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

Abstract

Due to their hardiness in severe weather, capability to yield milk and meat, fast generation times, and ability to endure the poor quality diets that restricted grazing on marginal lands affords, goats are an invaluable asset to rural agricultural systems. Gene flow, genetic distance, genotype frequencies, and alleles were investigated using two native goat breeds from Nigeria. Investigational goat populations included Red Sokoto (50) and WAD (45). For each animal, an ethylene di amine tetra acetic 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), genotype and allele frequency, expected and observed heterozygousities (H_e and H_o , respectively) and genetic distances between populations were calculated. The degree of genetic diversity was great. For instance, for Red Sokoto and West African Dwarf goats, the allele frequency of gene A varied between 7.2 and 7.5, while the allele frequency of gene C varied between 2.4 and 2.8. H_o values were 56% for West African Dwarf and 48.89% for Red Sokoto. The dwarf from West Africa (37.35%) has the lowest estimates of H_e stood at the top of the Red Sokoto population (40.73%). The Mean F_{is} , F_{it} , F_{st} and N_m^* were -0.3576, -0.3554, 0.0016 and 152.78 respectively for the populations studied. The results showed that WAD and Red Sokoto had the lowest genetic distance (0.0004) and the highest genetic similarity (0.9996) between the populations. This suggests that there is homology of alleles at the locus, low percentage of gene differentiation, and heterozygosis loss in the populations of native goat breeds from Nigeria, within the limits of the 95 goats analyzed. If more animals from different goat breeds are collected, improved genetic research and marker-assisted selections in goat improvement programs would be achievable.

Key Words; Genetic Distance, Gene flow, Allele frequency, Red Sokoto, West African

Dwarf(WAD).

INTRODUCTION

Goats comprise the largest group of small ruminant livestock in Nigeria, numbering over 53.8 million and make up 6.2% of all goats globally. Because of their adaptability to harsh climates, trypanotolerance in some breeds, ability to supply meat and milk, short generation interval, and capacity to thrive on subpar diets provided by scarce grazing on marginal lands, goats are important for increasing livestock productivity and animal protein in rural agricultural systems. Despite these advantages, little attention has been paid to the genetic composition and potential for genetic improvement of Nigerian small ruminants, including goats. Molecular markers are helpful tools 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 programs for animal species. Maudet et al (2002) utilizing genotype data from DNA markers, molecular genetics has made significant strides recently in its analysis of genetic variation and population dynamics. Genetic investigations of livestock species have made use of polymorphic markers, including random amplified polymorphic DNA (RAPD), amplified fragment length polymorphic (AFLP), and microsatellite. However, codominant alleles were seen with high genomic abundance due to their many advantages, which include their excellent reproducibility 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. The quantitative evaluation of genetic diversity within and between populations is an essential tool for decision-making in genetic conservation and usage programs. According to Kunene et al. (2009), the most widely used method for measuring these genetic diversities is the use of phenotypic features. Goats and other indigenous livestock breeds in Nigeria have been phenotypically defined, but genetic information about them is still lacking. Adebambo et al. (2011) and Okpeku et al. (2011) used molecular markers to characterize the West African dwarf (WAD) and Red Sokoto (RS) goat breeds; however, nothing is

known about the genetic diversity of other local goat breeds that are currently found in Nigeria. Studies on the genetic variety and similarity across and within goat breeds are needed to provide important genetic information that is needed to develop effective management plans for the protection and enhancement of their genetic resources. Examining the genetic diversity of two Nigerian goat breed populations was the aim of this study.

MATERIALS AND METHODS

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

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 and Management:

This study focused on the Red Sokoto and West African dwarf (WAD) goats, which appeared to be in good health. In the agriculture ecological zone, these goats are typically produced as livestock under extensive or semi-intensive husbandry. 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

Blood samples were drawn from the animals' jugular veins using a technique known as venipuncture. A 23-gauge sterile needle and syringe were used to aseptically extract roughly 5 milliliters of blood from each animal into an EDTA container while ethylene-diamine-tetra-acetic acid, or EDTA, was utilized as an anticoagulant. The blood was 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 carried 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 sample was used for DNA extraction using Bioline International's Isolate II Genomic DNA extraction Kits, in accordance with the manufacturer's instructions. The final elution was diluted using 100µL of elution buffer. The filtered DNA sample was also kept for long-term preservation at -20°C, 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 percent agarose gel that included ethidium bromide. It was determined how much separated DNA using PG Instruments Ltd.'s Ultra-Violet Spectrometer.

Primer Sequence:

HSP90 Forward 5' AAATAAGTCGACATGCCTGAGCAAACCCAG 3'

Reverse 5'CTTCATCTGCAGTTAGTTAGTCTACTTCTTCCAT 3'

Polymerase Chain Reaction (PCR):

Using a pre-programmed thermocycler, the amplification process was carried out in 200 µl 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 sterilized 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 94°C, the reaction was run through 40 cycles: denaturation for 30 seconds at 94°C, annealing for 30 seconds at 62°C, and extension for 30 seconds at 72°C. The final extension was done at 72°C for two minutes.

Gel electrophoresis

Then, according to Joseph and David (2001), 10 μ L of the PCR amplicon was electrophoresed at 100 volts in a 0.75 percent agarose gel with ethidium bromide in 1x TBE buffer with DNA ladder.

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 μ L 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 in order to differentiate between the A and B alleles. Following restriction digestion, the restricted fragments were examined and electrophoresed in an ethidium bromide-stained 4% 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)

Data Analysis

The banding pattern on the gel was given numerical values, where 1 indicated a band's presence and 0 its absence. The percentage of each identified allele in a population, or allele frequency, was calculated using gene counting and GENEPOP version 4.2 (Rousset, 2008). GENEPOP, version 4.2 (Rousset, 2008) was used to calculate the average heterozygosity, or the H_e , in a population assumed to be in HWE in order 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. Yeh & colleagues (1999). The genetic link between groups was measured using their genetic

distance from one another. The Nei standard genetic distance (Nei 1972), whose ⁸ value is proportional to evolutionary time, was determined using GENEPOP, version 4.2 (Rousset, 2008).

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 band size 400, 300, and 200 bp, respectively, for HSP 90 genes. The results revealed the variation in electrophoresis of the gene under study on 95 experimental animals comprised of 45WAD and 50RS such that the gel for the HSP 90 gene's broad ladders on lanes 39 through 58 show that the animal's HSP 90 genes are active and better adapted to the geographical area. 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 adapted to the area. The genotype frequencies of AA and AC in WAD were respectively, 0.511 and 0.489, are displayed in Table 1. Goats from the Red Sokoto region scored 0.44 and 0.56 for genotype AA and AC respectively. For ⁵ 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 allele A was somewhat more common in WAD (0.7556) than it was in Red Sokoto goats (0.7200). Allele C was more common in Red Sokoto 0.28 than in WAD 0.2444.

Table 1: ⁹ 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 (N_a). ² The effective number of alleles (N_e) for Red Sokoto and WAD was 1.6756 and 1.5857, respectively. The Shannon's Information index was 0.5561 in WAD and 0.5930 in RS goats. The variation in the effective number of alleles suggests that the genetic diversity of the two breeds was distinct.

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

HSP- 90

Sample Size

N_a

N_e

I

WAD

45

2

1.5857

0.5561

RS

50

2

1.6756

0.5930

Na = Observed number of alleles

Ne = Effective number of alleles [Kimura and Crow (1964)]

I = Shannon's Information index [Lewontin (1972)]HSP90

In contrast to the expected heterozygosity of 0.37, the observed heterozygosity for WAD goats was 0.48. In Red Sokoto goats, the mean expected heterozygosity was 0.40, but the mean observed heterozygosity was 0.56. It shown that the goats with WAD and RS had higher levels of heterozygosity than predicted. This demonstrated an isolate breaking effect(the mixing of two previously isolated population) and 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 4 provides a summary of the F-statistics and gene flow for each locus, whereas

Table 5 shows the genetic identity and distance between WAD and RS goats. The 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 (Nm) was 0.0004, while the mean genetic identity (Nei's) was 0.9996. This suggested that there was a high level of genetic similarity and minimal genetic divergence between pairs of populations. Genetic variation in WAD and RS goats should ideally be relatively high between populations and modest within populations.

Table 4. Summary of F-Statistics and Gene Flow for All Loci

Locus

Sample Size

Fis

Fit

Fst

Nm*

HSP 90

95 - 0.3576

-0.3554

0.0016

152.7813

* N_m = Gene flow estimated from $F_{st} = 0.25(1 - F_{st})/F_{st}$.

Table 5 Genetic Identity and Genetic distance between 2 WAD and Red Sokoto (RS)

Goats

Population

WAD

RS

WAD

0.9996

RS

0.0004

Above diagonal: Nei's genetic identity Below diagonal: Genetic distance WAD = West

African Dwarf goat RS = Red Sokoto goat

DISCUSSION

⁶ In Red Sokoto goats, the corresponding genotype values were 0.44, 0.56, and 0.00, while 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 ¹⁰ 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, ⁶ 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 estimated genetic distance between ² WAD and Red Sokoto goats was 0.0004. This is less than the range of 0.16 found by Oladepo et al. (2020) for Yankassa and WAD sheep, and 0.003 to 0.097 reported by Ali (2003) for 14 Spanish sheep breed. Though ³ 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 indicates that within the confines of the small populations under investigation, there is 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 results of the study show that the populations of native ² goat breeds in Nigeria have low genetic distance, low gene percentages, and high genetic similarity. Overall, the findings are quite consistent and fall between the 0.74 and 0.90 genetic similarity ranges reported by Li et al. (2006) and Xiang et al. (2007). Additionally, it supported Sulaiman's (2012) results, which found that local goat breeds in Iraq had genetic closeness ¹ of 0.97 and 0.96%, respectively, based on RAPD markers. Abdel-Rahman et al. (2010) reported 90% (0.9) genetic similarity between the Egyptian sheep breeds (Barki and Ossimi), which

is also less than the findings of this study. In contrast, Ali (2003) discovered 95% genetic similarity between ¹ four Egyptian sheep breeds, which is quite comparable to the findings of this investigation. The high genetic resemblance of 99.96% observed in this study could be attributed to the groups' possible common ancestor. ² 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 little genetic distance (0.0004). It was fewer than the genetic distances between the populations that ranged from 0.081 to 0.227, as reported by EsmaeelKhanian et al. (2007) in their ¹ evaluation of the genetic variability of six Iranian goat breeds. Li et al. (2006) reported that genetic distances across populations of black goats ranged from 0.1051-0.2978, which was likewise greater than the ranges observed in this investigation. In this study, the genetic distance between Sokoto Red and WAD goats was 0.0004, which was less than the 0.268 reported by Okpeku et al. (2011) and the 0.39 between RS and WAD goats previously reported by Adebambo (2003) from a smaller sample gathered from different states across Nigeria. 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 genetic distance (0.0004) between WAD and SR goats indicates the closest descent between the breeds.

This result was much lower than that of Balcioglu et al. (2008), who employed the RAPD-PCR method to obtain a G_{ST} value of 0.5117 (51.17%) in eight Turkish breeds; Chenyambuga et al. (2004), who employed microsatellite DNA markers to obtain a G_{ST} value of 0.157 (10.57%) in native goats of Sub-Saharan Africa; and El Hantati et al. (2012), who used Tunisian ovine breeds to obtain a G_{ST} value of 0.1922 (19.22%). The coefficient of differentiation among the population genes (G_{ST}) they evaluated indicated a rather low level of difference among the black goat populations. Li and associates (2007) claim that population genetic divergence only occurs when groups are completely or partially cut off from one another. Using neutral molecular markers, Hartl and Clark (1997) found ¹ that breed relationships were more closely related to the geographical locations of the breeds

than to their morphological differences, indicating that genetic drift is the process responsible for genetic differentiation between populations. The high genetic diversity found within population groups may be explained by natural selection favoring heterozygosity or subdivision combined with genetic drift, according to Toro and Maki-Tanila's 2007 proposal. This could happen as a result of overlapping generations and population mixtures from various geographic locations. Agha et al. (2008) claim 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 program serve as evidence for this. The study's low genetic distance (0.0004) ¹ suggested that there was no gene drift, and it might be connected to the haphazard mating that occurs when these breeds travel throughout the country for commercial purposes while being closely supervised. This provided credence to the idea put forth by Laval et al. (2000) that migration dramatically lessens the degree of genetic variation between groups. The main effect of gene flow is the homogeneity of allele frequencies among populations. 152.7813 is the equivalent of the study's estimate of gene flow between the two populations from (N_m), accounting for all three loci. Using microsatellite markers, Geng et al. (2008) investigated the genetic diversity of six sheep populations in China. They discovered that the mean value of gene flow was 11.25 and ranged from 2.74 to 44.39. As a result, the range reported by Geng et al. (2008) and the values acquired by Missohou et al. (2006), who reported ranges of 0.46–6.21 in seven West African goat breeds with microsatellite markers, were significantly lesser than the values observed in this work. In a 2007 study of three Chinese cow populations, Mao et al. found that the ² 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 have becoming more homogeneous. According to Wright (1931), populations become homogenized when the gene flow value is greater than one. Consequently, a considerable amount of genetic material was exchanged and these goats migrated around, according to the study's calculations of gene flow. These may be connected ¹ to the fact that some of

these animals are indigenous to northern Nigeria, where the majority of households and communities manage their livestock through pastoralist nomadic living. 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 neighbors to exchange animals for upkeep or breeding, or for related animals to congregate on pasture to breed. According to Laval et al. (2000), migration may be more important than drift or mutation in explaining the loss in genetic differentiation between populations.

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

These differences or discrepancies in anticipated heterozygosity 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, 2018). The observed heterozygosity in this study could be explained by overlapping generations, population mixing from different climatic locations, natural selection with heterozygosity in mind, subdivision with genetic drift, or other factors, according to Toro and Maki- Tanila (2007).

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 ¹¹ the genetic diversity of the two breeds differed. Sharman's Index of Information (Lewontin, 1972).

The inbreeding coefficient (F_{is}) 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 heterozygosity in the two goat breeds studied in this study.

The two breeds in this study's fixation index (F_{st}) value of 0.0016 and F_{is} value of -0.3576

show that inbreeding is occurring. Saitbekova et al. (1999).

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

According to Heridrick (2000), 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 (Frankham, 1995). Hedric (2013) suggests that higher Fis levels in sheep could be brought on by smaller populations, more intense selection pressure, or inappropriate measurement methods. Our analysis of the Fis value was consistent with that of Halima et al. (2016), who reported that it ranged from -0.02 in hybrid to -0.017 in Cashmere.

The population found by Saitbekova et al. (1999) had a negative Fis value, indicating decreased inbreeding, and our study's Fis value mirrored their findings. Additionally, research demonstrated that the three breeds and the 7 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 (1999) . 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 Omoteso et al. (2019), there may have been population mixing because of the high degree of gene flow (Nm) and low level of genetic differentiation (0.0030) in his findings. The results of the study demonstrated that groups with close geographic proximity had more genetic connections, most likely due to 1 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 ¹ 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 programs would be achievable.

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