POPULATION STRUCTURE OF NIGERIAN INDIGENOUS CATTLE BREEDS BASED ON ILLUMINA BOVINE50K SNP PANEL

[1,2]Oladepo, A. D.,[2]Okpeku, M., [3]Salako, A. E., [4]Adeoye, A. A., [1]Ayoola, M. A., [1]Ogunsipe, M. H., [1]Dada, O. A., and [1]Amaechi, C.I.

[1]Department of Agricultural Science, Adeyemi Federal University of Education, Ondo, Nigeria
[2]Discipline of Genetics, School of Life Science, College of Agriculture, Engineering and Sciences University of KwaZulu-Natal, Private Bag X54001, Durban 4000, South Africa
[3]Animal Breeding and Genetics Unit, Department of Animal Science, University of Ibadan, Nigeria
[4]Department of Animal Production and Health, Olusegun Agagu University of Science and Technology, Okitipupa, Ondo State, Nigeria
*Correspondence: oladepoad@aceondo.edu.ng; +234 (0) 7032502604

ABSTRACT

In this study, the genetic structure of Nigerian indigenous cattle breeds using the Illumina Bovine50K SNP array was investigated. A total of 96 DNA samples including White Fulani (n = 20), Red Bororo (n = 20), Sokoto Gudali (n = 20), Muturu (n = 20) and Bornu Kuri (n = 16) were genotyped. Statistical analysis was performed using PLINK version 2.0. Genetic diversity, measured as expected heterozygosity, was estimated at 0.502, 0.499, 0.510, 0.506 and 0.499 for White Fulani (WF), Red Bororo (RB), Sokoto Gudali (SG), Bornu Kuri (BK) and Muturu (MU) cattle breeds, respectively. The individual inbreeding coefficient varied from 0.013 ± 0.05, 0.008 ± 0.07, 0.014 ± 0.06, 0.017 ± 0.08 and 0.012 ± 0.04 for WF, RB, SG, BK and MU cattle breeds, respectively. The Principal component analysis clustered the populations according to agro-ecological zones and breed types. Linkage disequilibrium (LD) for shorter intervals (0–10 kb) ranged from 0.44 to 0.56 and WF had highest values. The cattle populations exhibited high level of genetic variation which holds potential for improved production in the agro-ecological zones. The results of this study demonstrated that 54,609 SNPs were polymorphic (MAF >0.05) in these breeds and indicate potential for application in Nigerian indigenous cattle populations. Genomic information generated from the BovineSNP50K can now be applied in genetic prediction; genetic characterization and genome-wide association studies. In addition,a national strategy is required to maintain genetic diversity in the cattle production systems through well-structured breeding and conservation programs.

Keywords: Bovine50K BeadChip, breed diversity, cattle populations, genetic variability, SNP

INTRODUCTION

Cattle production is one the major contributor to global agriculture. It is one of the major sources of high protein foods for human consumption and raw materials for industries. In Nigeria, some cattle breeds are classified as indigenous and transboundary animal genetic resources. (AnGR), this is because they manifest unique adaptation and productive abilities across the distinct agro-ecological zones within the country (Oladepo, 2016). Various advancements in Single Nucleotide Polymorphism-based methodologies have allowed for the identification of homozygosity-and-heterozygosity-rich regions to study both genome-wide levels of genetic uniformity and diversity. SNP features highly informative probes that is uniformly distributed across genomes of bovine from Bovine 50K genotyping and it was first made available in 2007 (Matukumalli *et al*., 2009). Biscarini *et al*. (2020) stated that Runs of Homozygosity (ROH) may be used to describe the inbreeding status in term of degree, age of populations or to identify genomic regions under selection. Similarly, Runs of Heterozgosity (ROHet) are genomic regions where diversity might be beneficial and may also indicate balancing selection events in a population (Biscarini *et al*., 2020). The Bovine50K SNP array has been used to quantify genetic diversity and population structure in indigenous cattle in South Africa (Lashmar *et al*., 2022) and Korea (Alam *et al*., 2021). However, few the genetic relationships among Nigerian indigenous cattle populations are under-documented and diversity studies among these populations using Bovine50K is scanty. The aim of this study was to use the Bovine50KSNP chips to investigate the genetic variability and population structure of Nigerian indigenous cattle breeds.

MATERIALS AND METHODS

Hair samples were obtained from 20 White Fulani, 20 Red Bororo, 20 Sokoto Gudali, 20 Muturu, 16 Bornu Kuri cattle breeds from different agro-ecological zones in Nigeria. Hair samples (≥20 strands of tail hairs) were collected by plucking from each animal using plier so as to ensure the hair follicle in the root is in the hair samples. The hairs were then put in a separate envelope and sealed. The hair samples were sorted out and labeled accordingly before the DNA extraction at the ARC-Biotechnology Platform. Genomic DNA was extracted at the ARC-Biotechnology Platform laboratory from the hair samples using the QiagenDNeasy extraction kit (Qiagen, South Africa) according to the manufacturer's protocol. The protocol was adapted for the hair samples where Dithiothreitol (DTT) was added with proteinase K in the first step. Genomic DNA for all samples was quantified using a Qubit® 2.0 fluorometer and the Nanodrop spectrophotometer (Nanodrop ND-1000). In addition, gel electrophoresis was performed to quantify the DNA.

Genotyping was conducted at the ARC-Biotechnology Platform with the Illumina BovineSNP50K BeadChip v2 which features 54 609 SNP probes distributed across the whole bovine genome with an average spacing of 49.9 kb (Matukumalli *et al*., 2009). Approximately 200 ng (12 µL of DNA loaded in each well of a BeadChip) of genomic DNA was used to genotype each sample. Samples were processed according to the Illumina Infinium–II assay protocol (Illumina, Inc. San Diego, CA 92122 USA). Briefly, each sample was whole-genome amplified for 20 hours at 37 °C. The samples were then fragmented, precipitated and re-suspended in an appropriate hybridization buffer. The samples were hybridized on the prepared BovineSNP50 BeadChip for 20 hours at 48 ºC. Following the hybridization, nonspecifically hybridized samples were removed by washing, while the remaining specifically hybridized loci were processed for the single-base extension reaction, stained and imaged on an Illumina iScan Reader.

Genotype data generated from the iScan system were loaded into Illumina Genome Studio version 1.9.0 software, which uses algorithms to perform primary data analysis, including raw data normalization, clustering and genotype calling. A final custom report was created from the genome studio using Plink Input Report 2.1.1, which created a ped (Pedigree file) and Map (SNP panel file) file to use for downstream analyses.

Basic genotype statistics for each marker, including call rate, minor allele frequency (MAF), Hardy-Weinberg Equilibrium (HWE), allele and genotype counts were calculated using the Quality Assurance Module from the SNP Variation Suite version 7 (SVS; Golden Helix Inc., Bozeman, Montana: www.goldenhelix.com). The following quality control criteria (filters) were used to remove from further analysis any SNPs with less than 95% call rate, and SNPs with less than 0.05 MAF. SNP were tested for HWE (P <0.001) to identify possible typing error. Samples with more than 10% missing genotypes were removed from the study.

RESULTS AND DISCUSSIONS

The average MAF varied from 0.394 (± 0.13), 0.429 (± 0.07), 0.394 (± 0.17), 0.428 (± 0.15) and 0.496 (± 0.18)for White Fulani (WF), Red Bororo (RB), Sokoto Gudali (SG), Bornu Kuri (BK) and Muturu (MU) cattle breeds respectively. The observed heterozygosity (H_o) for all populations was slightly lower than the expected heterozygosity (H_e), which could be attributed to a reduction in heterozygosity. Sokoto Gudali population exhibited the highest levels of genetic variability (0.506 ± 0.03). The inbreeding coefficients were generally low in all populations ranging from 0.008 ± 0.07 to 0.017 ± 0.08 (Table 1). The distribution of SNPs differed among chromosomes from 926 (CHI28) to 3256 (CHI1). The overall r^2 across chromosomes ranged from 0.12 ± 0.12 to 0.21 ± 0.22 and the Muturu population had the lowest r^2 values across chromosomes, while White Fulani population, had higher r^2 value (Table 2).

The Bovine50K SNP array was used to examine genetic diversity and population structure of Nigerian indigenous cattle breeds. Quality control procedures removed a higher number of SNPs in the White Fulani population due to low minor allele frequency (MAF < 0.05). This could indicate that the WF population sampled in this study was subjected to higher selection pressure compared to the other populations. The high levels of expected heterozygosity (He) in this study indicates genetic variation

within the population and are comparable to studies performed on South African (H_e = 0.498–0.508) (Lashmar *et al*., 2022).

These levels of inbreeding are similar to those reported previously in South African cattle breeds (0.07) (Matukumalli *et al*., 2009). It is important to maintain the current low levels of inbreeding and high genetic diversity in these populations to allow genetic improvement. The cattle populations were well differentiated according to agro-ecological zones and breed type, which consistent with several authors (Oladepo *et al*., 2016; Alam *et al*., 2021; and Lashmar *et al*., 2022). However, genetic links among the studied populations were observed, which could be due to co-ancestry, crossbreeding or gene flow. Linkage disequilibrium (LD) in this study persisted for a relatively shorter range and levels per chromosome varied slightly in Muturu breed, while no variation was observed among other indigenous breeds.

Table 1 Percentage of genetic diversity and inbreeding coefficient of the studied cattle populations

Population	N	Average MAF±SD	H_o±SD	H_e±SD	F_{IS}±SD
White Fulani	20	0.389 ± 0.16	0.495 ± 0.04	0.502 ± 0.02	0.013 ± 0.05
Red Bororo	20	0.394 ± 0.13	0.492 ± 0.06	0.499 ± 0.02	0.008 ± 0.07
SokotoGudali	20	0.429 ± 0.07	0.506 ± 0.03	0.510 ± 0.03	0.014 ± 0.06
Bornu Kuri	16	0.394 ± 0.17	0.494 ± 0.05	0.506 ± 0.01	0.017 ± 0.08
Muturu	20	0.428 ± 0.15	0.493 ± 0.07	0.499 ± 0.02	0.012 ± 0.04
Merged	96	0.496 ± 0.18	0.499 ± 0.03	0.504 ± 0.01	0.056 ± 0.09

MAF = minor allele frequency; H_0 = observed heterozygosity, H_e = expected heterozygosity, FIS = inbreeding coefficient

Table 2 Linkage disequilibrium (average r^2) per chromosome for the five selected Nigerian indigenous cattle breeds

CHI	CHI LEN(MB)	No. of SNPs	White Fulani	SokotoGudali	Red Bororo	Bornu Kuri	Muturu	Merged
1	156.67	3365	0.20 ± 0.22	0.17 ± 0.20	0.10 ± 0.20	0.11 ± 0.22	0.09 ± 0.12	0.08 ± 0.13
2	140.36	2940	0.20 ± 0.21	0.17 ± 0.21	0.10 ± 0.22	0.11 ± 0.21	0.08 ± 0.11	0.07 ± 0.10
3	120.54	2492	0.21 ± 0.22	0.17 ± 0.20	0.11 ± 0.21	0.10 ± 0.22	0.09 ± 0.12	0.08 ± 0.11
4	122.29	2527	0.18 ± 0.22	0.15 ± 0.20	0.10 ± 0.20	0.14 ± 0.22	0.10 ± 0.12	0.10 ± 0.10
5	115.06	2355	0.19 ± 0.22	0.16 ± 0.20	0.12 ± 0.21	0.13 ± 0.22	0.11 ± 0.12	0.09 ± 0.11
6	124.16	2549	0.25 ± 0.22	0.22 ± 0.20	0.10 ± 0.20	0.11 ± 0.22	0.08 ± 0.12	0.09 ± 0.13
7	113.27	2303	0.23 ± 0.22	0.19 ± 0.20	0.10 ± 0.21	0.15 ± 0.22	0.07 ± 0.12	0.08 ± 0.11
8	118.33	2463	0.23 ± 0.22	0.19 ± 0.20	0.11 ± 0.20	0.13 ± 0.22	0.09 ± 0.12	0.10 ± 0.10
9	100.02	1996	0.19 ± 0.22	0.17 ± 0.20	0.11 ± 0.22	0.14 ± 0.22	0.10 ± 0.12	0.11 ± 0.11
10	111.06	2210	0.18 ± 0.22	0.15 ± 0.20	0.10 ± 0.21	0.15 ± 0.22	0.11 ± 0.12	0.09 ± 0.10
11	114.54	2248	0.19 ± 0.22	0.18 ± 0.20	0.14 ± 0.21	0.12 ± 0.22	0.10 ± 0.12	0.11 ± 0.12
12	96.71	1860	0.22 ± 0.22	0.20 ± 0.20	0.11 ± 0.20	0.11 ± 0.22	0.09 ± 0.12	0.10 ± 0.10
13	92.15	1761	0.21 ± 0.22	0.20 ± 0.22	0.15 ± 0.22	0.13 ± 0.22	0.07 ± 0.12	0.09 ± 0.12
14	103.35	2013	0.21 ± 0.22	0.19 ± 0.20	0.12 ± 0.21	0.14 ± 0.22	0.09 ± 0.12	0.07 ± 0.11
15	90.76	1750	0.20 ± 0.21	0.16 ± 0.21	0.16 ± 0.21	0.13 ± 0.21	0.10 ± 0.11	0.12 ± 0.11
16	89.54	1694	0.21 ± 0.22	0.15 ± 0.22	0.13 ± 0.20	0.16 ± 0.22	0.10 ± 0.12	0.09 ± 0.10
17	84.46	1580	0.20 ± 0.22	0.16 ± 0.22	0.17 ± 0.22	0.16 ± 0.22	0.08 ± 0.12	0.11 ± 0.11
18	74.57	1394	0.21 ± 0.22	0.18 ± 0.22	0.14 ± 0.21	0.14 ± 0.22	0.11 ± 0.12	0.10 ± 0.10
19	72.34	1339	0.20 ± 0.21	0.17 ± 0.21	0.16 ± 0.21	0.11 ± 0.21	0.09 ± 0.11	0.08 ± 0.11
20	84.50	1597	0.19 ± 0.20	0.18 ± 0.20	0.19 ± 0.22	0.11 ± 0.20	0.07 ± 0.10	0.09 ± 0.13
21	81.75	1532	0.17 ± 0.22	0.15 ± 0.22	0.17 ± 0.20	0.10 ± 0.22	0.11 ± 0.12	0.10 ± 0.12
22	70.50	1280	0.21 ± 0.22	0.15 ± 0.22	0.19 ± 0.22	0.13 ± 0.22	0.10 ± 0.12	0.12 ± 0.10
23	65.45	1159	0.17 ± 0.18	0.16 ± 0.18	0.17 ± 0.19	0.12 ± 0.18	0.11 ± 0.11	0.10 ± 0.11
24	76.50	1435	0.21 ± 0.21	0.18 ± 0.21	0.17 ± 0.21	0.15 ± 0.21	0.09 ± 0.10	0.07 ± 0.12
25	57.85	967	0.20 ± 0.21	0.18 ± 0.20	0.16 ± 0.20	0.11 ± 0.21	0.09 ± 0.11	0.08 ± 0.10
26	66.04	1156	0.19 ± 0.22	0.16 ± 0.22	0.19 ± 0.22	0.14± 0.22	0.07 ± 0.12	0.09 ± 0.11
27	60.04	931	0.19 ± 0.22	0.15 ± 0.21	0.19 ± 0.21	0.12 ± 0.22	0.11 ± 0.12	0.11 ± 0.12
28	59.61	926	0.21 ± 0.22	0.16 ± 0.22	0.16 ± 0.22	0.11 ± 0.22	0.08 ± 0.12	0.09 ± 0.10
29	62.35	989	0.20 ± 0.21	0.17 ± 0.21	0.15 ± 0.20	0.14 ± 0.21	0.10 ± 0.11	0.11 ± 0.11
Overall	2,556.769	51,845	0.21 ± 0.22	0.17 ± 0.22	0.19 ± 0.21	0.15 ± 0.22	0.12 ± 0.12	0.09 ± 0.10

CHI = Chromosome; *LEN*= Length; *MB* = megabyte; SNPs = Single Nucleotide Polymorphisms

CONCLUSIONS

The results from these findings have practical implications on the implementation of genome-wide association studies or genomic selection. A denser SNP panel will be needed for the implementation of genomic selection, and the need for large sample sizes.

Acknowledgments

The authors would like to appreciate the management of Adeyemi Federal University of Education, Ondo, Nigeria; Tertiary Education Trust Fund (TetFund) of the Federal Government of Nigeria for sponsorship and the ARC-Biotechnology Platform, Pretoria, South Africa for making their laboratory resources available for the laboratory analysis of the samples.

REFERENCES

Alam, M. Z., Lee, Y., Hyo-Jung Son, H., Lauren H. Hanna, L. H., Riley, D. G., Mannen, H., Sasazaki, S., Park, S. P. and Kim, J. (2021). Genetic characteristics of Korean Jeju Black cattle with high density single nucleotide polymorphisms. Anim. Biosci. Vol. 34, No. 5:789-800

Biscarini, F., Mastrangelo, S., Catillo, G., Senczuk, G., and Ciampolini, R. (2020). Insights into genetic diversity, runs of homozygosity and heterozygosity-rich regions in Maremmana semi-feral cattle using pedigree and genomic data. Animals, 10 (12), 2285.

Lashmar, S. F., Visser, C. Okpeku, M., Muchadeyi, F. C., Mapholi, N. O. and van Marle-Köster, E. (2022) A within- and across-country assessment of the genomic diversity and autozygosity of South African and eSwatini Nguni cattle. Tropical Animal Health and Production 54:365 1-7

Matukumalli, L.K., Lawley, C.T., Schnabel, R.D., Taylor, J.F., Allan, M.F., Heaton, M.P., O'Connell, J., Moore, S.S., Smith, T.P., Sonstegard, T.S. and Van Tassell, C.P. (2009). Development and characterization of a high density SNP genotyping assay for cattle. PLoS ONE 4: e5350-5063.

Oladepo, A. D. (2016). Morphometric attributes and blood protein polymorphisms of selected indigenous cattle breeds in Nigeria. Ph.D. Thesis submitted to the University of Ibadan, Nigeria.