). Hardy-Weinberg equilibrium was tested using an exact probability test (Guo and Thompson, 1992). Heterozygosity (H) and gene diversity (D) were estimated as genetic variability parameters (Weir, 1996). Polymorphic information content (PIC) was estimated according to Botstein *et al*., (1980). Nei's genetic distance

Table 1 - Description of molecular markers analyzed.

Marker	Nature	Chromosome	Sequence 5'- 3'[1]	Reference
CSN3[2]	RFLP *Hinf*I	6	ATCATTTATggCCATTCCACCAAAC gCCCATTTCgCCTTCTCTgTAACAGA	1
LGB[3]	RFLP *Hae*III	11	gTACTTgTggTggACACCgACTACA CAgCAgACCggCTCCTggTATATgA	2
GH[4]	RFLP *Alu*I	19	gCTgCTCCTgAgggCCCTTCg gCggCggCACTTCATgACCCT	3
BM1224	MS	4	AggAACCACATTgggTAgTCC TCCCTCTCTCCCTgAggC	4
TEXAN15	MS	5	TCgCAAACAgTCAgAgACCACTC TggATgAgAAAgAAgAgCAgAgTTg	5
CSFM50	MS	2	AgTTCTCCTCTTgATTTCAgTAAC CCTACTTCCTgCCTTTgTAgCATA	6
BM7160	MS	7	TggATTTTAAACACAgAATgTgg TCAgCTTCTCTTTAAATTTCTCTgg	7

[1]Sequence of forward primer is given in the upper line of each pair. The bottom line corresponds to the reverse primer. RFLP = restriction fragment length polymorphism, MS = microsatellite. [2]κ-casein, [3]β-lactoglobulin, [4]Growth hormone.
1. Medrano and Aguilar-Cordova (1990), **2.** Ron *et al*. (1994), **3.** Schlee *et al*. (1994[a]), **4.** Bishop *et al*. (1994), **5**. Burns *et al*. (1995), **6.** Moore *et al*. (1994), **7** Stone *et al*. (1995).

(Nei, 1978) was used to investigate the relationship between the Aberdeen Angus breed and the cattle breeds Caracu, Canchim, Charolais, Guzerath, Gyr, Nelore, Santa Gertrudis and Simmental. This data was used for cluster analysis by the unweighted pair-group method using an arithmetic mean (UPGMA).

Results and Discussion

The allele frequencies observed for the seven markers are listed in Table 2, as are the frequencies reported for other breeds in the literature. The only significant departure from Hardy-Weinberg equilibrium ($p < 0.05$) was for the *GH* - *Alu*I locus, which resulted from an excess of homozygotes. This disequilibrium may reflect a series of events such as inbreeding, selection, genetic drift or population subdivision. The latter event could also result from sampling few individuals from each location, although this would be expected to affect more than one locus. Several reports on the effects of *GH* - *Alu*I polymorphism suggest that this is not a neutral mutation for production traits (Schlee *et al.*, 1994b; Pereira *et al.*, 2002). However, the data obtained here was insufficient to assess this hypothesis in our Aberdeen Angus sample. The microsatellite loci were polymorphic and the number of alleles ranged from 3

Table 2 - Allele frequencies for the seven markers in ten bovine populations.

Marker	Allele	An 52	Si 52	Ne 63	Cn[1] 30	Gi[1] 83	Gu[1] 25	Ch1[1] 30	Ch2 36	SG[1] 20	Ca[1] 30
CSN3	A	0.82	0.65	0.91	0.63	0.93	0.92	0.48	0.51	0.85	0.68
GH	L	0.77	0.82	1.00	0.90	1.00	1.00	0.72	0.74	0.97	0.80
LGB	A	0.17	0.46	0.40	0.40	0.37	0.34	0.55	0.54	0.17	0.57
TEXAN15	203	-	0.01	0.57	0.13	0.53	0.28	-	-	0.20	0.02
	205	0.02	0.05	0.03	-	0.19	0.05	-	-	0.32	0.07
	207	0.05	0.16	0.20	0.33	0.07	0.60	0.07	0.08	0.10	0.18
	209	0.19	0.36	0.03	0.17	0.06	0.05	0.08	0.13	0.20	0.25
	217	0.23	0.01	0.09	0.33	-	0.02	0.54	0.53	0.18	0.37
	219	0.14	-	-	-	0.07	-	0.07	0.02	-	-
	221	0.13	-	-	-	0.02	-	-	0.08	-	-
	others	0.24	0.41	0.08	0.04	0.06	0.00	0.24	0.16	0.00	0.11
CSFM50	168	-	0.01	0.32	0.18	0.38	0.47	0.05	0.04	0.15	0.15
	170	-	0.02	0.16	0.07	0.22	0.28	-	-	-	-
	172	0.63	0.11	0.22	0.23	0.07	0.07	0.17	0.19	0.15	0.15
	176	0.29	0.66	0.28	0.40	0.20	0.15	0.53	0.51	0.58	0.57
	others	0.08	0.20	0.02	0.12	0.13	0.03	0.25	0.26	0.12	0.13
BM1224	167	-	-	-	-	0.08	0.25	-	-	0.37	-
	169	0.02	0.11	0.07	0.43	0.20	0.15	0.02	0.04	-	-
	171	0.06	0.11	-	0.03	0.01	0.08	0.03	0.03	0.05	0.43
	173	0.12	0.23	0.13	-	-	0.03	0.15	0.18	0.15	0.03
	175	0.15	0.07	-	-	0.07	0.15	0.03	0.03	0.13	-
	177	0.42	0.11	0.30	0.35	0.04	0.07	0.43	0.42	-	0.35
	179	0.12	0.07	-	-	0.16	0.08	-	-	0.05	-
	181	0.08	0.02	0.45	0.03	0.30	0.10	0.13	0.14	0.15	0.03
	183	-	0.14	0.04	0.15	0.01	0.07	0.15	0.11	0.10	0.15
	others	0.03	0.14	0.01	0.01	0.13	0.02	0.06	0.05	0.00	0.01
BM7160	173	0.06	0.25	-	-	-	-	-	-	-	-
	175	0.12	0.03	-	-	-	-	-	-	-	-
	177	0.31	0.54	-	-	-	-	-	-	-	-
	179	0.33	0.16	-	-	-	-	-	-	-	-
	others	0.18	0.02	-	-	-	-	-	-	-	-

An - Aberdeen Angus, Si - Simmental (Regitano *et al*., 2000), Ch - Charolais (Ch1 - Garcia, 2001 and Ch2 - Regitano *et al*., 2000), Ca - Caracu, Cn - Canchim, Ne - Nelore, Gi - Gyr, Gu - Guzerath, SG - Santa Gertrudis. [1]Marker data for RFLP from Kemenes *et al*. (1999) and for microsatellites from Garcia (2001).

(CSFM50) to 12 (TEXAN15). Allele sizes and frequencies are shown in Table 2.

The parameters H and D have been used to assess the genetic variability of a population. The values obtained for Aberdeen Angus cattle based on the seven markers are shown in Table 3. The Aberdeen Angus breed is a highly specialized beef breed in which intense use is made of artificial insemination and embryo transfer, and this could result in a decrease in genetic variability. However, the average heterozygosity (H = 0.491) and the gene diversity (D = 0.554) determined here do not indicate such a reduction. The PIC values for each marker were estimated in order to assess the relevance of each locus for linkage analysis (Table 3). With the exception of CSFM50, the microsatellite loci would provide an excellent contribution to a genome scan with more than 70% of the meiosis expected to be informative in this population.

The genetic distances between the Aberdeen Angus population and eight other breeds used in Brazil were determined using published frequency data - Simmental (Regitano *et al.,* 2000), Charolais (Regitano *et al*., 1999), Nelore, Gyr, Guzerath, Charolais, Santa Gertrudis, Canchim and Caracu RFLP data from Kemenes *et al*. (1999) and microsatellite data from Garcia (2001) - (Table 4). The dendrogram obtained for these distances using the UPGMA clustering method (Figure 1) clearly distinguished the two main groups of cattle (*Bos indicus* and *Bos taurus*) and placed the crossbred Canchim (5/8 Charolais+3/8 *Bos indicus*) in the *Bos taurus* group. In this analysis, Aberdeen Angus was placed in an isolated group in the *Bos taurus* branch. Since Aberdeen Angus is a Scottish breed, it was expected to be less related to continental European breeds, such as Charolais and Simmental, because of its geographic separation.

In conclusion, genetic marker analysis showed that the genetic variability of the Brazilian population of Aberdeen Angus was comparable to that of other cattle

Table 3 - Genetic diversity (D), heterozygosity (H) and polymorphic information content (PIC) estimates for Aberdeen Angus cattle.

Marker	Number of alleles	H	D	PIC
CSN3	2	0.288	0.302	0.254
LGB	2	0.346	0.358	0.292
GH	2	0.192	0.289	0.245
TEXAN15	12	0.840	0.866	0.842
CSFM50	3	0.596	0.524	0.447
BM1224	8	0.608	0.769	0.736
BM7160	8	0.569	0.770	0.728

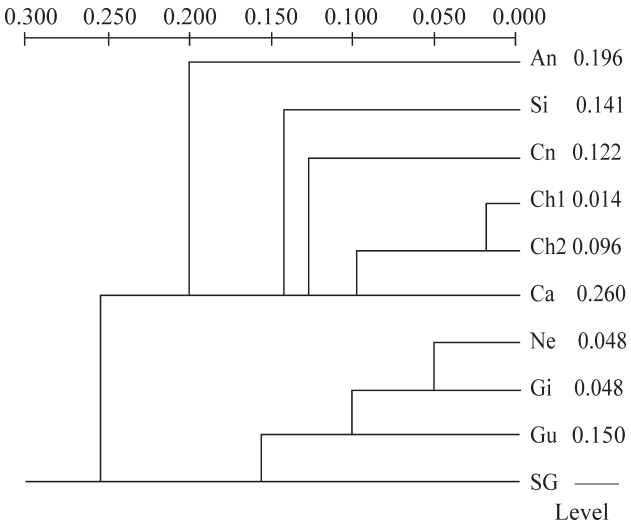

Figure 1 - Dendrogram obtained based on the UPGMA clustering of Nei's (1978) genetic distances. An -Aberdeen Angus, Si - Simmental, Ch1 - Charolais pop 1 (Garcia, 2001), Ch2 - Charolais pop 2 (Regitano *et al.* 2000b), Ca - Caracu, Cn - Canchim, Ne - Nelore, Gi - Gyr, Gu - Guzerath, SG - Santa Gertrudis.

Table 4 - Nei's (1978) genetic distances.

	An (52)	Si (52)	Ne (63)	Cn (30)	Gi (83)	Gu (25)	Ch1 (30)	Ch2 (36)	SG (29)	Ca (30)
An	0.00									
Si	0.20	0.00								
Ne	0.21	0.24	0.00							
Cn	0.17	0.14	0.16	0.00						
Gi	0.27	0.25	0.05	0.19	0.00					
Gu	0.29	0.26	0.11	0.17	0.09	0.00				
Ch1	0.22	0.17	0.29	0.13	0.39	0.42	0.00			
Ch2	0.20	0.16	0.27	0.12	0.36	0.39	0.01	0.00		
SG	0.18	0.15	0.16	0.19	0.13	0.16	0.28	0.26	0.00	
Ca	0.20	0.10	0.23	0.12	0.29	0.28	0.10	0.09	0.21	0.00

An - Aberdeen Angus, Si - Simmental, Ch - Charolais (Ch1 - Garcia, 2001 and Ch2 - Regitano *et al*., 2000), Ca - Caracu, Cn - Canchim, Ne - Nelore, Gi - Gyr, Gu - Guzerath, SG - Santa Gertrudis. Sample size in parentheses.

breeds in Brazil. Genetic distance analysis separated the Aberdeen Angus breed from other taurine breeds, a result consistent with their geographic origins.

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