Genetic Diversity of Three Indigenous Cattle Breeds Reared in Benin

Mahugnon Santoze Adido, Mathew Gitau Gicheha, Mahougnon Camus Adoligbe and Kodzo Atchou

Pan African University Institute for Basic Sciences, Technology and Innovation, P.O. Box 62000-00200 Nairobi, Kenya
Jomo Kenyatta University of Agriculture and Technology, P.O. Box 62000-00200 Nairobi, Kenya
Research Unity on Communicable Disease, Polytechnic School of Abomey-Calavi, University of Abomey-Calavi 01 BP 2009 Cotonou, Benin

Article history
Received: 15-12-2018
Revised: 25-02-2019
Accepted: 13-03-2019

Corresponding Author:
Mr. Mahugnon Santoze Adido
Department of Molecular Biology and Biotechnology,
Jomo Kenyatta University of Agriculture and Technology,
Kenya
Email: santosadido@gmail.com

Abstract: Livestock production is an important component of the Benin economy contributing an upward of 25% of the agricultural Gross Domestic Products (GDP). Indigenous cattle sector contributes more to the GDP compared to other livestock species. Despite the economic role played by the sector, there has been little or no efforts to genetically improve the indigenous cattle in the country. Recently, the government and other development partners have embarked on projects to improve the sector performance. The first step would be to morphologically and genetically characterize the cattle populations so as to match them with the available resources for optimal conservation and utilization. There exist no genetic diversity information for the different cattle types in Benin. The objective of this study was thus to determine the genetic diversity of the three most abundant indigenous cattle types. A total of 86 cattle from all three breeds were genotyped at the 14 loci. High levels of allelic and gene diversity were observed with an overall mean of 8.67 and 0.76 respectively. The mean inbreeding estimate within breeds was found to be negative at -0.124, -0.111 and -0.146 in Azawak, Borgou and Somba cattle breeds respectively. The global F statistics and AMOVA resulted in low genetic differentiation among the breeds with 1.14% of total variation being attributed to between-breed differences. Neighbor-joining tree revealed Azawak and Borgou clustered together while Somba breed being relatively distinct from the aforementioned. High levels of admixture were evident from the distribution of pairwise inter-individual allele sharing distances. Besides, the STRUCTURE analysis confirmed the tight genetic linkage between the breeds. High genetic diversity and poor genetic structure among the cattle breeds investigated could be due to historic zebu–taurine admixture and unstructured breeding practices. This results will aid in design of sustainable indigenous cattle genetic improvement programmes.

Keywords: Biodiversity, Conservation, Genetic Structure, Livestock, Microsatellites Markers

Introduction

The economy of the Republic of Benin is mainly based on the rural sector, which is home to more than 70% of the population (Tidjani et al., 2006). The livestock sector contributes 25% of the country’s agricultural GDP, acts as cushion against emergencies in rural households and has been used as a tool for eradicating poverty. Despite such significant contribution, the livestock sector in Benin is still characterized by the traditional production, breeding and marketing practices which have continually stagnated the sector (Amadou et al., 2012). Pastoral and agro-pastoral systems are the main production systems practiced by smallholder farmers with herds mainly dominated by indigenous livestock species (Bradley et al., 1994). There are no records to indicate that any attempt has been made to genetically improve the livestock species.
This is despite the existence of detectable differences in performance between and among individuals within a breed. Genetic variation is the basis of animal genetic improvement (Mwai et al., 2015) and characterization of domestic animals is the first step in design and implementation of sustainable genetic resources use and conservation programmes. Various studies have identified the potential in indigenous cattle breeds in increasing rural households’ income thus alleviating poverty while enhancing food and nutritional security (Amadou et al., 2012).

There has been a focus on selecting animals for high performance in controlled environments while ignoring the ability of the individuals to produce and reproduce in the uncontrolled environment in which such animals have lived for long periods of time. Such biases value the prevailing production market in terms of quantity and quality, but the consequent effects have resulted to the extinction of some breeds. Nyamushamba et al. (2017) noted that there is rapid decline in the purity of indigenous breeds due to uncontrolled crossbreeding and breed replacements with non-native breeds. This is despite the obvious consequences of climate change which has negatively impacted on the supply of good quality natural pasture while also encouraging the emergence of new diseases epidemiology. Animals selected in such environments have potential to develop response mechanisms to respond to the new threats compared to those selected to perform in different environments. Furthermore, there is negative relationship between high performance and the animal ability to respond to environmental challenges.

The extensive and random distribution of exotic cattle breed by governmental and non-governmental organization is also believed to dilute the indigenous genetic stock (Mogesse, 2007). If this trend continues, the gene pool of indigenous cattle could be lost in the near future (Rischkowsky and Pilling, 2007). This threat is in line with the FAO report of the year 1999 which states that animal genetic resources in developing countries in general, are being eroded through the rapid transformation of the agricultural system. The main cause of the loss of indigenous Animal Genetic Resources (AnGR) being identified as the indiscriminate introduction of exotic genetic resources, before proper characterization, utilization and conservation of indigenous genetic resources. This study provides information on genetic variation of cattle population in Benin. This information will be used by scientists and researchers in implementing breeding programmes that result to sustainable utilisation and conservation of the important indigenous cattle genetic resources.

Materials and Methods

Animal Sampling

Blood sampling was carried out from February to March 2018 in 3 different regions of Benin namely: Natitingou (10°17’ 60.00” N and 1°21’ 59.99” E), Tchaourou (9°20’ 48.06” N and 2°36’ 32.42” E) and Cové (7°13’ 97” N and 2°20’ 92” E) as presented in Fig. 1. These regions are located in 3 different agro-ecological zones.

Fig. 1: Localization of sampling sites in three agro-ecological areas of Benin
Samples were collected in 9 localities through the study areas with the choice of localities in each region being done according to the availability of targeted breed. In each geographical area, different sites were considered in order to have a representative dataset. A major consideration was collection of sample with the least possible relation between animals. A total of 86 adult animals representing the 3 cattle breeds under investigation (Azawak, Borgou, Somba) were sampled.

**Blood Samples Collection**

About 8 to 10 mL of blood were collected from the jugular vein puncture in vacuum tubes containing EDTA as an anticoagulant and stored at -20°C till transportation to Kenya for further analysis. Recommended measures were taken during the blood collection to minimize pain and discomfort to the animals as much as possible.

**Microsatellites Markers**

Genomic DNA was extracted using DNeasy Blood and Tissue Kit developed by QIAGEN® as per the manufacturer’s instructions. DNA typing was performed by Polymerase Chain Reaction (PCR) using 14 FAO and ISAG recommended microsatellites markers. Each of the markers with forward primer was conjugated to one of the four fluorescent dyes FAM (Blue), NED (Yellow), PET (Red) and VIC (Green). The markers were selected based on their technical characteristics (good aptitude to amplification and easy interpretation of typing) and their genetic characteristics (number of alleles, localization and repartition through the genome). Table 1 summarizes the characteristics of the microsatellite markers used.

**PCR Amplification and Genotyping**

Microsatellites were amplified by PCR in simplex with reactions for 14 markers being carried out in a 10 µL reaction volume containing 1.5 µL of DNA template and 8.5 µL of total PCR mix. The mix composed of 2 µL of 5 X Green Buffer, 0.2 µL of dNTPs (2.5 mM), 1 µL of MgCl2 (25 mM), 0.25 µL of FWD primer (10 µM), 0.25 µL of REV primer (10 µM), 0.05 µL of Qiagen Taq DNA polymerase (5 U/µL) and 5.25 µL of H2O. The amplifications were carried out in a thermal cycler (ABI 9700) using the following conditions: initial denaturation at 94°C for 3 min, followed by 35 cycles of 30s at 94°C, 30 sec at annealing temperature of 50, 55 or 60°C (depending on the microsatellite) and 30 sec extension at 72°C, then final extension at 72°C for 10 min ended the reactions. Amplified fluorescent PCR products were multiplexed and electrophoresed in an automated DNA analyser ABI3100 with LIZ500 as an internal lane control. Geneious 11.1.5 (https://www.geneious.com, Kearse et al., 2012) were then used to extract the allele size data for each sample.

**Table 1: Characteristics of the microsatellite markers**

| Locus | Chr. number | Primer sequences (5’ – 3’) | Detected size range (bp) |
|-------|-------------|---------------------------|-------------------------|
| BM1818 | 23          | F: AGCTTGGGAATATAACCAAAGG  | 248-278                 |
|        |             | R: AGTGCCTTCA AGGTCCATGC   |                         |
| BM2113 | 2           | F: GCTGCTTCTACCAAATACCC    | 122-156                 |
|        |             | R: TTAGACAACAGGGGTTGG      |                         |
| INRA023 | 3            | F: GAGTAGAGCTACAAGATAAACTTC | 195-225                 |
|        |             | R: TAACTACAGGGGTTAGTGAACCTCA |                       |
| INRA035 | 16          | F: ATCCCAGGCCTCCACATTTG    | 100-124                 |
|        |             | R: TTGTGCTTATGACACTAATCGC  |                         |
| HEL9   | 8           | F: CCCATTCATGCTTCAGAGGT    | 141-173                 |
|        |             | R: CACATCCATGTTCTCACACC    |                         |
| ETH3   | 19          | F: GAACCTGCCCTCTCGATCTTGG  | 103-133                 |
|        |             | R: AATCTGCTTCTGGCCAAGTGG   |                         |
| SPS115 | 15          | F: AAAATGACACCACAGCTCTCCAG | 234-358                 |
|        |             | R: AAGCAGTGCTCTCTGTGGTGG   |                         |
| ILSTS005 | 10         | F: GAAAGCAATGAAAATCTAAGGCC | 176-194                 |
|        |             | R: TGTCCTGACGTTCTGAAGCG    |                         |
| ILSTS059 | 13         | F: AGTATGTAAGGCAAGGGG      | 105-135                 |
|        |             | R: CGACTTGTTGTTCTCAAAGC    |                         |
| INRA063 | 18          | F: ATTTGTCACAAGCTTAAATCTAACC |         |
|        |             | R: AACCAACAGAATGCTTGGAGAAGG | 167-189                |
| TGLA126 | 20          | F: CTAAATATGAGTGGAGGGCTCTTC | 115-131                |
|        |             | R: TTGTTGCTCTATTTGCTGAATATCCC |                  |
| TGLA227 | 18          | F: GGATTCTAAAATCTTGTTAAC   | 75-105                  |
|        |             | R: ACAGACAGAAATCAATGGAAGAC |                         |
| TGLA053 | 16          | F: GTTCCTCAGAAATGTTGCAATTC | 143-191                 |
|        |             | R: ATCTCGCATGTAATTACACAGA  |                         |
| ILSTS028 | 15          | F: TCCAGATTTTTGTACCAAGACC  | 105-135                 |
|        |             | R: GTGATGCTCATACCTTTTGGAGC |                         |
Statistical Analysis of Data

The frequency of null alleles was the first to be checked from the dataset using MicroChecker 2.2.3 (Van Oosterhout et al., 2004). This was followed by an adjustment of the allele and genotype frequencies of the amplified alleles so as to permit their use in further population genetic analysis. The observed heterozygosity (Ho), expected heterozygosity (He), observed and effective number of alleles representing basic genetic diversity measures were calculated using Weir and Cockerman's method using GENEPOP software (Rousset, 2008). Deviations from Hardy-Weinberg equilibrium and heterozygosity deficiency were estimated using the GENEPOP software package (Rousset, 2008). A hierarchical analysis of the variance was carried out using the analysis of molecular variance (AMOVA) implemented in the GenAlEx 6.5 (Peakall and Smouse, 2012) package following the definition of the breeds groups based on prior information and origin. Pairwise genetic distances (DS) (Nei, 1972) between subpopulations was estimated using GenAlEx 6.5 (Peakall and Smouse, 2012) software. The Unweighted Pair Group Method with Arithmetic mean (UPGMA) tree and Neighbour-Joining tree based on inter-individual allele sharing distances among population were constructed using DARWin 6.0.17 software (Perrier and Jacquemoud, 2006) to investigate the relationships between the three populations.

The genetic population structure analysis of the three cattle population was assessed using Bayesian admixture procedure was implemented in STRUCTURE 2.3.4 (Pritchard et al., 2000) to infer the most likely number of clusters. The software was programmed to run using the admixture model and correlated allele frequency. The number of assumed populations (K) was estimated for K ranging from 2 to 12. Five repetition were routed per K with a burn-in period of 100000 followed with 500000 iterations to obtain the corresponding Ln Pr (X|K). The values for the number of clusters (K) were assessed following Evanno et al. (2005), by comparing the estimated posterior probability of data for different K values and the standard deviation between runs for the same K. The data were entered into CLUMPAK (Kopelman et al., 2015) program to provide a graphic display.

Results

Genetic Diversity within Cattle Population under Investigation

Table 2 presents the allelic diversity of the 3 cattle populations considered in this study. A total number of 136 alleles were observed across the 14 loci in all 3 populations with a mean number of 8.66 alleles per loci. In overall, within breed, the mean observed number of alleles per locus was 7.64, 9.5 and 8.85, respectively, in Azawak, Borgou and Somba. The MicroChecker analysis of the cattle population at all loci revealed no significant presence of null alleles.

Table 2: Allelic diversity in Azawak, Borgou and Somba cattle population

| Population Markers | Azawak | Borgou | Somba |
|--------------------|--------|--------|-------|
| INRA35             | 4.000  | 3.069  | 5.000 | 3.004 | 6.000 | 3.163 |
| BM1818             | 10.000 | 7.511  | 12.000| 7.258 | 12.000| 8.134 |
| HEL9               | 9.000  | 5.323  | 10.000| 6.426 | 9.000 | 5.714 |
| ETH3               | 10.000 | 7.680  | 15.000| 9.533 | 12.000| 7.806 |
| BM2113             | 7.000  | 5.551  | 12.000| 7.101 | 10.000| 7.278 |
| INRA23             | 8.000  | 5.682  | 15.000| 7.164 | 11.000| 7.333 |
| TGLA126            | 5.000  | 3.516  | 8.000 | 4.787 | 7.000 | 4.722 |
| INRA063            | 8.000  | 3.571  | 7.000 | 4.520 | 7.000 | 3.153 |
| TGLA227            | 9.000  | 5.551  | 12.000| 5.598 | 12.000| 5.628 |
| ILSTS005           | 6.000  | 3.823  | 6.000 | 3.551 | 5.000 | 3.722 |
| SPS115             | 12.000 | 4.426  | 11.000| 3.712 | 10.000| 3.585 |
| ILSTS059           | 6.000  | 1.731  | 8.000 | 1.644 | 9.000 | 2.688 |
| ILSTS028           | 7.000  | 4.302  | 6.000 | 4.017 | 9.000 | 4.323 |
| TGLA053            | 6.000  | 3.213  | 6.000 | 2.669 | 5.000 | 2.839 |
| Mean               | 7.643  | 4.639  | 9.500 | 5.070 | 8.857 | 5.006 |
| SD                 | 0.589  | 0.450  | 0.906 | 0.537 | 0.670 | 0.525 |

Na: Observed number of Alleles
Ne: Effective number of Alleles
Table 3: Observed heterozygosity (Ho), expected heterozygosity (He) and estimated heterozygosity deficit (FIS) at different loci in the three cattle populations

| Population | Loci     | Azawak Ho | Azawak He | Azawak FIS | Borgou Ho | Borgou He | Borgou FIS | Somba Ho | Somba He | Somba FIS |
|------------|----------|-----------|-----------|------------|-----------|-----------|------------|----------|----------|-----------|
|            | INRA35   | 0.852     | 0.674     | -0.245     | 0.848     | 0.667     | -0.257     | 0.970    | 0.684    | -0.443    |
|            | BM1818   | 0.769     | 0.867     | 0.131      | 0.767     | 0.862     | 0.127*     | 0.955    | 0.877    | -0.065    |
|            | HEL9     | 0.846     | 0.812     | -0.022     | 0.893     | 0.844     | -0.039     | 0.950    | 0.825    | -0.126    |
|            | ETH3     | 0.890     | 0.870     | -0.132     | 0.980     | 0.895     | -0.102     | 0.960    | 0.872    | -0.124    |
|            | BM2113   | 0.860     | 0.820     | -0.203     | 0.971     | 0.859     | -0.116     | 0.890    | 0.863    | -0.136    |
|            | INRA23   | 0.960     | 0.824     | -0.196     | 0.971     | 0.860     | -0.114     | 0.870    | 0.864    | -0.135    |
|            | TGLA126  | 0.964     | 0.716     | -0.331     | 0.941     | 0.791     | -0.175     | 0.980    | 0.788    | -0.247    |
|            | INRA063  | 0.950     | 0.720     | -0.373     | 0.920     | 0.779     | -0.270     | 0.956    | 0.683    | -0.378    |
|            | TGLA227  | 0.870     | 0.820     | -0.203     | 0.941     | 0.821     | -0.131     | 0.960    | 0.822    | -0.193    |
|            | ILSTS005 | 0.966     | 0.738     | -0.291     | 0.943     | 0.718     | -0.299     | 0.906    | 0.731    | -0.346    |
|            | ILSTS015 | 0.931     | 0.774     | -0.185     | 0.886     | 0.731     | -0.198     | 0.909    | 0.721    | -0.238    |
|            | ILSTS059 | 0.500     | 0.422     | -0.166     | 0.394     | 0.392     | 0.009      | 0.471    | 0.628    | 0.278**   |
|            | ILSTS028 | 0.667     | 0.768     | 0.155*     | 0.704     | 0.751     | 0.081      | 0.579    | 0.769    | 0.272*    |
|            | TGLA053  | 0.480     | 0.689     | 0.321**    | 0.686     | 0.625     | -0.082     | 0.773    | 0.648    | -0.170    |
| Mean       |          | 0.821     | 0.751     | -0.124     | 0.846     | 0.757     | -0.111     | 0.866    | 0.770    | -0.146    |
| SD         |          | 0.049     | 0.030     | 0.20       | 0.045     | 0.035     | 0.126      | 0.046    | 0.023    | 0.21      |

*p<0.05, ** p<0.01

Table 4: F-statistics (FIS, FIT and FST) and gene flow (Nm) for overall populations

| Locus     | FIS    | FIT    | FST    | Nm    |
|-----------|--------|--------|--------|-------|
| INRA35    | -0.3041| -0.3098| -0.0044| 39.980|
| BM1818    | 0.0742 | 0.0690 | -0.0056| 23.524|
| HEL9      | -0.0568| -0.0210| 0.0339 | 6.177 |
| ETH3      | -0.1181| -0.0859| 0.0288 | 7.642 |
| BM2113    | -0.1499| -0.1191| 0.0268 | 8.267 |
| INRA23    | -0.1469| -0.1131| 0.0295 | 7.877 |
| TGLA126   | -0.2432| -0.2209| 0.0180 | 7.991 |
| INRA063   | -0.3301| -0.3215| 0.0065 | 19.527|
| TGLA227   | -0.1718| -0.1734| -0.0013| 27.193|
| ILSTS005  | -0.3083| -0.3179| -0.0074| 75.922|
| SPS115    | -0.2040| -0.1960| 0.0067 | 16.361|
| ILSTS059  | 0.0315 | 0.0404 | 0.0092 | 11.184|
| ILSTS028  | 0.1597 | 0.1566 | -0.0036| 16.652|
| TGLA053   | 0.0253 | 0.0333 | 0.0082 | 12.313|
| Mean      | -0.127 | -0.112 | 0.0114 | 20.270|

The mean observed heterozygosity was 0.821, 0.846 and 0.866 respectively in Azawak, Borgou and Somba cattle breed while the mean expected heterozygosity was estimated to be 0.751, 0.757 and 0.770 respectively (Table 3). Comparatively, Borgou cattle had higher allelic diversity when the three cattle populations were considered. Similarly, Somba cattle population had higher heterozygosity estimates than Azawak and Borgou breeds.

Test for Hardy-Weinberg Equilibrium

Results for the F-statistics and gene flow for the three cattle populations are presented in Table 4. The overall mean inbreeding estimate (FIS) was -0.127. The respective mean estimates of inbreeding within breeds were -0.124, -0.111 and -0.146 for the Azawak, Borgou and Somba cattle populations. The overall loci estimate of the FIS was moderate and negative averaging -0.127 which is an indicator of low level of inbreeding.

The test for Hardy-Weinberg Equilibrium revealed that some of the loci had significant deviation (p<0.05) indicating heterozygosity deficiency at 2, 1 and 2 loci in Azawak, Borgou and Somba cattle respectively (see Table 3). The test of linkage disequilibrium indicated that there was no significant association (p>0.05) indicative of linkage disequilibrium between any pair of microsatellite loci for any population.

The coefficient of genetic differentiation estimated through the estimator described by Weir and Cockerham (1984) had FST values ranging from -0.0074 in ILSTS005 to 0.0339 in HEL9 with an average value of 0.0114. This implied that 1.14% of the total genetic variation exists among the three cattle populations whereas 98.86% depicted differences among individuals within the populations. This is an indication that individuals from the three populations are genetically more closely related than within population. These findings were further confirmed...
by the results obtained from the analysis of Molecular Variance (AMOVA) as presented in Table 5.

**Genetic Variation and Relationship between Breeds**

The Nei’s unbiased genetic distance (Nei 1978) estimates between pairs of the three populations of cattle breeds are presented in Table 6. The respective genetic distance between Azawak and Borgou, Azawak and Somba and Borgou and Somba populations were 0.013, 0.075 and 0.017. The findings indicate that the Azawak and Borgou population are closely related when compared to the Somba population.

| Source of Variation | Sum of squares | Variance component | Percentage of variation | P-Value |
|---------------------|----------------|--------------------|------------------------|---------|
| Between Population  | 16.686         | 0.061              | 1.14                   | 0.001   |
| Within Population   | 518.00         | 6.023              | 98.86                  | 0.001   |
| Total               | 534.686        | 6.084              | 100                    |         |

Phylogenetic relationship between the breeds was established through construction of a Neighbor-Joining tree shown in Figure 2a using the unweighted pair group method which uses arithmetic averages (UPGMA). The Azawak and Borgou breeds tended to cluster together, while Somba breed appeared to be relatively distinct from them. However, the Neighbor-Joining tree derived from pairwise inter-individual allele sharing distances revealed admixture of individuals from all the three breeds as shown in Figure 2b. This was expected considering the level of zebu–taurine crossbreeding that has been occurring in the region among cattle keepers.

**Fig. 2:** Neighbour-joining tree based on pairwise (a) population and (b) inter-individual allele sharing distances among Azawak (red), Borgou (blue) and Somba (green) breeds
Fig. 3: Summary plot of estimated membership coefficient for each individual, in each cluster for K=3 and 4 obtained with a 100,000 burn-in under the admixture model for the breed analysis. Each individual is represented by a single vertical line broken into K coloured segments, with lengths proportional to each of the inferred clusters.

Table 6: Genetic distance between the 3 population of cattle breeds

| Population | Azawak | Borgou | Somba |
|------------|--------|--------|-------|
| Azawak     | ***    | 0.013  | ***   |
| Borgou     | 0.013  | ***    | 0.017 |
| Somba      | 0.075  | 0.017  | ***   |

Table 7: Proportion of membership of each of the three cattle breeds, Azawak, Borgou and Somba in each of the three inferred clusters

| Inferred clusters | 1  | 2  | 3  |
|-------------------|----|----|----|
| Azawak            | 0.528 | 0.314 | 0.157 |
| Borgou            | 0.201 | 0.427 | 0.372 |
| Somba             | 0.108 | 0.346 | 0.547 |

Bayesian Identification of Genetic Clusters

In order to estimate the number of genetic clusters among all of the examined breeds, analysis for population structure was done and consistent results were obtained and are presented in Table 7. The corresponding graphics are displayed in Figure 3. Between 2 to 12 clusters (K values) were tested using the admixture model, assuming that each individual did not necessarily have a genetic background originating from one of the K populations. Results indicated that 3 was the optimal K following Evanno’s test. This corresponds to the number of breeds used in the current study analysis. Every cluster was associated with a breed: Somba breed to cluster 3, highlighting the highest proportion of membership (54.7%), Azawak breed was associated to cluster 1, while most of Borgou animals were in cluster 2 with the lowest proportion of membership at 42%. Approximately 37% of Borgou individuals were found in the same cluster as Somba evidencing the crossbreeding between zebu and taurine that has been occurring between and among cattle populations.

When K was set to 3 (optimal K value based on Evanno’s test), none of the breed studied were well differentiated (distinguished) evidencing a strong similarity between the breeds and suggested a certain degree of genetic admixture. As envisaged from Evanno’s test, increasing the K value above 3 did not add more information. This result confirms the close genetic linkage between the breeds.

Discussion

Genetic Diversity within Cattle Population

A total of 136 alleles were observed across the 14 loci for all three populations studied. The number averaged 8.66 alleles per loci and allele frequency proportion ranging from 0.014 to 0.773 which is an indication of high level of allelic diversity. The values obtained were comparable to those obtained in Senegal (Ndiaye et al., 2015; 7.5) and in Niger (Grema et al., 2017; 7.86) but higher than those obtained in cattle breeds from Mozambique (Besa et al., 2009; 5.9). A higher mean number of alleles were previously reported for different African cattle breeds genetic diversity studies (Ema et al., 2014; Cameroon cattle at 10.7, Okomo-Adhiambo, 2003; Kenya cattle at 11.6, Ndumu et al., 2008; African Great Lakes Region Ankole longhorn cattle at 13.8, Kugonza et al., 2011; Ankole cattle of Uganda at 10.5).

Results from this study indicate that the indigenous cattle breed of Benin have a high level of genetic diversity which confirms the observation by Freeman et al. (2004) that the breeds located near the perimeter of tsetse zone tend to display highest values of allelic diversity. Furthermore, the high level of allelic diversity found in Borgou (“hybrid” zebu x taurine) population is similar to that found in Djakore breed (Ndiaye et al., 2015) indicating that hybrid population tend to have a high value of allelic diversity. This suggests that a large allelic richness may
Genetic Differentiation among the Cattle Population

Genetic differentiation estimated through FST and the Analysis of Molecular Variance (AMOVA) revealed little differentiation between the three cattle populations with a variation of 1.14%. Similar values were obtained from Niger cattle breeds (0.026) by Grema et al. (2017). Higher FST levels were reported in Cameroon breeds (0.061) (Ema et al., 2014), Ankole Longhorn cattle (0.090) (Ndumu et al., 2008). The values implied higher genetic variation within than between populations. Comparatively, Somba breed displayed the lowest within breed variability amongst the cattle breeds investigated. This was expected as the Somba breed is reared in an isolation from the other cattle (geographically) and has therefore not been exposed to much uncontrolled crossings with the other breeds. However, some level of interbreeding between the Somba and Borgou occurs as the latter is reared by nomadic pastoralists who seasonally graze their cattle in areas where Somba cattle are kept implying that interbreeding between the two breeds have probability of occurring.

Genetic Relationship and Population Structure among Cattle Population

Unbiased Nei’s genetic distance pairwise matrix estimates revealed close genetic relationship among the three cattle population. The shortest distance was found between Azawak and Borgou breeds while a little more genetic distance exists between Somba and these two breeds. The closer relationship between Azawak and Borgou breeds can be explained by the proximate geographical distance that exist between the two breeds. Additionally, Borgou cattle breed is a stabilised crossbreed between the Bos indicus (Zebu) and the Bos taurus (Taurine). Similar trend was detected between Zebu Arabe, Zebu Bororo and Kuri (Niger cattle) (Grema et al., 2017). The cluster analysis performed using STRUCTURE revealed populations clustering together confirming relatedness and evidencing a certain level of genetic admixture between the cattle breeds.
Acknowledgments

The authors recognize the financial support provided by the African Union Commission through the Pan African University Institute for Basic Sciences, Technology and Innovation.

Author’s Contribution

All authors contributed to the design and the implementation of the research, to the analysis of the results and to the writing of the manuscript.

Conflict of Interest

The authors declare that in this study there are no conflicts of interest.

References

Amadou, H., L.H. Dossa, D.J.P. Lompo, A. Abdelkadir and E. Schlecht, 2012. A comparison between urban livestock production strategies in Burkina Faso, Mali and Nigeria in West Africa. Tropical Animal Health Production, 44: 1631-1642.

Bessa, I., I. Pinheiro, M. Matola, K. Dzama, A. Rocha and P. Alexandrino, 2009. Genetic diversity and relationships among indigenous Mozambican cattle breeds. South African J. Anim. Sci.

Bradley, D.G., D.E. MacHugh, R.T. Loftus, R.S. Sow and C.H. Hoste et al., 1994. Zebu-taurine variation in Y chromosomal DNA: A sensitive assay for genetic introgression in West African trypanotolerant cattle populations. Animal Genetics, 25: 7-12.

Dorji, N. and M. Daugjinda, 2014. A fundamental statistical tools application for livestock diversity studies from microsatellite data-A Mini Review. Open Access Library J.

Ema, P.N., Y. Manjeli, F. Meutchieyé, C. Kambou and B. Wanjala et al., 2014. Genetic diversity of four Cameroonian indigenous cattle using microsatellite markers. J. Livestock Sci., 5: 9-17.

Evanno, G., S. Regnaut and J. Goudet, 2005. Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. Molecular Ecology, 14: 2611-2620.

FAO, 1999. Animal Genetic Resource Information. http://www.fao.org/docrep/004/y1100m/y1100m01.htm

Freeman, A.R., C.M. Meghen, D.E. Machugh, R.T. Loftus and M.D. Achukwi et al., 2004. Admixture and diversity in West African cattle populations. Molecular Ecology, 13: 3477-3487.

Grema, M., A. Traoré, M. Issa, M. Hamani and M. Abdou, 2017. Short Tandem Repeat (STR) based genetic diversity and relationship of indigenous Niger cattle. Archives Animal Breeding, 60: 399-408.

Kearse, M., R. Moir, A. Wilson, S. Stones-Havas and M. Cheung et al., 2012. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics, 28: 1647-1649.

Kopelman, N.M., J. Mayzel, M. Jakobsson, N.A. Rosenberg and I. Mayrose, 2015. Clumpak: A program for identifying clustering modes and packaging population structure inferences across K. Mol Ecol Resour., 15: 1179-1191.

Kugonza, D.R., H. Jianlin, M. Nabasirye, D. Mpairwe and G.H. Kiwuwa et al., 2011. Genetic diversity and differentiation of Ankole cattle populations in Uganda inferred from microsatellite data. Livestock Sci., 135: 140-147.

Mogesse, H.H., 2007. Phenotypic and genetic characterization of indigenous chicken populations in Northwest Ethiopia. Doctoral Dissertation, University of the Free State.

Mwai, O., O. Hanotte, Y.J. Kwon and S. Cho, 2015. African indigenous cattle: Unique genetic resources in a rapidly changing world. Asian-Australasian J. Anim. Sci., 28: 911.

Ndaiye, N.P., A. Sow, G.K. Dayo, S. Ndaiye, G.J. Sawadogo and M. Sembène, 2015. Genetic diversity and phylogenetic relationships in local cattle breeds of Senegal based on autosomal microsatellite markers. Vet. World, 8: 994-1005.

Ndumu, D.B., R. Baumung, O. Hanotte, M. Wurzinger and M.A. Okeyo, 2008. Genetic and morphological characterisation of the Ankole Longhorn cattle in the African Great Lakes region. Genetics Selection Evolution, 40: 467.

Nei, M., 1972. Genetic distance between populations. Am. Naturalist, 106: 283-292.

Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics, 89: 583-590.
Nyamushamba, G.B., C. Mapiye, O. Tada, T.E. Halimani and V. Muchenje, 2017. Conservation of indigenous cattle genetic resources in Southern Africa’s smallholder areas: Turning threats into opportunities-A review. Asian-Australasian J. Anim. Sci., 30: 603.

Ojango, J.M., N. Mpofo, K. Marshall and L. Andersson-Eklund, 2011. Quantitative methods to improve the understanding and utilization of animal genetic resources. Animal Genetics Training Resource, Version.

Okomo-Adhiambo, M., 2003. Characterisation of genetic diversity in indigenous cattle of East Africa: Use of microsatellite DNA techniques: A case study.

Peakall, R. and P.E. Smouse, 2012. GenALEX 6.5: Genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics, 28: 2537-2539.

Perrier, X. and J.P. Jacquemoud-Collet, 2006. DARwin software. http://darwin.cirad.fr/

Pienaar, L., J.P. Grobler, F.W.C. Nesper, M.M. Scholtz and H. Swart, 2014. Genetic diversity in selected stud and commercial herds of the Afrikaner cattle breed. South African J. Anim. Sci., 44: 80-84.

Pritchard, J.K., M. Stephens and P. Donnelly, 2000. Inference of population structure using multilocus genotype data. Genetics, 155: 945-959.

Rischkowsky, B. and D. Pilling, 2007. The state of the world’s animal genetic resources for food and agriculture. Food Agriculture Org.

Rousset, F., 2008. Genepop’007: A complete re-implementation of the genepop software for Windows and Linux. Mol. Ecol. Resour., 8: 103-106. DOI: 10.1111/j.1471-8286.2007.01931.x

Sanarana, Y., C. Visser, L. Bosman, K. Nephawe and A. Maiwashe et al., 2016. Genetic diversity in South African Nguni cattle ecotypes based on microsatellite markers. Tropical Animal Health Production, 48: 379-385.

Tidjani, A.D., D. Djegga, A. Lothore and P. Delmas, 2006. Les marchés de bétail autogérés: Un exemple béninois. – SOS Faim Belgique. SOS Paysanne.

Van Oosterhout, C., W.F. Hutchinson, D.P. Wills and P. Shipley, 2004. MICRO-CHECKER. Software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes, 4: 535-538.

Waples, R.S., 2014. Testing for Hardy–Weinberg proportions: have we lost the plot? J. Heredity, 106: 1-19.

Weir, B.S. and C.C. Cockerham, 1984. Estimating F-statistics for the analysis of population structure. Evolution, 38: 1358-1370.