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Research Article
Genetic Distance and Gene Flow in Five Populations of Nigerian Local Breeds of Goat Using Random Amplified Polymorphic DNA Markers

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Abstract

Background and Objective: The ability of goats to tolerate harsh climates, supply meat and milk, short generation interval and ability to thrive on poor quality diets provided by scarce grazing on marginal lands, make goats strategic to increasing livestock productivity and animal protein in rural agricultural systems. The study was aimed to investigate the genetic distance and gene flow in 5 populations of the Nigerian local breeds of goat using random amplified polymorphic DNA markers. **Materials and Methods:** The populations studied were: Sokoto Red, Sahel, Kano Brown, Bornu White and West African Dwarf goats. The 120 blood samples were randomly collected from various locations across the 4 geographical zones in Nigeria, from the jugular vein of the animals. Approximately 5 mL of blood was collected aseptically from each animal into an ethylenediaminetetraacetic acid (EDTA) container. The DNA samples were isolated and purified from the samples and the random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) was carried out. **Results:** The result showed that highest genetic similarity (0.9995) and lowest genetic distance (0.0005) were recorded between Sokoto Red and Sahel, while the lowest genetic similarity (0.9505) and highest genetic distance (0.0507) were recorded between Bornu White and WAD. The closest relationship was observed between Sokoto Red, Sahel and Bornu White goat populations and the farthest relationship was observed between WAD and Sokoto Red populations. **Conclusion:** It was concluded that there were high genetic similarity, low genetic distance, low percentage gene differentiation and loss of heterozygosis in the studied populations of Nigerian local breeds of goat.

Key words: Genetic distance, gene flow, RAPD markers, goat breeds, dendrogram, genetic similarity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Goats constitute the largest group of small ruminant livestock in Nigeria totaling about 53.8 million and also constituting 6.2% of the World's goat population[1]. The ability of goats to tolerate harsh climates, trypanotolerance in some breeds, supply meat and milk, short generation interval and ability to thrive on poor quality diets provided by scarce grazing on marginal lands, make goats strategic to increasing livestock productivity and animal protein in rural agricultural systems[2-4]. Despite these advantages, not much attention has been laid on the genetic characterization[5] and possible genetic improvement of small ruminants (goats) in Nigeria.

Molecular markers are important tools in tagging desirable loci underlying the traits which have breeding importance. The study of genetic variation plays an important role in developing rational breeding strategies for economical animal species[6]. In recent years, a range of innovations in molecular genetics have been developed for the study of genetic variation and evolution of populations using DNA marker genotype information. Genetic analysis of livestock species have been performed using polymorphic markers such as restriction fragment length polymorphisms (RFLPs) and microsatellites[2,7], but their use is limited since designation of these genetic markers is expensive, technically demanding and is time consuming[8]. However, random amplified polymorphic DNA (RAPD) marker which uses short oligonucleotide primers of arbitrary sequence to amplify genomic DNA by polymerase chain reaction (PCR) enables an approach for identifying polymorphic and genetic markers faster[9]. The RAPD technique has also been used in analysis of genetic diversity between different breeds of animals such as cattle[10], goat[11] and sheep[12].

Quantitative assessment of genetic diversity within and among populations is an important tool for decision making in genetic conservation and utilization plans. The most widely used method to quantify these genetic diversities is by utilizing phenotypic characters[12]. In Nigeria, goats and other local breeds of livestock have been characterized phenotypically but their genetic characterization are still lacking. However, Adebambo *et al.*[13] and Okpeku *et al.*[14], have both employed molecular markers in characterization of West African Dwarf (WAD) and red Sokoto (RS) breeds of goat, but, there is little information on the genetic diversity of other existing local breeds of goat in Nigeria. Investigation on genetic diversity and similarity between and within breeds of goat is necessary to provide useful genetic information essential for developing effective management plans for the conservation and improvement of their genetic resources. Therefore, this study was aimed to investigate the genetic diversity of five populations of Nigerian local breeds of goat.

MATERIALS AND METHODS

Location of the study: The experiment was conducted within four geographical zones of Nigeria: South East, North West, North East and North Central. Nigeria is located in West Africa on the Gulf of Guinea (latitude 10°00'N, longitude 8°00'E) with a total area of 923,768 km^2 (twice the size of California). Nigeria is bounded by Niger, Benin and Cameroon Republics on the North, West and East, respectively[15]. The study was conducted in 2017 and it lasted from May to December, 2017.

Experimental animals: Five breeds of goat were used for this study: Sahel goat (SH), Kano Brown (KB), West African Dwarf goat (WAD), Red Sokoto goat (RS) and Bornu white goat (BW). One hundred and twenty blood samples were randomly collected at various locations across the four geographical zones in Nigeria namely: Sokoto Red (23), Kano Brown (21), Bornu White (23), WAD (26) and Sahel (27). The blood samples were collected from the jugular vein of the animals through a process known as venipuncture. Approximately, 5 mL of blood was collected aseptically from each animal into an EDTA container, using 23 gauge sterile needle and syringe and was stored at-20°C using (ethylene-di-amine-tetra-acetic acid, EDTA) as anticoagulant. The laboratory analysis was carried out at Department of Animal Science, University of Port Harcourt, Port Harcourt.

DNA extraction/isolation and RAPD-PCR (polymerase chain reaction) conditions: Total DNA was isolated from whole blood samples using a ZymoBead™ Genomic DNA Kit, following the procedure as recommended by the manufacturer (Zymo Research Corporation, website: www.zymoresearch.com). The RAPD-PCR reaction followed the procedure described by El Hentati *et al.*[16]. Amplifications were performed using a thermal cycler (PTC-100TM Programmable Thermal Controller, MJ Research, Inc., MA, USA) using the primers shown in Table 1.

Table 1: Sequences of the random primers selected for the individual typing of the studied animals

Primers	Sequence 5'-3'
GOA1	CCGCGCCGGT
GOA2	CAGCCTCGGC
GOA3	ACGTCGAGCA

The 20 µL amplification reactions contained 50 ng template DNA, 1.0 µM of each primer, 16 µL nuclease free water in a Bioneer AccuPower® TLA PCR Premix. The Thermal cycler was programmed for 40 cycles of denaturation at 94°C for 1 min, annealing at 36°C for 45 sec and extension at 72°C for 1 min. An initial denaturation step of 5 min at 94°C and a final extension step of 5 min at 72°C were included in the first and last cycles, respectively. The results were analyzed quantitatively by visualizing the gel with Ultra Violet (UV) light and a gel imaging device. The image was captured with a computer operated camera attached to the UV transilluminator (Fig. 1) and the intensity of the bands of interest was measured and compared against standards (markers) loaded on the same gel.

Analysis of gene diversity (gene differentiation) and gene flow in subdivided population: The absolute magnitude of gene differentiation among subdivided populations are measured by Gst. The Gst is defined as the proportion of genetic diversity that resides among populations. It is a measure of population differentiation. Values of Gst range from zero to one, with low values indicating that little variation is found among populations and high values denotes that a large amount of variation is found among populations. The Ht and Hs are indices for measuring Gst, as, Gst is calculated from Ht and Hs:

- Ht = Total genetic diversity in the pooled population
- Hs = Mean diversity within each population
- Nm = Estimate of gene flow from Gst or Gcs
  e.g. Nm = 0.5 (1-Gst)/Gst

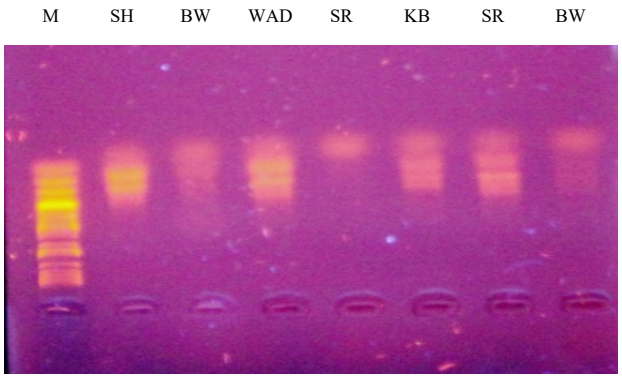

Fig. 1: RAPD profile of 5 Nigerian local goat breeds
M: Molecular marker, SH: Sahel goat, BW: Bornu White, WAD: West African Dwarf, SR: Sokoto Red, KB: Kano Brown

RESULTS

Measure of genetic similarity and genetic distance: Genetic similarity and genetic distance were used to access the genetic similarity (above diagonal) and genetic distance (below diagonal) across the 5 populations of Nigerian local breeds of goat as shown in Table 2. The result shows that genetic similarity was very high ranging from 0.9506-0.9995 while genetic distance in the goat populations was very low ranging from 0.0005-0.0507.

The highest similarity value of 0.9995 occurred between the Sokoto Red and Sahel populations, followed by WAD and Kano Brown populations with value of 0.9971. Sokoto Red and Bornu White, Kano Brown and Sahel populations had similarity value of 0.9895 on the average and the lowest similarity was obtained between Bornu White and WAD (0.9506). On the other hand, the lowest genetic distance of 0.0005 was recorded between Sahel and Sokoto Red populations. Between WAD and KB populations, genetic distance of 0.0029 was obtained. The highest genetic distance in the whole population under consideration existed between WAD and Bornu White populations with value of 0.0507.

Analysis of gene diversity (gene differentiation) and gene flow in subdivided population: The gene diversity in subdivided population was accessed using genetic differentiation and gene flow as presented in Table 3. The gene diversity in subdivided population, Gst, across the three loci combination showed that the variation across the loci are very small, with loci GOA3, GOA1 and GOA2 having varied values of 0.0102, 0.0150 and 0.1067, respectively and an

Table 2: Genetic similarity (above diagonal) and genetic distance (below diagonal)

Population	SR	KB	BW	WAD	SH
SR	-----	0.9886	0.9895	0.9834	0.9995
KB	0.0114	-----	0.9618	0.9971	0.9895
BW	0.0105	0.0390	-----	0.9506	0.9901
WAD	0.0167	0.0029	0.0507	-----	0.9822
SH	0.0005	0.0105	0.0100	0.0179	-----

SR: Sokoto Red, KB: Kano Brown, BW: Bornu White, WAD: West African Dwarf, SH: Sahel

Table 3: Analysis of genetic diversity in subdivided population across all the Loci

Locus	Ht	Hs	Gst	Nm (Estimated of gene flow)
GOA1	0.4992	0.4899	0.0150	32.8752
GOA2	0.4914	0.4832	0.1067	29.5253
GOA3	0.4996	0.4944	0.0102	48.3663
Mean	0.4960	0.4891	0.0139	35.3710

Gst: Proportion of genetic diversity, Ht, Hs: Measuring Gst, Nm: Estimate of gene flow

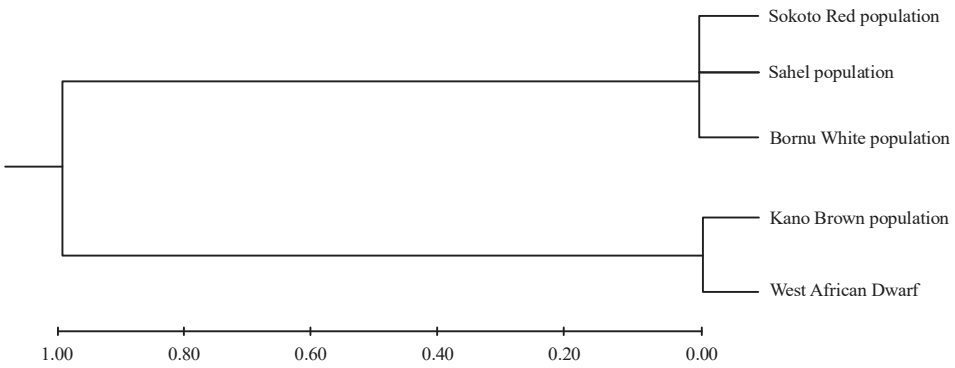

Fig. 2: Dendrogram measure of genetic distance

average value of 0.0139. The mean coefficient of gene differentiation (Gst) was 0.0139, which could mean that 98.61% of the total variation is within breeds, whereas, diversity between the 5 studied populations was 1.39%. The measure of gene flow across the loci showed that locus GOA3 had the highest value of 48.3663, followed by loci GOA1 and GOA2 having 32.8752 and 29.5253, respectively with an average of 35.3710 recorded across the entire loci. Gene flow (Nm) represents the number of effective migrants per generation.

Dendrogram measure of genetic distance: The Dendrogram based on original measure of genetic distance was analyzed using Unweighted Pair Group Method of Arithmetic Means (UPGMA). Depending on banding pattern resulted from using RAPD primers, the Dendrogram was constructed as shown in Fig. 2. The diagram showed how breeds were clearly separated from each other and were grouped according to geographic locations of origin.

The goat populations were separated into two distinct clusters. Cluster I comprised of Sokoto Red, Sahel and Bornu White, whereas, cluster II comprised of Kano Brown and West African Dwarf goat. The WAD goat stood out clearly as a breed while SR, BW and SH showed a measure of close relationship. Regardless of location, KB and WAD goats clustered closely together, while exhibiting a sharp difference between other populations. It could be clearly seen that the Kano Brown and WAD populations are very special and must have been closely related in the past, even though their link with the other populations were quite weak as a result of genetic communication.

DISCUSSION

The results of current study showed high genetic similarity, low percentage gene, low genetic distance in studied populations of Nigerian local breeds of goat. The results are in agreement and were within the same range of genetic similarity documented by Li *et al.*[17] and Xiang *et al.*[18], who reported ranges of 0.74-0.90. It also agreed with the report of Sulaiman[19], who recorded genetic similarity of 0.97 and 0.96%, respectively in Iraqi local breeds of goat using RAPD markers. Abdel-Rahman *et al.*[20] reported genetic similarity of 90% (0.9) between Barki and Ossimi breeds of Egyptian sheep, which is also, in tandem with the result of this work. However, Ali[21] obtained much higher genetic similarity value of 95% in 4 breeds of Egyptian sheep. The high genetic similarity obtained in this study may be attributed to the descent of the populations which may be presumed to have common ancestor. Or it could be attributed to incessant inbreeding and uncontrolled mating practices prevalent in local livestock production in Nigeria.

The genetic distance obtained in this study (0.0005-0.0507) was very low. It was lower than the findings of Esmaeelkhanian *et al.*[22], who assessed the genetic variability among six Iranian goat breeds and reported that genetic distances varied between 0.081 and 0.227 in the populations. Li *et al.*[17] obtained genetic distances among the black goat populations ranging from 0.1051-0.2978 which were also higher than the ranges recorded in this study. Genetic distance of 0.0167 between WAD and Sokoto Red obtained in this study was lower than 0.39 reported earlier between RS and WAD goats by Adebambo[23] from a smaller sample drawn from several states across Nigeria and 0.268 by Okpeku *et al.*[14], which was an indication of higher level of cross-breeding among goats in southern Nigeria concomitant with higher population of humans and by extension higher population density of reared goats with less inbreeding among goat populations. The genetic distance between SH and SR goats (0.0005) was an indication that the breeds have closest descent, while the genetic distance between SR and WAD (0.0507) showed farther descent.

This result was far below the findings of Balcioglu *et al.*[24] who obtained a Gst value of 0.5117 (51.17%) in eight breeds of Turkey by using RAPD-PCR method, 0.157 (10.57%) by Chenyambuga *et al.*[25] in indigenous goats of Sub-Saharan Africa using microsatellite DNA markers and El Hantati *et al.*[16] who obtained Gst value of 0.1922 (19.22%) in Tunisian Ovine breeds. The coefficient of differentiation among population genes (Gst) they studied was 0.2766 (27.66%), indicating a comparatively low degree of differentiation among the black goat populations[17]. The authors opined that genetic differentiation of population occurs only when populations are partially or completely isolated from each other. Hartl and Clark[26], who applied neutral molecular markers noted that it was genetic drift process that causes genetic differentiation between populations, they observed from their study that breed relationships were more related to geographical locations of the breeds than to the morphological differences between the breeds. Toro and Maki-Tanila[27] in agreement, suggested that the high genetic diversity observed within population groups could arise from overlapping generations and population mixtures from different geographical locations, with natural selection favouring heterozygosity or subdivision accompanied by genetic drift. Agha *et al.*[28] stated that the effect of these factors is more pronounced when the effective population size is very large, which is supported by the poor infrastructure on ground presently for livestock improvement and lack of proper breeding policy in Nigeria. The low value of percentage gene differentiation 1.39% obtained in this suggested that there was no gene drift and could be attributed to indiscriminate mating that occur among these breeds as they roam about under extensive management and moved across the entire country for marketing purposes. This authenticated the opinion of Laval *et al.*[29] who stated that migration has a great effect on the reduction of genetic differentiation between populations.

The main effect of gene flow is homogenization of allele frequencies between populations. The estimation of gene flow in this study among the 5 populations under consideration from Gst, showed that mean (Nm) is equal to 35.3710 across all the three loci considered. Geng *et al.*[30] used microsatellite markers to study genetic diversity in 6 sheep populations in China, found value of gene flow ranging from 2.74-44.39 with a mean value equal to 11.25. Thus, the values obtained in this study fall within the range reported by Geng *et al.*[30], but higher than the values obtained by Missohou *et al.*[31], who reported ranges of 0.46-6.21 in Seven West African goat breeds with microsatellite markers. Mao *et al.*[32] studied 3 Chinese cattle populations, reported that values of gene flow between pair of populations were 0.509 and 1.149. The higher value of gene flow in this study is an indication of homogenization of populations. According to Wright[33], a gene flow value greater than one, leads to homogenization of populations. Therefore, gene flow estimates in this study suggested mobility and considerable exchange of genetic material among these goats. These could be attributed to the fact that some of these animals originated from northern Nigeria where nomadic pastoralism is the dominant livestock management system and also, the extensive system of management which allows the animals to roam freely and fend for themselves in most rural households and communities in the South East. These enable and reinforce the ability of related animals to meet on pasture to breed or for neighbours to exchange related animals for upkeep or breeding. According to Laval *et al.*[29], migration may exert a greater effect than mutation or drift on the reduction in genetic differentiation between populations.

On the clustering pattern of the dendrogram, similar observation of population clustering according to their geographic origin has been reported in cattle[13,34] and chicken[35]. This result was in agreement with the report of Rahman *et al.*[36], who reported that Dendrogram based on Nei[37] genetic distance using UPGMA method indicated segregation of black Bengal goat and Jamunapari goat. They reported that an UPGMA dendrogram showed that two populations clustered together and the other seven populations formed another group. Esmaeelkhanian *et al.*[22] had similar observation with the result of this study. They stated that the phylogenetic tree in their study showed two main separate clusters. The result of this study demonstrated that geographically adjacent populations were more genetically related, probably because of founder effects and interbreeding, especially around bordering areas. The result indicated that Sokoto Red and Sahel had closest (0.02435) genetic relationship while WAD and Sokoto Red had the farthest (1.07183) genetic relationship. This could be attributed to the geographical adaptation of these breeds, WAD goats were well adapted to southern humid area of Nigeria while Sokoto Red goats were adapted to dry arid and semi-arid zone of the country.

CONCLUSION

It was concluded that there were high genetic similarity, low genetic distance, low percentage gene differentiation and loss of heterozygosis in the studied populations of Nigerian local breeds of goat.

SIGNIFICANCE STATEMENT

The study discovers the genetic distance, gene diversity and gene flow status of the Nigeria local breeds of goat. The information can be beneficial for the conservation of genetic resources of Nigerian breeds of goats. The results of this study can offer some crucial scientific data useful for breeding programme of Nigerian local breeds of goats. Thus, a new approach on the breeding plans of Nigerian local breeds of goat may have been discovered.

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