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MOLECULAR EVIDENCES OF MUTATION-DRIFT EQUILIBRIUM BY MICROSATELLITE MARKERS IN FOUR MEAT PRODUCER BOVINE BREEDS IN BRAZIL

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ABSTRACT

This study aimed to assess possible deviations in mutation-drift equilibrium in bovine breeds. A total of 178 blood samples were collected from Curraleiro Pé-Duro, Tabapuã, Guzerat and Senepol breeds for DNA extraction and genotypic analysis by eleven microsatellite markers. The deviation loci number to mutation-drift equilibrium was 1 according to allele infinity model (IAM) and 2 by the stepwise mutation model (SMM) for Curraleiro Pé-Duro breed. For Tabapuã breed, there were 5 loci by IAM, and 3 loci by SMM. Although no locus was found by IAM for Guzerat breed, 2 loci were found by SMM. 3 loci by IAM and 2 by SMM were found in Senepol breed. Sign test showed deviation of the number of loci with Heterozygosity excess applying IAM only in Tabapuã breed. In Wilcoxon breed sign test showed deviation of the number of loci with heterozygosity excess in IAM only on Curraleiro Pé-Duro, Tabapuã and Senepol breeds. Overall, the results showed presence of mutation-drift equilibrium. No potential bottleneck effect was detected by mode-shift allele distributions, therefore all breeds were in normal L-shaped. All breeds have shown evidences of mutation-drift equilibrium due more to high loci number of heterozygosity excess than deficiency of heterozygosity.

Key words: Meat cattle; IAM; SMM; Livestock production; Heterozygosity excess.

INTRODUCTION

Brazil is the largest cattle producer, and is also the second largest producer of beef of the world behind the United States of America. It has an effective approximation number of 212 million animals, based on the official number of cattle registered in Brazil according to “Pesquisa da Pecuária Municipal” from the Brazilian Institute of Geography and Statistics (IBGE, 2011). Therefore, several studies are needed regarding the genetic diversity either within or between different bovine breeds existing in Brazil (FAO, 2007). Additionally results of such studies would contribute to the reconstruction of history of the herds, which can also be used as tool for maintaining them for future strategies in breeding programs (Groeneveld et al., 2010).

Several studies of molecular characterization of cattle breeds mainly comprised studies of genetic diversity (Silva Filho et al., 2012; Silva Filho et al., 2013a, Silva Filho et al., 2014) and genetic relationships among breeds (Egito et al., 2007). However, there were a few studies related to domestic animals that actually evaluated the mutation-drift equilibrium in the different populations as a way to justify some loss of genetic variability through the bottleneck effect.

In the present study, we chose four bovine breeds that have been considered as excellent beef husbandry adapted to distinct environmental conditions, which are found throughout the Brazilian territory. The Curraleiro Pé-Duro breed, which is most adapted to semi-arid conditions is representing the northeast of Brazil (Silva Filho et al., 2014); Tabapuã and Guzerat breeds, despite of their ubiquitous geographic distribution in the country, are highlighted as the major beef breed in the Amazon region (Northern Brazil) (Rosa et al., 1992.); and Senepol breed, found mostly on Brazil’s Southeast (Ribeiro et al., 2009).

The aim of this work was to detect possible evidences of mutation-drift equilibrium of those breeds mentioned above by using microsatellite markers, often applied in routine work of bovine paternity.

MATERIALS AND METHODS

178 animals from four breeds were randomly selected from different farmers (Curraleiro Pé-Duro n=60, Tabapuã n=60, Guzerat n=29 and Senepol n=29). A 5-mL blood sample was collected from the animal's jugular vein using Vacutainer tubes. These samples were used for DNA extraction by the phenol/chloroform/isoamyl alcohol method (25:24:1) in 1.5-mL tubes following the protocol described by Sambrook et al. (1989).

A set of eleven microsatellite loci recommended by the International Society of Animal Genetics (ISAG) were selected: BM1818, BM1824, BM2113, ETH10, ETH225, ETH3, INRA023, SPS115, TGLA122, TGLA126 and TGLA227. PCR was performed in multiplex, in a final volume of 15 µL, according to the following conditions: 1X STR 10X buffer (Promega, Brazil); 1.5 mM $MgCl_2$ (Promega, Brazil); 1.4 pM each forward primer labeled with one of the fluorochromes FAM, HEX or TAMRA and 1.4 pM each reverse primer not labeled; 0.5 U *T*aq DNA Polymerase Platinum® (Life Technology, Brazil) and 50-100 ng of genomic DNA.

Reactions were performed in a PCR Mastercycler (Eppendorf, Germany). PCR conditions were as follow: initial heating at 94°C, 4 min; 30 cycles of denaturation at 94°C for 1 min, annealing at 58°C for 1 min and extension 72°C for 1 min; followed by final extension at 72°C for 4 min. PCR products were separated in an ABI 3100 automated DNA sequencer (Applied Biosystems), and fragments were analyzed by Genotyper (Applied Biosystems).

Observed allele number (N_A), expected Heterozygosity (H_E) and Heterozygosity in equilibrium (H_{EQ}) analyzed under both Infinity Allele Model (IAM) and Stepwise Mutation Model (SMM) performed by BOTTLENECK v1.2.02 software (Piry *et al*. 1999). Statistical analyses SIGN TEST and WILCOXON TEST were performed to determine the loci number with excess Heterozygosity, which was based on the theory of a locus assuming mutation-drift equilibrium from 1,000 interactions and that allele status changes was under IAM and SMM. The proportions of different allele classes were determined by using quantitative graphic method by MODE SHIFT TEST (Luikart et al., 1998) that detected whether the distributions of allele frequencies follow L-shaped or not. All tests were evaluated by BOTTLENECK v1.2.02 software (Piry *et al*. 1999).

RESULTS AND DISCUSSION

In total, 272 alleles were observed and analyzed from the four breeds. 70 of them were found in Curraleiro Pé-Duro(CPD) breed, 74 in Tabapuã (TA) breed, 69 in Guzerat (GU) breed and 59 in Senepol (SE) breed. Among all microsatellite loci evaluated in this study, both models (IAM and SMM) demonstrated that a few loci showed deviation of mutation-drift equilibrium related to the expected Heterozygosity within the different breeds (Table 1).

SIGN TEST demonstrated presence of mutation-drift equilibrium (P>0.05) in all breeds analyzed in this study under both IAM and SMM models. However, an exception was found on Tabapuã breed, which presented significant deviation (P<0.05) of mutation-drift equilibrium according to IAM (Table 2). The results of WILCOXON TEST were quite different from SIGN TEST, which showed that Curraleiro Pé-Duro, Guzerat and Senepol breeds had significant deviations (P<0.05) of mutation-drift equilibrium under IAM, based on the probability of one tail for Heterozygosity excess, except for Guzerat breed. Meanwhile, there was no significant differences (P>0.05) among all breeds for SMM in mutation-drift equilibrium (Table 2).

Table 1. Comparisons of expected heterozygosities numbers and heterozygosities in equilibrium numbers under mutation models for microsatellite markers (IAM and SMM).

Loci	Curraleiro Pé-Duro				Tabapuã				Guzerat				Senepol			
	N_A	H_E	H_{EQ}		N_A	H_E	H_{EQ}		N_A	H_E	H_{EQ}		N_A	H_E	H_{EQ}	
			IAM	SMM			IAM	SMM			IAM	SMM			IAM	SMM
BM1818	7	0.768	0.611	0.762	7	0.814	0.609^*	0.767	8	0.749	0.710	0.812	5	0.753	0.547^*	0.685
BM1824	5	0.692	0.495	0.665	6	0.768	0.557^*	0.718	4	0.624	0.469	0.600	4	0.551	0.466	0.604
BM2113	6	0.653	0.569	0.723	6	0.766	0.559^*	0.721	7	0.807	0.667	0.782	7	0.823	0.673^*	0.783
ETH10	5	0.698	0.496	0.666	4	0.675	0.416^*	0.581	5	0.624	0.550	0.688	6	0.779	0.620	0.743
ETH225	6	0.697	0.552	0.723	7	0.625	0.616	0.766^*	7	0.616	0.669	0.779^*	5	0.547	0.557	0.682
ETH3	7	0.675	0.608	0.766	5	0.603	0.492	0.661	5	0.718	0.553	0.683	5	0.716	0.552	0.686
INRA23	7	0.519	0.608	0.765^*	9	0.789	0.688	0.822	8	0.549	0.714	0.813^*	3	0.251	0.358	0.466
SPS115	8	0.632	0.652	0.796^*	7	0.657	0.613	0.768^*	6	0.763	0.623	0.743	7	0.626	0.674	0.780^*
TGLA122	7	0.791	0.611^*	0.768	10	0.842	0.717^*	0.840	7	0.773	0.668	0.782	6	0.685	0.620	0.745
TGLA126	5	0.506	0.492	0.666	7	0.774	0.610	0.765	6	0.754	0.617	0.741	7	0.725	0.672	0.782
TGLA227	7	0.731	0.604	0.764	6	0.500	0.560	0.725^*	4	0.411	0.475	0.594	4	0.754	0.470^*	0.603^*

N_A= Allele number; H_E= Expected Heterozygosity; H_{EQ}= Heterozygosity in equilibrium; IAM= Infinity Allele Model; SMM= Stepwise Mutation Model. * Deviation from the mutational-drift equilibrium $P < 0.05$.

Figure 1 represents the results in graphic format of the allele distribution frequency in ten classes based MODE SHIFT TEST. All breeds demonstrated L-shaped distribution, where most allele frequencies are in 0.0-0.1 class (less than 10%). This represents no evidence of recent bottleneck in both breeds.

Table 2. Sign and Wilcoxon tests analysis of the mutation-drift equilibrium for expected and observed numbers of loci with Heterozygosity excess in beef bovine breeds from Brazil.

Breeds	Models	SIGN test			WILCOXON test
		Exp. Het. Excess	Obs. Het. Excess	Prob.	Prob. one tail for Het. Excess
Curraleiro Pé-Duro	IAM	6.48	9	0.104	0.004*
	SMM	6.54	4	0.106	0.989
Tabapuã	IAM	6.40	10	0.023*	0.001*
	SMM	6.51	6	0.491	0.711
Guzerat	IAM	6.45	8	0.264	0.074
	SMM	6.55	5	0.257	0.880
Senepol	IAM	6.30	8	0.236	0.008*
	SMM	6.51	5	0.265	0.861

IAM= Infinity Allele Model; SMM= Stepwise Mutation Model; Exp. H. Excess= Expect number of loci with Heterozygosity excess; Obs. Het. Excess= Observed number of loci with Heterozygosity excess; Prob.= Probability; * Deviation from the mutational-drift equilibrium $P < 0.05$.

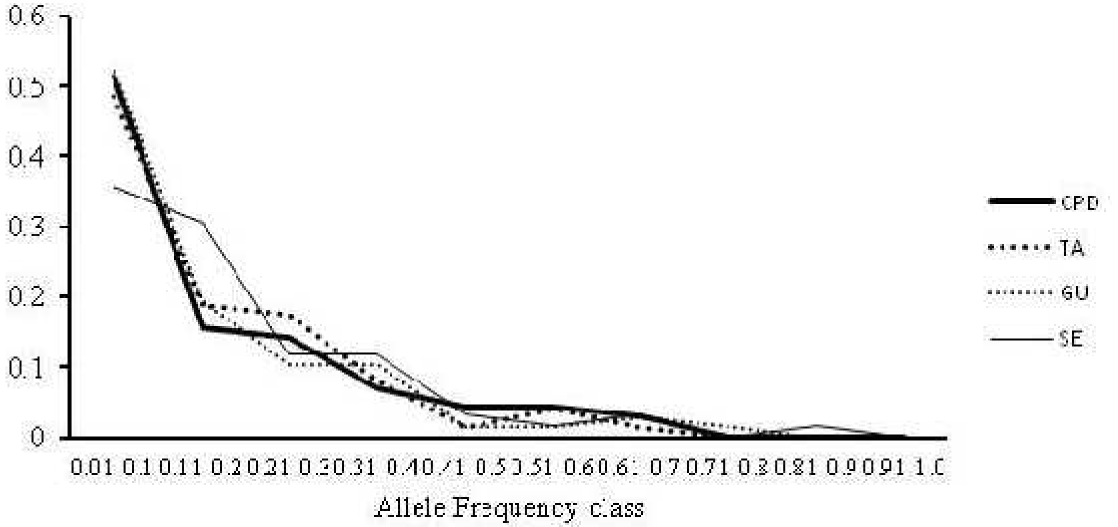

Figure 1. Mode shift in the allele frequency for Curraleiro Pé-Duro (CPD), Tabapuã (TA), Guzerat (GU) and Senepol (SE) breeds.

Both statistical tests used in this study have demonstrated rarely significant effect for deviation of mutation-drift equilibrium, being the majority to IAM, whereas all breeds were not significant by SMM. Manatrinon et al. (2008) evaluated 25 microsatellite loci in some Austrian and Hungarian cattle breeds using SIGN and WILCONXON tests. The results revealed that no potential bottleneck was occurring in all screened breeds, probably due to mutation-drift equilibrium. Sodhi et al. (2006) also analyzed 25 microsatellite loci in Tharparkar breed with mutation-drift equilibrium. Studies performed with Icelandic cattle using eleven microsatellite loci also demonstrated no evidence of deviation in mutation-drift equilibrium (Ásbjarnardóttir et al., 2010). Regarding to Bargus cattle breed from South India, the population size has decreased dramatically over the three past decades, which could be demontrated by significant differences in number of loci with H_E excess per number of loci with H_E deficiency (Ganapathi et al., 2012). Similarly, it also could be observed in one tail test for H_E excess under IAM and SMM (Ganapathi et al., 2012).

Recently, absences of bottleneck evidences were demonstrated in four breeds by plotting the proportion of rare alleles, which were distributed in class with less than 10%. These observations were similar with those found in Icelandic cattle (Ásbjarnardóttir et al., 2010), Austrian and Hungarian cattle breeds (Manatrinon et al., 2008) and

Tharparkar breed as well (Shodi et al., 2006). According to the mode shift test, the four cattle breeds evaluated in present work have not shown any loss of low frequency alleles, as it has occurred in Bargus cattle breed (Ganapathi et al., 2012).

Therefore, there was no evidence of mutation-drift equilibrium for the four bovine breeds in this study. An exception to this statements was found in some statistical tests when those were analyzed using IAM, which have already been reported as inappropriate in studies based on microsatellite markers (Clark, 1998). On the other hand, the SMM is more reliable to analyze the mutation-selection balance by using these molecular markers, as already have been reported elsewhere (Ohta and Kimura, 1973; Bürger, 1988).

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