Genetic relationships among five zebu breeds naturalized in America accessed with molecular markers

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Abstract

This study aims to analyze the within-breed genetic diversity and determine the genetic relationships among the most important zebu populations introduced in the American continent from Asia. Gyr (GYR), Red Sindhi (SIN), Nelore (NEL), Guzerat (GUZ), and the synthetic Brahman (BRH) breeds have been established especially in American tropical regions. Estimates of genetic structure and diversity were carried out within and among the five populations studied. The results of within-breed genetic diversity showed medium to high medium values of diversity in all genetic parameters observed heterozygosity (\(H_e\)=0.622±0.022; expected heterozygosity (\(H_e\))=0.662±0.023; number of alleles (\(N_a\))=5.71±1.63; and allelic richness (\(A_r\))=4.097±0.958. The lowest value of \(H_e\) was observed in NEL (0.569±0.019) and the highest in BRH (0.688±0.015). Lowest value of \(H_e\) was also observed in NEL (0.612±0.024) and the highest in BRH (0.700±0.020). Gyr, GUZ and NEL showed high estimations of inbreeding, 9.98, 7.92 and 6.83% respectively. Values of \(N_a\) varied between 4.93±1.52 in GUZ and 7.04±1.99 in BRH and the \(A_r\) values ranged between 3.687±0.895 in NEL and 4.42±1.91 in SIN. On the other hand, although phenotypically, the five breeds are clearly distinguishable, the genetic analysis of structure and kinship demonstrates a total independence among GYR, GUZ and NEL, while BRH and Red Sindhi show a closed relationship. These facts support that GYR, GUZ and NEL have had a diverse origin but also different models of introgression in America. Brahman and SIN could be explained by the intervention of the Sindhi ancestors in the formation of the American synthetic Brahman.

Introduction

The domestication of cattle, both Bos taurus and Bos indicus, from its precursor, aurochs (Bos primigenius) was of great importance in the history of mankind, leading to extensive modifications, such as type of food, behaviour and socio-economic structure of many populations from approximately 11,000 years ago (Clutton-Brock, 1999). Genetic divergence between Bos taurus and Bos indicus populations suggest that there were two independent domestication processes in different geographical areas and from two distinct groups of aurochs (Bradley et al., 1998). In the particular case of Bos indicus, archaeological evidence suggests that domestication probably occurred in the Indus Valley (now Pakistan), with a major proportion of these breeds in India and later secondary introduction in Africa (Beja-Pereira et al., 2006). Most part of the cattle Bos indicus imported into the United States, Mexico, and Brazil was composed of breeds that originated in India and to a lesser extent, in the region of Pakistan.

Guzerat (GUZ), Nelore (NEL) and Gyr (GYR) breeds have had the greatest influence in the United States, Mexico and Brazil cattle with Asian origin (Sanders, 1980). Until mid-1920, most Zebu in the United States were of the NEL type. Thus, these contributed greatly to the formation of the American Brahman (BRH), though some pure bulls were also used in the large BRH herds, after 1925. It is important to emphasize that the cattle from which the Gray Brahman descends, came from Brazil during the 1920 decade. The Grey Brahman is, unlike the GYR, GYR, Red Sindhi (SIN) and NEL, a synthetic breed, mix GUZ, NEL, with some influence from other Zebu breeds and some Europeans breeds (Sanders, 1980).

The basic approach in the zebu populations is its use in cross-breeding, where their contribution is the adaptation to the tropical climate; however, in some Latin American countries they are used as direct meat producers. They have also developed dairy fitness lines such as GYR dairy from Brazil (Ardila, 2010).

The introduction of cattle in America has not followed a stable process for all breeds. Some of them, as shown by mitochondrial DNA (Ginja et al., 2010), were introduced through backcrossing on aurochs breeds of European origin, that functioned as a mother base. In other cases, the introduction was carried out with small groups that subsequently spread, suffering the consequent bottleneck.

The autosomal microsatellites are molecular markers generally recommended to estimate within and among populations genetic diversity, to investigate the relationships between among breeds and set conservation priorities (Lenstra et al., 2012). In addition to its own value in assessing the genetic diversity and the structure of domestic breeds, microsatellites are useful in the analysis of conservation, since this is expected to be linked with genes associated with important features, including the adaptation (Hall et al., 2012; Medugorac et al., 2011). The aim of this paper is to analyze the within-breed American Zebu genetic diversity accessed with microsatellite markers and identify the genetic relationships among populations of GVR, BRH, SIN, GUZ and NEL, established in the American continent.

Materials and methods

A database of 1525 pairs of alleles of 113 available randomized samples of five Zebu breeds, GYR (23), BRH (36), SIN (11), GUZ (15) and NEL (28), was analyzed. The database was obtained from the sample bank BIOBOVIS consortium, established within the
Medium to high values are observed in all estimates (on average, $H_e=0.622\pm0.022$; $H_o=0.662\pm0.023$; $N_a=5.71\pm1.63$; $A_e=4.097\pm0.958$), the lowest values of $H_o$ were observed in NEL (0.569±0.019) and the highest in BRH (0.688±0.015). In a similar way, the lowest values of $H_e$ were observed in NEL (0.612±0.024) and the highest in BRH (0.700±0.020), the latter being considered as high (Ott, 1992). Values of high $f$ were observed, in GYR, GUZ and NEL with 9.98, 7.92 and 6.83% respectively (P<0.05), when the maximum acceptable level of inbreeding within which there is no negative effect on the production capacity is 6.25% and the critical level is 12.5% (Parland et al., 2007).

Higher values of $f$ (16.42%) were found by Cervini et al. (2006) in NEL of various regions of Brazil. Egito et al. (2007) reported in Brazil f values of 10.61, 12.14 and 11.40% in NEL, GYR and GUZ, respectively. Chaudarhi et al. (2009) working with the Zebu breeds of India, Gaolao and Kenkatha reported 21.21% and 22.48% of $f$ respectively.

Various factors have been associated with the increased value of $f$ in populations, such as the levels of inbreeding, selection hitchhiking, presence of null alleles and the appearance of substructure or Wahlund effect (Nei, 1987; Sodhi et al., 2000) in India, with values of 5.2, 6.5 and 5.9 in the Sahiwal, Hariana and Deoni breeds, respectively. However, Chaudarhi et al. (2009) report $N_a$ of 9.52 and 7.92 in the Bos indicus Gaolao and Kenkatha cattle, respectively. Data of $A_e$ found in this group of breeds is similar to those reported in Bos taurus Latin American native breeds, with 4.67±0.51 (Delgado et al., 2012). The high values of $f$ found in most of the breeds studied in the present paper (except for SIN) could be attributed to the effects of the genetic improvement programmes to which they have been subjected for many years. If control programmes of mating were not established to prevent the increase of inbreeding, there could be a detrimental effect on the productivity of these populations, as observed in other breeds such as the Brown Swiss (da Silva et al., 2001). Studies of the breeds GYR by Aidar De Queiroz et al. (2000) and SIN by Garnero et al. (2008) both in Brazil, demonstrated that there is a decrease in live weight at different ages in inbred animals compared with non-inbred animals which supports our findings in these breeds.

The results of the correspondence analysis (Figure 1) indicated that the first three axes explain 82.27% of the variability. The first axis accounted for 32.59% of the variability, separating NEL from the other breeds; the second axis accounted for 27.16% of the variability, separating GUZ from the other breeds and the third component accounted for 22.52% of the total variability, separating GYR from other breeds. All populations were presented in separate groups but BRH and SIN breeds were found in closest cloud data. This can be explained by a divergent genesis of NEL, GUZ and GYR breeds in its American naturalization process, which might be more homogeneous and independent of maternal Creole freely Iberian origin for BRH and SIN, thus maintaining an apparent common origin.

AMOVA results indicated that the percentage of variation within groups was 93.17% and the differences between groups were 6.83%. This value agrees with the differentiation values reported among breeds by Cañón et al. (2001) and Villalobos et al. (2010) in Bos taur-
**rus** breeds and sufficient to consider an evident genetic separation among the breeds studied here, as stated in international conventions (FAO, 2007). The values of \( F_{ST} \) (Table 2, below the diagonal) correspond to the value of genetic differentiation by pairs of breeds, ranging from a low of 0.036, pair BRH/GYR to 0.106 pair GUZ/GYR. \( N_{m} \) values are also presented in Table 2 (above the diagonal); the lowest values of \( N_{m} \) were observed in the pair GUZ/NEL (3.03) and the highest in the pair BRH/SIN (10.80), which corroborates our hypothesis about the different models of naturalization followed by the studied breeds.

Reynolds genetic distances (Table 3) of the five breeds ranged from 0.046 in the pair SIN/BRH to 0.154 in the pair NEL/GUZ. The NEL population was the most distanced from all the zebu populations in this study. The dendrogram of distances presented (Figure 2) shows NEL and GYR breeds related in a cluster, GUZ in another separate cluster and SIN and BRH, sharing a different cluster. Within studies of bovine genetic diversity and applying the methodology described above, Martínez et al. (2012) and Villalobos et al. (2012) observed that the five Zebu populations at stake presented values of \( Q>0.84 \) and shared the same cluster in different calculated values of \( K \), when compared with other populations of *Bos taurus*, probably masking the intrarracial realtionships associated only to the Zebu populations.

The method based on Bayesian clustering models allowed us to evaluate with higher resolution the genetic structure of the five zebu (Figure 3).

The results for \( K=2 \) indicate a clear separation between NEL and other Zebu breeds confirming other observations in the AFC and the analysis of genetic distances of Reynolds. When \( K=3 \), NEL and GUZ separated from the rest of Zebu strains. Similar to what was observed on axis 1 of AFC, GUZ breed shows a separation compared to other breeds on axis 2, adding both (NEL and GUZ) 59.75% of the total inertia. \( K=4 \), value considered the optimal number of ancestral populations of American zebu as calculated by Evanno et al. (2005), shows that the remaining breeds were mostly related to BRH and SIN, confirming the observed data in the cloud in the AFC. The same pattern of behaviour was observed at Reynolds dendrogram of distances. Similarly, observing the values of \( N_{m} \) and \( F_{ST} \) revealed that these two populations will have the highest gene flow in relation to other breeds (10.80) and one of the lowest values of genetic differentiation; therefore it can be inferred that there would still be some representation of alleles of the two breeds that still share, since the Brahman breed was created in the nineteenth century (Sanders, 1980) or recent crossbreed made between the two populations. However, though phenotypically the five breeds have clearly different characteristics, the value of \( K=4 \) (Figure 3) represents the optimum value of ancestral populations.

When the value of \( K \) reached the value of 5, all populations within the study are fully separated. This phenomenon could be attributed to the synthetic origin of the Brahman breed originated from the Guzerat, Gyr, Nelore and Red Sindhi breeds themselves involved in the present study.

![Figure 1. Factorial analysis of correspondences of five contemporary zebu breeds. GYR, Gyr; BRH, Brahman; SIN, Red Sindhi; GUZ, Guzerat; NEL, Nelore.](image)

**Table 2. Values of average number of migrants (above the diagonal) and the F-statistic \( F_{ST} \) (below the diagonal) of five zebu contemporary populations.**

| Population | GYR | BRH | SIN | GUZ | NEL |
|------------|-----|-----|-----|-----|-----|
| GYR        | -   | 7.340 | 4.530 | 4.200 | 3.820 |
| BRH        | 0.036 | -   | 10.800 | 5.110 | 4.390 |
| SIN        | 0.074 | 0.042 | -   | 5.270 | 3.370 |
| GUZ        | 0.106 | 0.071 | 0.071 | -   | 3.030 |
| NEL        | 0.075 | 0.060 | 0.089 | 0.102 | -   |

**Table 3. Reynolds genetic distance matrix of five contemporary zebu breeds.**

|         | GYR | BRH | SIN | GUZ | NEL |
|---------|-----|-----|-----|-----|-----|
| GYR     | -   | 0.065 | 0.102 | 0.112 | 0.123 |
| BRH     | 0.046 | -   | 0.087 | 0.095 | 0.134 |
| SIN     | 0.095 | -   | -   | 0.154 | -   |
| GUZ     |       |     |     |     |     |
| NEL     |       |     |     |     |     |

**Table Notes: GYR, Gyr; BRH, Brahman; SIN, Red Sindhi; GUZ, Guzerat; NEL, Nelore.**
Conclusions

The zebu populations GYR, GUZ and NEL have shown a clear genetic differentiation, justified by a diverse origin and by a different model of introgression in America. Brahma and SIN have shown a strong genetic relation, probably due to the intervention of the Sindhi ancestors in the formation of the American synthetic Brahma. The other populations showed values above the acceptable maximum inbreeding, which put them in the risk of having a negative impact on productive capacity.

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