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Genetic Diversity of Three Indigenous Cattle Breeds Reared in Benin

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Abstract: Livestock production is an important component of the Benin economy contributing an upward of 25% of the agricultural Gross Domestic Products (GDP). Indigenoues 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 Covè (7°13'7.97" N and 2°20'21.92" E) as presented in Fig. 1. These regions are located in 3 different agro-ecological zones.

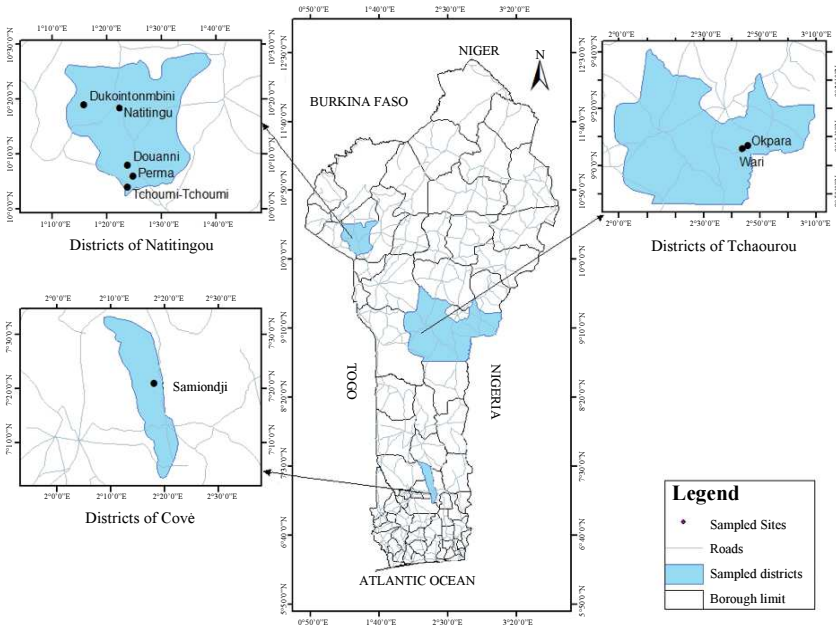


Fig. 1: Localization of sampling sites in three agro-ecological areas of Benin

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: AGCTGGGAATATAACCAAAGG	
		R: AGTGCTTTCA AGGTCCATGC	248-278
BM2113	2	F: GCTGCCTTCTACCAAATACCC	
		R: CTTAGACAACAGGGGTTTGG	122-156
INRA023	3	F: GAGTAGAGCTACAAGATAAACTTC	
		R: TAACTACAGGGTGTTAGATGAACTCA	195-225
INRA035	16	F: ATCCTTTGCAGCCTCCACATTG	
		R: TTGTGCTTTATGACACTATCCG	100-124
HEL9	8	F: CCCATTCAGTCTTCAGAGGT	
		R: CACATCCATGTTCTCACCAC	141-173
ETH3	19	F: GAACCTGCCTCTCCTGCATTGG	
		R: ACTCTGCCTGTGGCCAAGTAGG	103-133
SPS115	15	F: AAAGTGACACAACAGCTTCTCCAG	
		R: AACGAGTGTCCTAGTTTGGCTGTG	234-358
ILSTS005	10	F: GGAAGCAATGAAATCTATAGCC	
		R: TGTTCTGTGAGTTTGTAAGC	176-194
ILSTS059	13	F: AGTATGGTAAGGCCAAAGGG	
		R: CGACTTGTGTTGTTCAAAGC	105-135
INRA063	18	F: ATTTGCACAAGCTAAATCTAACC	
		R: AAACCACAGAAATGCTTGGAAG	167-189
TGLA126	20	F: CTAATTTAGAATGAGAGAGGCTTCT\	
		R: TTGGTCTCTATTCTCTGAATATTCC	115-131
TGLA227	18	F: CGAATTCCAAATCTGTTAATTTGCT	
		R: ACAGACAGAAACTCAATGAAAGCA	75-105
TGLA053	16	F: GCTTTCAGAAATAGTTTGCATTCA	
		R: ATCTTCACATGATATTACAGCAGA	143-191
ILSTS028	15	F: TCCAGATTTTGTACCAGACC	
		R: GTCATGTCATACCTTTGAGC	105-135

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 GenAlEx 6.5 (Peakall and Smouse, 2012) software. Wright's F-statistics (FIT, FIS and FST) for each locus 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 from Nei's DS genetic distances 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.

Results for the observed heterozygosity (Ho), expected heterozygosity (He) and estimated heterozygosity deficit (FIS) at different loci in the three cattle populations are presented in Table 3. In overall population, the mean value of observed and expected heterozygosity are 0.844 and 0.759 respectively across the fourteen loci.

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

Population	Azawak		Borgou		Somba	
Markers	Na	Ne	Na	Ne	Na	Ne
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	0.587	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	Azawak			Borgou			Somba		
Loci	Ho	He	FIS	Ho	He	FIS	Ho	He	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
SPS115	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	11.091
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.

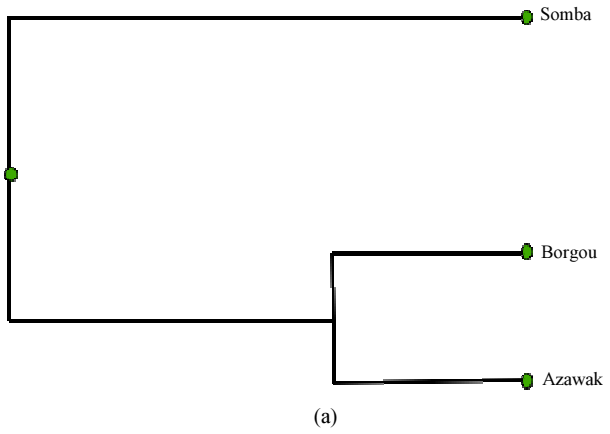
Genetic Variation and Relationship between Breeds

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.

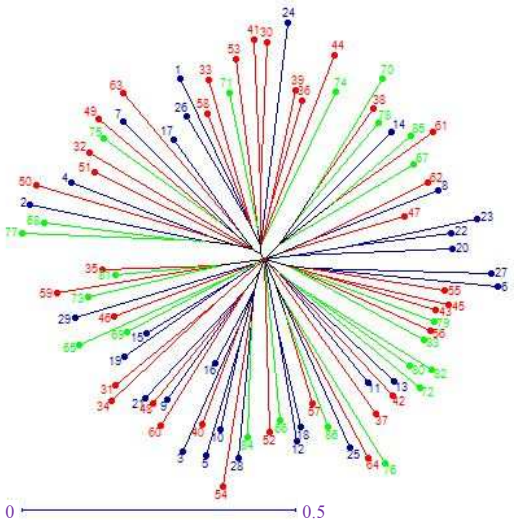
Phylogenetic relationship between the breeds was established through construction of a Neighbor-Joining tree shown in Figure 2a using the unweighted pair group method which uses arithmetic averages (UPGMA). The Azawak and Borgou breeds tended to cluster together, while Somba breed appeared to be relatively 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.

Table 5: Analysis of molecular variance for the three cattle 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	



(a)



(b)

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

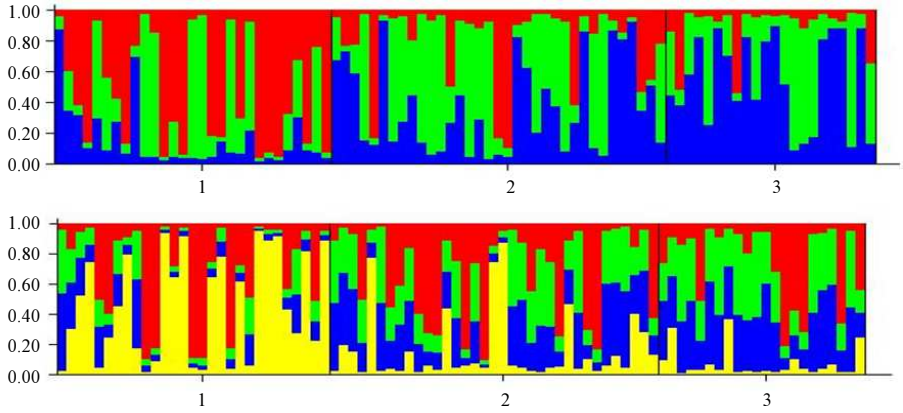


Fig. 3: Summary plot of estimated membership coefficient for each individual, in each cluster for K=3 and 4 obtained with a 100,000 burn-in under the admixture model for the breed analysis. Each individual is represented by a single vertical line broken into K coloured segments, with lengths proportional to each of the inferred clusters

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

Population	Azawak	Borgou	Somba
Azawak	***		
Borgou	0.013	***	
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

reflect the heterogeneity of the breed. Additionally, African zebu breeds have been influenced by historical zebu-taurine crossbreeding and the high allelic diversity observed is undoubtedly a result of admixture and the consequent contribution of the taurine and zebu alleles.

All three cattle breed of Benin showed significantly negative mean of FIS indicating an increase in heterozygosity. Locus wise comparison of inbreeding coefficient (FIS) within breeds showed reduced heterozygosity in three markers (ILSTS059, ILSTS028 and BM1818) with an overall inbreeding coefficient showing low positive inbreeding value. All markers considered were in Hardy-Weinberg Equilibrium except for three loci (INRA35, INRA063 and TGLA053). The effect of these markers (78.57%) adhered to HWE is an indication that the allele frequency among Benin cattle breeds studied has remained constant from generation to generation (Dorji and Daugjinda, 2014). The deviation from HWE of the three markers could be associated to presence of null alleles, intense artificial selection and/or use of few breeding bulls in the region, selective forces operating at certain loci, non-random sampling and age structure of samples used, assortative mating, sex linkage as well as the Wahlund effect which is the presence of fewer heterozygotes in a population than predicted on account of population subdivision (Waples, 2014). The role of null alleles for the observed heterozygosity deficit can be discounted based on the results of MicroChecker analysis.

Natural mating is dominant among native Benin cattle populations reared in smallholder pastoral and agro-pastoral production system with very low level of use of artificial insemination. The few animals that are artificially served are mainly exotic or improved local breeds mainly kept for milk production in the less extensive grazed systems.

Structured genetic improvement programs where genetic gain is deliberately generated relative to the breeding goal seldom exist in Benin implying that reduction in heterozygosity due to intense artificial selection and use of few breeding sires does not arise. Population subdivision is a possible cause of the observed heterozygosity deficit considering that samples were collected from different geographical locations in Benin. However, it is noteworthy that much higher deviations from HWE have been reported in South African Nguni cattle type (Sanarana *et al*., 2016). Expected heterozygosities expressing gene diversity values obtained among studied populations were high and varied among the breeds (75% for Azawak and Borgou, 77% for Somba). The findings concur with those reported for Senegal cattle populations (Ndiaye *et al*., 2015; 76%) as well as those for Niger cattle breed (Grema *et al*., 2017; 70%). However, they were higher than those reported in Afrikaner cattle populations (Pienaar *et al*., 2014; 57%). It is notable that the observed heterozygosity in the three populations was higher than the expected heterozygosity, pointing out lower inbreeding and selection in these populations (see Table 4). These high levels of gene diversity can be explained by a combination of their hybridized status and the absence of selection for any particular trait. The high genetic variation that exists among the cattle population studied is useful in the breeds genetic improvement through within and between breeds selection and mating. High gene diversity levels are normally associated with long-term natural selection for adaptation and the historic mixing of different populations (Ojango *et al*., 2011).

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.

Conclusion

The findings from this study confirm the results of phenotypic characterization that identified the Azawak, Borgou and Somba as three distinct breeds. This research further indicated that the Azawak and Borgou breeds are more closely related genetically than they are with the Somba breed. Much of the genetic differentiation occurred among individuals within populations than among populations. Low values of inbreeding coefficients are an indication of low levels of artificial selection which would have favoured use of few breeding animals. A critical analysis of this study finding indicates that there is significant genetic variation between and within the Azawak, Borgou and Somba that can be utilized in implementation of an indigenous cattle selection program without immediate threat to rise in inbreeding levels in the cattle population in Benin.

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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.

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