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Genetic Distance and Gene flow in Two Breeds of Nigerian Indigenous Goats Using Restriction Fragment Length Polymorphic Marker

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DOI 10.53974/unza.jabs.7.3.1200

ABSTRACT

Goats constitute an indispensable part of the rural agricultural systems because of their capacity to withstand severe weather, potential to yield milk and meat, fast generation times. Additionaly, they can withstand the substandard diets that are provided by restricted grazing on marginal soils. Gene flow, genetic distance, genotype frequencies, and alleles were investigated using two native goat breeds from Nigeria. The investigational goat populations included Red Sokoto (50) and WAD (45). For each animal, an ethylenediaminetetraacetic acid (EDTA) container was filled with approximately 5 milliliters of aseptic blood taken. Polymorphism of restriction fragment length and Agarose gel electrophoresis were carried out after the DNA samples were isolated and purified. Hardy-Weinberg equilibrium (HWE), gene allele frequency, anticipated and observed heterozygousities (He and Ho, respectively), and populations were compared based on their genetic distances. The degree of genetic diversity was great. According to the findings, Red Sokoto and WAD exhibited the highest genetic similarity (0.9996) and the lowest genetic distance (0.0004) between the two populations. If more animals from different goat breeds are collected, improved genetic research and marker-assisted selections in goat improvement programmes would be achievable.

KEYWORDS: *Genetic Distance, Gene Flow, Allele frequency, Red Sokoto, West Africa Dwarf (WAD) goats.*

INTRODUCTION

Goats are the largest group of small ruminant livestock in Nigeria and 6.2 per cent of all goats worldwide with a population of over 53.8 million. Because of their short generation interval, ability to thrive on poor diets provided by sparse grazing on marginal lands adaptability to harsh climates, trypanotolerance in some breeds, and capacity to supply milk and meat, goats are important for increasing livestock the productivity and animal protein in rural agricultural systems. Notwithstanding these benefits, not much research has been done on the genetic makeup and prospects for genetic advancement of Nigerian goats and other small ruminants (Adebambo *et al.*, 2 ; Li *et al* 20; Maudet *et al.*, 22 ; Okpeku *et al.*, 25; Omotoso *et al.* 27;).

Molecular markers are useful for pinpointing the desired loci underlying the traits that are essential for successful reproduction. A solid grasp of genetic variation is necessary to develop cost-effective breeding programmes for the animal species. Maudet *et al.* 22 utilising genotype data from DNA markers, molecular genetics, has made significant strides recently in its analysis of genetic variation and population dynamics. Polymorphic markers, such as Amplified Fragment Length Polymorphism (AFLP) and random amplified polymorphic DNA (RAPD), have been used in genetic studies of livestock animals, and microsatellites. However, codominant alleles were seen with high genomic abundance due to their many advantages, which include their excellent repeatability and stability when compared to other markers, as well as their random distribution, high genomic

abundance, and generally moderate polymorphism. This study employed Restriction Fragment Length Polymorphisms, or RFLPs, for these reasons.

A crucial tool for decision-making in genetic conservation and utilisation initiatives is the quantitative assessment of genetic diversity both within and between populations. The most popular technique for calculating these genetic diversities, according to Kunene *et al.*17, is the use of phenotypic traits. Nigerian native animal breeds, including goats, have been phenotypically characterised, but genetic data on them is still missing. The West African Dwarf (WAD) and Red Sokoto (RS) goat breeds were characterised using molecular markers by Adebambo *et al.* 2 and Okpeku *et al.* 25; however, the genetic diversity of other local goat breeds that are now found in Nigeria is unknown. Important genetic data required to create efficient management strategies for the preservation and improvement of goat breeds' genetic resources can only be obtained through studies on the genetic diversity and similarity across and within breeds. The purpose of this study was to analyse the genetic diversity of two breed populations of Nigerian goats.

MATERIALS AND METHODS

Experimental Location

The experiment was conducted in two distinct geographic zones of Nigeria, namely the North Central (Kwara State) and South West (Osun State and Oyo State) regions, between August 2018 and February 2019.

Experimental Animal

For this research, a total of 95 experimental animals—50 Red Sokoto and 45 WAD goats—were employed.

Ethical Consideration

In conducting research on genetic distance and gene flow in two breeds of Nigerian indigenous goats using restriction Fragment Length Polymorphic Markers (RFLPs), all procedures involving animals were conducted with utmost respect for their welfare, in compliance with national and international guidelines for the ethical treatment of animals in research.

Experimental Animal and Management

This study focused on the Red Sokoto and West African dwarf (WAD) goats, which seemed to be in a better state. These goats are usually raised as livestock under substantial or semi-intensive husbandry in the agricultural ecological zone. The animals are fed mountains of trash, discarded agricultural products, household scraps, and cassava peels. The residents of these towns have embraced this goat farming technique in an effort to lessen disputes between humans and goats over food. The animals' records were not kept up to date. Ethno-veterinary medicine was a popular specialty at the time.

Collection of Blood Samples

Using a procedure called venipuncture, 95 blood samples were taken from the animals' jugular veins. Using an Ethylene-Diamine-Tetra-Acetic Acid (EDTA) container as an anticoagulant, about 5 milliliters of blood were aseptically extracted from each animal using a 23-gauge sterile needle and syringe. Blood samples were then placed in a refrigerator set at -20°C. The samples were kept at -20 degrees Celsius after being delivered on ice to the lab. The laboratory analysis was conducted out at the Federal University of Technology in Akure, Ondo State, Nigeria, in the Bio-safety Research Laboratory.

LABORATORY ANALYSIS

Extraction of DNA

200µL of the blood samples were used for DNA extraction using Bioline International's Isolate II Genomic DNA extraction Kits, in compliance with the guidelines provided by the manufacturer. The final elution was diluted using 100µL of elution buffer. The filtered DNA sample was also kept for long-term preservation at -20ºC, per protocol.

Confirmation and Quantification

The last DNA extraction stage's final eluted solution's existence of genomic DNA was confirmed by agarose gel electrophoresis. In close proximity to a DNA ladder, the samples were run for 30 minutes at 100 volts on a 0.75 per cent agarose gel that included ethidium bromide. It was determined how much separated DNA there was using PG Instruments Ltd.'s Ultra-Violet Spectrometer.

Primer Sequence

HSP90 Forward 5' AAATAAGTCGACATGCCT-GAGCAAACCCAG 3'

Reverse 5'CTTCATCTGCAGTTAGTTAGTC-TACTTCTTCCAT 3'

Polymerase Chain Reaction (PCR)

Using pre-programmed rmocycler, amplification process was carried out in 200 uL microcentrifuge tubes (Mastercycler pro by Eppendorf). 15 microliters of PCR master mix, 1 microliter each of forward and reverse primers, 3 microliters of DNA template, and 10 microliters of sterilised distilled water were used to make a 30 microliter reaction mix. After the materials

were thoroughly mixed, they were centrifuged at 11,000 revolutions per minute for 5 seconds. After denaturation for four minutes at 94ºC, the reaction was run through 40 cycles: thirty seconds of 94ºC denaturation, thirty seconds of 62ºC annealing, and thirty seconds of 72ºC extension. The last extension was carried out for two minutes at 72ºC.

Gel Electrophoresis

Then, according to Joseph and David 16 10µL of the PCR amplicon was electrophoresed at 100 volts in a 0.75 per cent agarose gel in 1x TBE buffer containing a DNA ladder and ethidium bromide.

Gel Documentation

The gel was placed in a gel documentation machine (VWR's Genosmart2) so that the bands could be seen under Ultra-Violet light. Segment Length of Limitation Electrophoresis on an agarose gel and polymorphism Ten units of restriction enzyme (Invitrogen, USA) specific to each gene were used to digest twenty uL of the PCR results, resulting in a final reaction volume of twenty-five microliters. The reaction mixture was incubated for five hours in a water bath at 37°C. In an agarose gel, the restriction fragments were separated to distinguish between the A and B alleles. Following constraint digestion, the restricted fragments were examined and electrophoresed in an ethidium bromide-stained 4 per cent agarose/1X TBE gel. Molecular sizing was performed using a 100-bp ladder. When exposed to UV radiation, the bands and the gel documentation system photographed the gels (Enduro, Inc)

Statistical Analysis

The banding pattern on the gel was given numerical values, where 1 indicated a band's presence and 0 was its absence. The percentage of each identified allele in a population, or allele frequency, was calculated using gene counting and GENEPOP version 4.2. GENEPOP, version 4.2 was utilised to calculate the average heterozygosity, or the He, in a population assumed to be in HWE to ascertain the genetic diversity of Nigerian goats. Using chi-square analysis and the exact test for POPGENE software, version 1.31, the degree of population deviance from Hardwinberg—a proxy for the intensity of external influences was determined. The genetic link between groups was measured using their genetic distance from one another. The Nei standard genetic distance (D ST) (Nei5), whose value is proportional to evolutionary time, was determined using GENEPOP, version 4.2.

RESULTS

Using the PCR-RFLP method, polymorphisms of the HSP 90 gene were discovered through genotyping two breeds of Nigerian goats. Using specific primers derived from goat heat adaptation genes, the PCR analysis of all goat DNA samples (as animals, i.e., 45 WAD & 50 Red Sokoto goats) resulted in unique amplified fragments of the predicted brand size 400, 300, and 200 bp, respectively, for HSP 90 genes. The electrophoresis gel for the HSP 90 gene's broad ladders on lanes 39 through 58 shows that the animal's HSP 90 genes are active and better adapted to situations involving heat stress. Ladders 1 through 38 and 58 through 95 of the electrophoresis gel for the HSP 90 genes have thin and normal ladders, suggesting that these genes are downregulated and less able to withstand heat stress. The genotype frequencies of AA and AC in WAD when they were, respectively, 0.511 and 0.489, are displayed in Table 1. Goats from the Red Sokoto region scored 0.44 and 0.56, respectively. For Red Sokoto and West African dwarf goats, the allele frequency of a gene per locus varies from 2.4 to 2.8 for Allele C, and from 7.2 to 7.5 for Red Sokoto and West African dwarf goats for Allele A.

The A allele was somewhat more common in WAD (0.7556) than it was in Red Sokoto goats (0.752). Allele C was more common in Red Sokoto 0.28 than in WAD 0.2444.

Table 1: Genotype and Allele frequencies of genes in Nigerian indigenous WAD and RS goats

Marker	Genotype	WAD (n=45)	RS (n=50)
HSP-90	AA	0.511	0.440
	AC	0.489	0.560
	Allele		
	A	0.7556	0.7200
	C	0.2444	0.2800

WAD = West African Dwarf goat
RS = Red Sokoto goat
HSP 90
AA = Homozygous genotype
AC = Heterozygous genotype
A=Allele A

Table 2 provides an overview of the genetic variation data for WAD and RS goats. It proved that the goats with WAD and RS had the same number of alleles

(Na). The effective number of alleles (Ne) for Red Sokoto and WAD was 1.6756 and 1.5857, respectively. The Shannon's Information index was 0.5561 in Red Sokoto goats and 0.5930 in WAD goats. The difference in the effective number of alleles indicates that the two breeds' genetic diversity was different.

Table 2: Summary of Genetic Variation Statistics in WAD and RS goats

HSP- 90	Sample Size	Na	Ne	I
WAD	45	2	1.5857	0.5561
RS	50	2	1.6756	0.5930

Na = Observed number of alleles HSP90
Ne = Effective number of alleles
I = Shannon's Information index (Lewontin26)

In contrast to the expected heterozygosity of 0.37, the observed heterozygosity for WAD goats was 0.48. In Red Sokoto goats, Compared to the mean actual heterozygosity of 0.56, the mean expected heterozygosity was 0.40. It showed that the goats with WAD and RS had higher levels of heterozygosity than predicted. This demonstrated that both WAD and RS goats exhibited genetic variation at the trans HSP locus. This implies that the chosen group will gain from improved performance genetically if the selection process is carefully planned and carried out (Table 3).

Table 3: Summary of Heterozygosity for all loci in WAD and RS Goats.

Marker	Sample size	Ho	He	Average heterozygosity	Nei
WAD	45	0.4889	0.3735	0.3863	0.3694
RS	50	0.5600	0.4073	0.3863	0.4032

Ho: Observed heterozygosity
He: Expected heterozygosity
Nei: Nei's (1973) expected heterozygosity

Table 3 is the summary of the F-statistics and gene flow for each site, while Table 4 displays the genetic identity and distance between WAD and RS goats. Values of Fis, Fit, Fst, and Nm were -0.3576, -0.3554, 0.0016, and 152.7813, in that order. Between Red Sokoto and WAD goats, the mean genetic distance (Nei's) was 0.0004, whereas genetic identity (Nei's) was 0.9996. This suggested that there was a high level of genetic similarity and minimal genetic divergence between the groupings. Genetic variation in WAD and RS goats should ideally be relatively high within populations and modest between populations.

Table 4: Genetic Identity and Genetic distance between WAD and Red Sokoto (RS) Goats

Population	WAD	RS
WAD	****	0.9996
RS	0.0004	****

Above diagonal: Nei's genetic identity
Below diagonal: Genetic distanceWAD = West African Dwarf goatRS = Red Sokoto goat****Similarity index

DISCUSSION

In Red Sokoto goats, the corresponding values were 0.44, 0.56, and 0.00, respectively; in WAD, the genotype distribution for AA, AC, and CC was 0.511, 0.489, and 0.00, respectively (Table 1). The Chi-square analysis for the discrepancies between the observed and expected genotype frequencies showed no significant divergence ($P<0.05$), suggesting that the flocks under investigation do not follow the Hardy-Weinberg equilibrium for the gene locus. Conversely, in Red Sokoto goats, allele C showed a greater frequency (0.28) and a lower frequency (0.2444) in WAD. In WAD, the A allele frequency was 0.75, which was higher than 0.72 in those goats. The genetic distance between Red Sokoto goats and WAD goats was calculated to be 0.0004. This is less than the range of 0.16 found by Oladepo *et al.*26 for Yankassa and WAD sheep, and 0.003 to 0.097 reported by Ali5 for 14 Spanish sheep breed. Though WAD and Yankassa sheep are essentially of different breeds within the same species, this is to be expected as Spanish sheep belong to the same breed. This indicated that within the confines of the small populations under investigation, there was significant allele commonality at the gene locus. This suggests that the populations under study were composites of smaller populations. Still, the extremely significant ($P<0.001$) differences seen in Yankassa and WAD sheep suggest that the samples contained a range of subpopulations with different gene frequencies. This clarified the reason for the temporary decrease in heterozygotes and the increase in homozygotes in both populations.

The study's findings demonstrate the strong genetic similarity, low gene percentages, and low genetic distance among Nigeria's native goat breed populations. Overall, the findings are quite consistent

and fall between the 0.74 and 0.90 genetic similarity ranges reported by Li *et al*20 and Xiang *et al*31. Additionally, it supported Omotoso *et al*27 results, which found that local goat breeds in Iraq had genetic closeness of 0.97 and 0.96 per cent, respectively, based on RAPD markers.

Abdel-Rahman *et al*1 investigated 90 per cent (0.9) genetic similarity between the Egyptian sheep breeds Barki and Ossimi, which was also less than the analysis's findings. In contrast, Ali7 discovered 95 per cent genetic similarity between four Egyptian sheep breeds, which is quite comparable to the findings of this investigation. The study's extraordinary genetic similarity of 99.96 per cent may have resulted from a shared ancestor between the populations. On the other hand, it could be connected to the unregulated inbreeding and mating habits that define the indigenous livestock agriculture of Nigeria. The result of this investigation was a very smallgenetic distance (0.0004).When Esmaeel Khanian *et al*10 evaluated the genetic variability of six Iranian goat breeds, they found that the genetic distances across the populations ranged from 0.081 to 0.227. This is less than the genetic distances between the populations. Li *et al*20 reported that genetic distances across populations of black goats ranged from 0.1051 to 0.2978, which was likewise greater than the collections of experimental goats in current study investigation. In this research, the genetic distance between Sokoto Red and WAD goats was 0.0004, which was less than the 0.268 reported by Okpeku *et al.*4 and the 0.39 between RS and WAD goats previously reported by Adebambo3 from a smaller sample collected from various Nigerian states. This showed that there was less inbreeding across goat populations and greater cross-breeding between goats and humans in southern Nigeria, resulting in a larger population density of reared goats. The closest descent between the breeds is indicated by the genetic distance (0.0004) between WAD and SR goats.

This result was much lower than that of Balcioglu *et al*6, who employed the RAPD-PCR method to achieve the aim of Eight Turkish breeds, having a GST score of 0.5117 (51.17%); Chenyambuga *et al.*8 , employed microsatellite DNA markers to obtain a Gst value of 0.157 (10.57%) in native goats of Sub-Saharan Africa. Also, El Hantati *et al.*9, used Tunisian ovine breeds to obtain a Gst value of 0.1922 (19.22%). A relatively low degree of variation among the black goat populations was suggested by the coefficient of differentiation among the population genes (Gst) that they analysed, which was 0.2766 (27.66%) (Li *et al.*20). The authors claimed that population genetic divergence only occurs when groups are completely or partially cut off from one another. Breed relationships were found to be more strongly correlated with the geographical locations of the breeds than with their morphological differences by Hartl and Clark14 using neutral molecular markers. This suggests that genetic differentiation between populations is primarily caused by genetic drift. According to Toro and Maki-Tanila's28 suggestion, natural selection favoring heterozygosity or subdivision paired with genetic drift may explain the considerable genetic diversity found within population groups. Overlapping generations and demographic mixes from different geographic areas could cause this. Agha *et al.*4 claimed that these traits have a stronger influence on the size of the effective population. The existing state of Nigeria's poor infrastructure for improving livestock and the lack of a suitable breeding programme serve as evidence for this. The low proportion of gene differentiation (1.39%) in the study indicated that there was no gene drift, and it may have something to do with the random mating that happens when these breeds tour the nation under strict supervision for commercial objectives. This provided credence to the idea put forth by Laval *et al.*18 that migration dramatically lessens the degree of genetic variation among groups. The uniformity of allele frequencies across populations is the primary result of gene flow. The estimate of gene flow between the two populations from Gst, (Nm), accounting for all three loci, is 152.7813. Geng *et al.*12 examined the genetic diversity of six sheep populations in China using microsatellite markers. They found that the range of gene flow values was 2.74 to 44.39, with a mean of 11.25. The range obtained by Missohou *et al.*23, who reported ranges of 0.46–6.21 in seven West African goat breeds with microsatellite markers, and the range reported by Geng *et al.*12 were therefore much greater than the values found in this work.

In a 2007 study of three Chinese cow populations, Mao *et al.*21 found that the gene flow between the two populations was 1.149 and 0.509 times greater, respectively. The higher gene flow value found in the study suggests that the populations were becoming more homogeneous. According to Wright 24, populations become homogenised when the gene flow value is more than one. Consequently, considerable amount of genetic material was exchanged and these goats migrated around, according to the study's calculations of gene flow. This could have to do with the fact that some of these animals are native to northern Nigeria, where pastoralist nomadic living is the primary means of livestock management for most households and communities. They may also be related to the extensive management systems of the vast majority of

rural South West households and communities, which allow animals to roam freely and survive on their own. This makes it easier and more robust for neighbours to interchange animals for conservation or rearing, or for allied animals to congregate on grasses to breed. According to Laval *et al.*18, migration may be more important than drift or mutation in explaining the loss in genetic variation between populations.

In this study, the heterozygosity values for the RS and WAD goats were 0.56 and 0.4889, respectively. The expected heterozygozity for RS and WAD goats was 0.4073 and 0.3735, respectively.

These differences or discrepancies in anticipated heterozygozity could be caused by breed variances in goats as well as population structure variables. An isolation-breaking effect may exist because of the union of two previously isolated populations (Bayrem *et al.*7). According to Toro and Maki- Tanila18, there are a few possible explanations for the observed heterozygosity in this study, such as overlapping generations, population mixing from different climatic locations, natural selection with heterozygosity in mind, subdivision with genetic drift, and other reasons. The number of functional alleles varied for the HSP 90 gene, but the number of identified alleles stayed constant. The effective number of alleles was 1.6756 in RS goats and 1.5857 in WAD goats. It was possible to ascertain that RS's effective allele count (1.5857) exceeded the WAD value. This demonstrated that the genetic diversity of the two breeds differed. Sharman's Index of Information (Lewontin19).

The inbreeding coefficient (Fis) for both goat breeds showed a low degree of heterozygosity, indicating that a high degree of homozygosity would have been the result. This could be the reason for the absence of predicted heterozygosityin the two goat breeds studied in this study.

The two breeds in this study's fixation index (Fst) value of 0.0016 and Fis value of -0.3576 show that inbreeding is occurring.

Fis's dimensions show how dissimilar to Hardy Wein berg it is. Genetic equilibrium: when Fis is positive, the locus is in heterozygozity deficiency (HWE); otherwise, it is 0.

According to Heridrick15, a negative Fis score indicated that the heterozygote level was higher than predicted by HWE. The study's findings show that inbreeding occurs frequently in small populations. However, population and selection against inbred individuals may account for these results (Frankham29). Hedrick28 suggested that higher Fis levels in sheep could be caused by smaller populations, more intense selection pressure, or inappropriate measurement methods. Present study analysis of the Fis value was inline with that of Halima *et al.*31, who stated that it ranged from -0.02 in hybrid to -0.017 in Cashmere.

The population found by Saitbekova *et al.*29 had a negative Fis value, indicating decreased inbreeding, and this study's Fis value mirrored their findings. Additionally, research demonstrated that the three breeds and the populations of Saanen, Toggenburg, and British Alpine have minimal genetic variation.

Little population variation was detected both within and between the experimental animals (breeds), as demonstrated by the low Fst value (Saitbekova *et al.*29). The individual's mean inbreeding coefficient compared to the subpopulation (Fis) was -0.3185, indicating an excess of heterozygosity in goat populations; more research is required to confirm this. According to Omotoso *et al.*27, there may have been population mixing because of the high degree of gene flow (Nm) and minimal genetic differentiation (0.0030). The study's findings demonstrated that groups with close geographic proximity had more genetic connections, most likely due to founder effects and interbreeding, especially close to borders. The closest genetic relationship between Sokoto Red and WAD goats was found in the results (0.9996). These breeds' adaptation to their respective regions may help to explain this: While WAD goats thrived in the country's humid southern region, Sokoto Red goats were more suited to the country's dry, semi-arid regions.

CONCLUSION

The results of the study demonstrated that groups with close geographic proximity had more genetic connections, most likely due to founder effects and interbreeding, especially close to borders. The closest genetic relationship between Sokoto Red and WAD goats was found in the results (0.9996). These breeds' adaptation to their respective regions may help to explain this: While WAD goats thrived in the country's humid southern region, Sokoto Red goats were more suited to the country's dry, semi-arid regions. If more animals from different goat breeds are collected, improved genetic research and marker-assisted selections in goat improvement programmes would be achievable.

Significance Statement

Gene flow, genetic distance, genotype frequencies, and alleles were investigated using two native goat breeds from Nigeria. According to the findings, Red Sokoto and WAD goats which are the native goat breeds investigated exhibited the highest genetic similarity and the lowest genetic distance between

the two populations. Within the confines of the ninety five goats examined. This implies that there is homology of alleles at the locus, low percentage of gene differentiation, and heterozygosis loss in the populations of native breeds of goats from Nigeria.

Author's contribution: The research, manuscript writing and editing was done solely by the corresponding author, Adetunmbi Tella.

Conflict of interest: There is no conflict of interest.

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