Genetic differentiation of Guzerat (*Bos indicus*) metapopulation in Minas Gerais state, Brazil

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Introduction

Guzerat breed is originated from a semi-arid region in India called Kankrej. Its evolution in a harsh environment resulted in its adaptation to Brazilian extreme tropical conditions after being introduced in the country at the end of the XIX century. This breed was prevalent among Zebu cattle until 1939. However, the broad utilization of this breed for crossing resulted in an expressive reduction in the purebred population size (Peixoto et al., 2009). In 1995, FAO included Guzerat breed in the list of the domestic genetic resources to be conserved by management. Guzerat is regarded in Brazil a double proposal (milk and meat) breed. Beyond the productive potential, this breed stands out for its parasites resistance, ability to consume gross forage and thermal tolerance. Therefore, in 1994, it was started the National Program for the Improvement of Guzerat Dairy Cattle based in two important selection tools: progeny testing and MOET nucleus scheme. Up to now, 10 sire summaries were published and almost 400 sires were proven. Since the beginning, concern on the genetic variability has been taken into account at the moment of choosing candidate bulls and determining MOET matings. Farmers have been also planning matings to minimize inbreeding and to avoid losses in genetic variability (Peixoto et al., 2009). For the genetic diversity studies, microsatellites have been chosen among DNA-based genetic markers because their large genome distribution, highly polymorphic and easy genotyping. Microsatellites have been used to estimate the magnitude of genetic variation between and within populations (Dadi et al., 2008, Ibeagha-Awemu and Erhardt, 2009). The objective of this study was to assess genetic diversity of Guzerat cattle using molecular data in herds located in Minas Gerais state, Brazil, where there is the large population effective, in order to support breeding and management decisions and avoid genetic variability losses.

Material and methods

A total of 744 blood samples, representing 10 % of the animals from each one of the 15 major herds of Guzerat cattle in Minas Gerais state, Southeastern Brazil, were used in this study. Each herd was regarded as a subpopulation of this state metapopulation. Genomic DNA was extracted from leukocytes using a modified phenol/chloroform

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method. DNA was quantified and qualified trough nanospectrophotometry (NanoDrop ND-1000). A total of 11 microsatellite markers were selected based on their polymorphic skills or number of alleles in a smaller sample. Markers were selected from the bovine genetic linkage map available at MARC/USDA (Meat Animal Research Center/ United States Department of Agriculture). Microsatellite marker alleles were detected by capillary electrophoresis in the MegaBACE 1000 DNA sequencer (GE Healthcare, Buckinghamshire, United Kingdom). Fisher´s exact test was used to determine Hardy-Weinberg departures in each Locus. Unbiased exact P-values were estimated using Markov Chain Monte Carlo method with 1000 iterations. To estimate genetic differentiation and relationship among subpopulations F statistics (Fis, Fit, Fst) were obtained based on allele identity method (Weir and Cockerham, 1984) and allele size (Rho-statistics). The computational package GENEPOP 4.0 (Rousset, 2008) was used for analyses.

Results and discussion

Allele numbers over all loci ranged from 9 to 25 and frequencies varied herd by herd, sufficiently for evaluating genetic differentiation. Highly significant H-W departures were observed for the majority of loci ($P<0.05$) within subpopulations, mainly for microsatellites M25-1 and M26-2 ($P<0.0001$). Probably, they are linked to other loci influencing reproduction and/or production traits undergoing some degree of natural or artificial selection pressure. There was a deficiency of heterozygotes in seven of the eleven loci investigated ($P<0.05$). It was reflected by the multilocus F estimates shown in Table 1. Low values were estimated for overall Fis (WC) and Fit (WC), but differed significantly from zero ($P<0.0001$) pointing out the occurrence of inbreeding within subpopulations and in the total population, respectively. These results are supported by Dadi et al. (2008) and Ibeagha-Awemu and Erhardt (2009) with other Zebu purebred and crossbred populations. The four loci significantly ($P<0.05$ to $P<0.001$) influencing overall Fis were M11-3, M20-1, M25-1 and M26-2. Microsatellite M26-2 was the most inbred locus, and its Fis value was highly significant ($P<0.0001$) in all subpopulations. Rho-statistics found a little bit larger estimates. Fst values were low but significant and became evident the similarity among subpopulations. Results obtained for Fst indicates that subpopulations are sharing many multilocus genotypes. It can be related to the high level of gene flow between subpopulations due to the increasing of artificial insemination within herds occurred after the publication of the Guzerat sire summaries, allowing the introduction of new lineages within herds. Accordng to Ibeagha-Awemu and Erhardt (2009), significant Fst reflects the homogeneity of population with positive (increased within herd variation, spread of interesting genes, etc.) and negative (reduced between herd variation, reduced chances of improvement, etc) consequences. So, genetic parameters are strongly useful to decisions on conservation and improvement strategies.

Table 1: F-statistics in the Guzerat metapopulation based in microsatellite loci.

Locus	Fis (WC)	Fst WC)	Fit (WC)	Fis (Rho)	Fst (Rho)	Fit (Rho)
M6-1	-0.0406	0.0241	-0.0155	-0.0108	0.0502	0.0399
M28-2	-0.0342*	0.0222	-0.0112	0.0836	0.0612	0.1397
M21-1	0.0350	0.0293	0.0633	0.0449	0.0129	0.0572
M25-1	0.0546***	0.0233	0.0766	0.1170	0.0372	0.1499
M15-2	-0.0385	0.0322	-0.0051	-0.0321	0.0430	0.0122
M12-2	-0.0723*	0.0249	-0.0456	-0.0871	0.0260	-0.0588
M11-3	-0.0188*	0.0335	0.0154	-0.0355	0.0086	-0.0266
M26-2	0.3914***	0.0162	0.4013	0.4912	0.0146	0.4986
M22-2	-0.0238**	0.0299	0.0068	-0.0593	0.0472	-0.0093
M20-1	-0.0227	0.0410	0.0193	0.0991	0.0182	0.1156
M10-1	0.0149	0.0189	0.0335	-0.0655	0.0020	-0.0633
ALL:	0.0224	0.0269	0.0487	0.0376	0.0360	0.0722

Conclusion

Results of this study shows that this Guzerat metapopulation can be at risk of becoming genetically uniform. Appropriate breeding and management strategies such as the mating planning as well as a constant genetic diversity monitoring should be adopted to minimize risks. Further loci should be genotyped to increase power of significance test.

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