UNVEILING HIDDEN TREASURES IN ZAMBIAN INDIGENOUS CATTLE USING 32 MICROSATELLITES

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ABSTRACT

Selection has created a range of diverse breeds, important for genetic studies. To date studies have been conducted to evaluate genetic diversity in cattle, but this has not been undertaken in Zambia. Our study used genetic information from 32 microsatellites in three Zambian Bos indicus breeds (Tonga, Tonga and Barotse) to assess genetic diversity and population structure. Results demonstrate that the Angoni breed exhibited slightly excess average observed heterozygosity (1.0%), while the Barotse and Tonga breeds exhibited slight deficit observed heterozygosity of 0.09% and 3.6% respectively. Global heterozygosity deficit across populations (Fit=4.2%), differed significantly from zero ($p<0.001$), because of observed inbreeding within breeds (Fis=1.0%).These breeds were slightly genetic differentiation (Fst= 3.2%), significantly different from zero ($p<0.001$). High gene flow (Nm=11.3%) was evident between populations. Although, these breeds didn't exhibit a high and unique breed's purity, cattle exhibited higher level of genetic diversity within breeds than between breeds, despite the evidence of close gene flow between the three populations going by Bayesian cluster at K=2. This suggests evidence of existing divergent and multi-loci genetic admixtures between and within breeds. This uniqueness of population clustering offers valuable information on the available gene pool for utilisation, improvement and conservation.

INTRODUCTION

Unquestionably, miscellany has generated different diverse cattle breeds worldwide. However, programs for the conservation of local and well-adapted genetic material for future use are required, because in the last 30 years, there has been a loss in genetic diversity (Rege and Tawah 1999; Thornton *et al*. 2010). The evidence indicates that unsystematic breeding and poor management of animal genetic resources are the main causes of this genetic erosion (Rege 1999; Hanotte *et al*. 2002). Hence, one of the strategic priorities for the Global Plan of Action for Animal Genetic Resources (FAO 2007) is to ensure proper genetic characterisation of various animal species. Studies have been conducted on African cattle breeds (Ibeagha-Awemu and Erhardt 2005; Edea, *et al.* 2013). Therefore, the aims of this study were to assess the genetic diversity and population structure of Zambian indigenous *Bos indicus* cattle in order to design sustainable breeding programs.

MATERIALS AND METHODS

A total 147 blood samples were randomly collected representing three *Bos indicus* breeds Angoni (n=53), Tonga (n=37) and Barotse (n=57) from unrelated individuals in three locations in Zambia (Figure 1). The collection of blood samples followed FAO guidelines and stored on Whatman® FTA cards for DNA isolation by standard protocols of ZyGEM for livestockGEM™ Storage Cards (Blood). Most microsatellite loci recommended by the International Society for Animal Genetics for evaluating cattle genetic diversity were analysed (Hoffman *et al*. 2004) and DNA was extracted from the samples and genotyped using the 32 microsatellites.

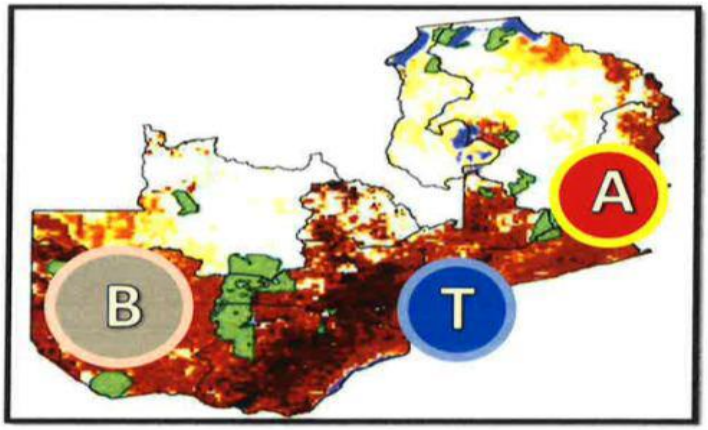

Figure 1. Sites in Zambia where blood samples for three *Bos indicus* breed were collected. A=Angoni (n=53), T= Tonga (37) and B= Barotse (57)

RESULTS AND DISCUSSION

Genetic diversity analysis. In genetic diversity analysis, a total of number of 200 common alleles and 74 private alleles were obtained (Peakall and Smouse, 2012) was used to calculate various statistics as described in Table 1. There was a global deficit of observed heterozygosity across populations (Fit) of 4.2%, which was significantly different from zero. The inbreeding (Fis) was 1.0% across the loci within the populations (Table 1). The overall multiple locus mean (Fst) value of 3.2% obtained across breeds indicates that 96.8% of genetic diversity is from genetic differentiation within each breed, which was moderately low but significantly different from zero. The overall mean (Fis) value of 1.0% obtained across all loci was highly significant ($P<0.01$), indicating that there was inbreeding within the overall population (Wright 1965). However, this Fis value of 1.0% may not reveal the true inbreeding that occurs in the population. Furthermore, the results showed the global deficit of heterozygotes across populations (Fit) was 4.2%, which is also significantly different ($P<0.01$) from zero suggesting that there was random mating across the population or demonstrating that the animals sampled were not closely related due to geographic distance between sampling sites. The Shannon tests of gene flow migration (Nm) between pairwise populations at each locus were highly significant across breeds and suggesting there was a distinct gene flow between the three Zambia indigenous cattle.

Table 1. Genetic diversity analysis for Bos indicus Zambian indigenous cattle using 32 microsatellites DNA information delivered from 274 alleles

Frequency distribution of number of alleles, heterozygosity (observed and expected) and fixation (F) for each breed							F-Statistics and Shannon migration estimates across all loci			
Breeds		Na	Ne	Ho	He	F	Fit	Fis	Fst	Nm
Angoni (n=30)	Mean Se	7.875 ±0.555	4.433 ±0.341	0.741 ±0.025	0.735 ±0.021	-0.01 ±0.02	0.042	0.01	0.032	0.113
Tonga (n=14)	Mean Se	7.063 ±0.406	4.224 ±0.27	0.705 ±0.028	0.727 ±0.021	0.036 ±0.021				
Barotse (n=28)	Mean Se	7.406 ±0.406	3.36 ±0.286	0.692 ±0.027	0.711 ±0.022	0.009 ±0.026				
Total	Mean Se	7.448 ±0.277	4.172 ±0.173	0.713 ±0.15	0.72 ±0.012	0.012 ±0.013				

Se= Standard Error, Na = No. of Different Alleles, Ne = No. of Effective Alleles, Ho = Observed Heterozygosity, F = Fixation Index = (He - Ho) / He = 1 - (Ho / He), Fis = (Mean He - Mean Ho) / Mean He (**Expected Heterozygosity), ,Fit = (Ht - Mean Ho) / Ht, Fst = (Ht - Mean He) / Ht, Nm = [(1 / Fst) - 1] / 4 and Ht = Total Expected Heterozygosity. Fis is the inbreeding coefficient within individuals, Fit measures the global heterozygosity deficiency, Fst is the inbreeding coefficient within subpopulation and Nm is gene migration.**

Population differentiation using Molecular Analysis of Variance, Shannon statistics of analysis of molecular variance (AMOVA) was used to partition genetic diversity among breeds, within populations and with breeds (Table 2). The results revealed 2.3% genetic variation among the three breeds and 41% within breeds. Furthermore, the Angoni breed had a higher proportion of genetic diversity of 50.3%, followed by Barotse with 42.9% and Tonga with 26.7%. This is likely to due to the Angoni's geographical location in the Eastern Province where is the majority of the population is exposed to the wider gene pool from other populations. There are no definite physical boundaries in the Eastern Province (Figure 1).

Table 2. **Shannon statistical analysis of molecular variance in Zambian indigenous cattle breeds using co-dominant microsatellite genotype data**

Source of Variation	DF[1]	Diversity (%)	Standard divergence[2]
Between Breeds	2	2.33	0.880
All Breeds	141	41.86	0.996
Within Angoni	61	50.27	0.996
Within Tonga	27	26.65	0.998
Within Barotse	53	42.86	0.995
Total	143	97.73	0.997

[1] DF= Degrees of freedom and [2] Standard divergence = divergence time (coefficients) at each node

Bayesian structure analysis. Structure analysis for the ancestry clustering of the population structure (Pritchard *et al*. 2000) and structure clustering and triangle plots (Figure 3) showed that the Tonga and Barotse grouped into one cluster and the Angoni in another cluster. This suggests that there are most likely to be 2 distinct genetic populations rather than 3 populations.

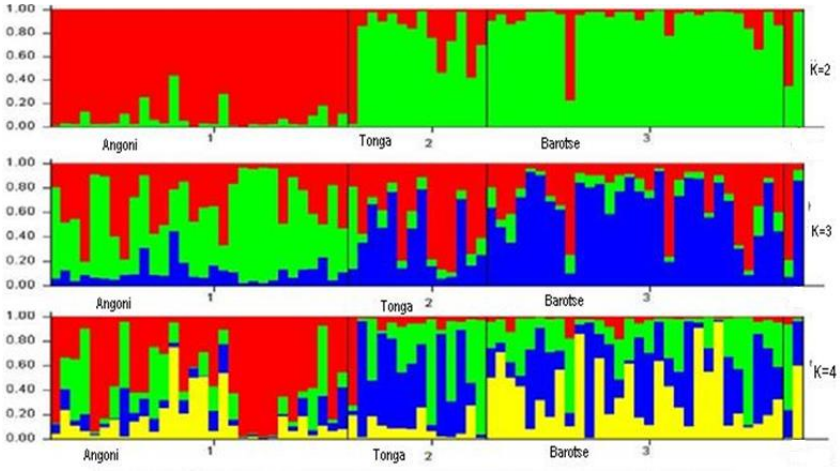

Figure 2. Summary of Bayesian plot estimates of population structure for the three Zambian Bos indicus indigenous breeds. A single vertical line in the cluster represents each individual animal. Genotype contribution into K coloured segments adds up to 1 for each of the inferred clusters assuming K = 2 , 3 and 4 separate populations

Conclusion. Although the Zambian indigenous cattle breeds did not exhibit high or unique purity, these breeds offer a valuable genetic resource relevant to breed conservation. There was evidence of some gene flow between the three populations and inbreeding was largely insignificant. The results suggest existing divergent and multi-loci genetic admixtures between and within breeds. The most striking feature is the close relationship between the Tonga and Barotse breeds. However, the population structure clustering indicates there is a distinct separation from the Angoni breed. The clustering patterns can be explained by the coexistence of breeds in some regions of Zambia.

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