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Studies of genetic distance and gene flow in Red Sokoto and West African Dwarf goats using restriction fragment length polymorphic marker

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ABSTRACT. Due to their hardiness in severe weather, capability to yield milk and meat, fast generation times, capacity to withstand the substandard diets, goats constitute an indispensable part of rural agricultural systems. Gene flow, genetic distance, and alleles were investigated in Red Sokoto (50) and West African Dwarf (45). For each animal, approximately 5 milliliters of aseptic blood taken. Restriction fragment length polymorphism and electrophoresis were carried out after the DNA samples were isolated and purified. Gene flow, anticipated (He) and observed heterozygosity (Ho), degree of genetic diversity and Hardy-Weinberg equilibrium (HWE) were determined. The results revealed that for Red Sokoto and West African Dwarf goats, the allele frequency of gene A and C varied between (7.2 &7.5) and (2.8 & 2.4) respectively. Ho values were 56% for Red Sokoto and 48.89% for West African Dwarf. The West African dwarf (37.35%) had the lowest estimates of He compared to Red Sokoto population (40.73%).The Mean Fis, Fit, Fst and Nm* were -0.3576, -0.3554, 0.0016 and 152.78 respectively for the populations studied. According to the findings, the goat populations exhibited the highest genetic similarity (0.9996) and the lowest genetic distance (0.0004), It was deduced that there is homology of alleles at the locus, low percentage of gene differentiation, and heterozygosis loss in the populations of native goats studied.

Keywords: Genetic distance;gene flow;allele frequency; Red Sokoto;West African Dwarf goat.

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Introduction

Goats make up the majority of small ruminant livestock in Nigeria, numbering approximately 53.8 million, and account for 6.2% of all goats globally. Because of their adaptability to harsh climates, trypano tolerance 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 is known about the genetic composition and future genetic development of Nigerian goats and other small ruminants. Molecular markers are helpful instruments for identifying the desired loci underlying the characteristics necessary for successful reproduction. Developing efficient breeding strategies for animal species requires a firm understanding of genetic diversity. The analysis of genetic diversity and population dynamics in molecular genetics has advanced significantly in recent years, thanks to the use of genotype data from DNA markers, as demonstrated by Aboul-Naga et al. (2022). Genetic studies of livestock animals have made use of polymorphic markers, such as amplified fragment length polymorphism (AFLP), microsatellite systems and random amplified polymorphic DNA (RAPD). Codominant alleles, high genomic abundance, which include their random distribution, typically mild polymorphism, remarkable repeatability and stability are their benefits when compared to other markers. Restrictions fragment length polymorphisms, or RFLPs, were used in this investigation for these purposes. The quantitative evaluation of genetic diversity within and between populations is an essential tool for decision-making in genetic conservation and usage projects. Encina et al. (2021) state that using phenotypic features is the most often used method for determining these genetic diversities. Goats and other local animal breeds in Nigeria have had their phenotypic characteristics studied, but genetic information about them is still lacking. Adigun et al. (2021) and Akintunde et al. (2024) used molecular markers to define the West African dwarf (WAD) and Red Sokoto (RS) goat breeds; however, it is

uncertain how diverse other indigenous goat breeds that are now present in Nigeria are genetically related. Studies on the genetic variety and similarity across and within breeds are the only ways to gather important genetic data needed to develop effective management strategies for the preservation and improvement of goat breeds' genetic resources. Analyzing the genetic diversity of two breed of Nigerian goats was the aim of this study.

Materials and methods

Experimental animal

A total of 95 goats comprising of 50 Red Sokoto and 45 WAD goats were used for this research.

Experimental site

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

Management of experimental animal

This study focused on the Red Sokoto and West African dwarf (WAD) goats, which seemed to be in better state. These goats are raised as livestock under substantial or semi-intensive husbandry in the agriculture ecological zone. The animals are fed with hays, discarded agricultural products, household scraps, and cassava peels. The residents of these study locations have embraced this goat farming technique in an effort to lessen disputes between humans and goats over food.

Blood samples collection

Blood samples were taken from the animals' jugular veins. Using an ethylene-diamine-tetra-acetic acid (EDTA) 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 laboratory analysis was conducted at the Bio-safety Research Laboratory of Federal University of Technology ,Akure, Ondo State, Nigeria.

Laboratory analysis

DNA isolation

A total of 200µL of the blood sample was 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

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.

Primer Sequence

HSP90 Forward 5’ AAATAAGTCGACATGCCTGAGCAAACCCAG 3’
Reverse 5’CTTCATCTGCAGTTAGTTAGTCTACTTCTTCCAT 3’(Andrea et al. 1991)

Polymerase chain reaction (PCR)

Using a pre-programmed thermocycler, the 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 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 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

10 µL of the PCR amplicon was electrophoresed at 100 volts in a 0.75 percent 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.

Restriction Fragment Length Polymorphism and agarose gel electrophoresis

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 in order to distinguish between the A and B alleles. Following constraint 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)

Result 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 (Rous-set, 2008)was utilized to calculate the average heterozygousity, or the He, 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. The genetic link between groups was measured using their genetic distance from one another. The Nei standard genetic distance (D ST) (Arain, 2022), whose value is proportional to evolutionary time, was determined using GENEPOP, version 4.2 (El-tarras et al. 2015).

Results

Polymorphisms of the HSP 90 gene were found by genotyping two breeds of Nigerian goats using the PCR-RFLP technique. A unique amplified fragment of the estimated band size of 400, 300, and 200 bp, respectively, for HSP 90 genes was obtained from the PCR analysis of all goat DNA samples (i.e., 45 West African Dwarf& 50 Red Sokoto goats), using particular primers developed from goat heat adaptation genes. The animal's HSP 90 genes are active (upregulated) and better suited to conditions including heat stress, as seen by the broad ladders on lanes 39 through 58 of the electrophoresis gel for HSP 90 gene. The thin and normal ladders on Ladders 1 through 38 and 58 through 95 of the electrophoresis gel for HSP 90 gene indicate that these genes are down regulated and animals possessed it are resistant or tolerate heat stress. Table 1 presents genotype frequencies of AA and AC in WAD at 0.511 and 0.489, respectively. Red Sokoto goats had scores of 0.44 and 0.56, respectively. The allele frequency of a gene per locus ranges for West African dwarf goats and Red Sokoto from 2.4 to 2.8 for Allele C, and from 7.2 to 7.5 for Allele A. Compared to Red Sokoto goats (0.72), the West African Dwarf population (0.7556) had a somewhat higher frequency of the A allele. Compared to West African Dwarf 0.2444, Red Sokoto 0.28 had a higher frequency of allele C.

Table 1. Genotype and Allele frequencies of genes in 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.

The genetic variation data for West African Dwarf and Red Sokoto goats are presented in Table 2. It demonstrated that there was an equal number of alleles (Na) in WAD and RS. Red Sokoto had an effective

number of alleles (Ne) of 1.6756, while West African Dwarf had a Ne of 1.5857. For Red Sokoto goats, the Shannon's Information index was 0.5930, whereas for West African Dwarf goats, it was 0.5561. The two breeds' genetic diversity was different, as seen by the variation in the effective number of alleles. For WAD goats, the observed heterozygosity was 0.48, compared to the anticipated heterozygosity of 0.37. In Red Sokoto goats, the mean anticipated heterozygosity was 0.40, as opposed to the mean actual heterozygosity of 0.56. It revealed that the observed heterozygosity levels of the goats with WAD and RS were greater than expected. This proved that the trans HSP locus showed genetic variation in both WAD and RS goats. This suggests that if the selection procedure is well designed and executed, the selected group will benefit from enhanced performance genetically.

Table 2. Genetic Variation Statistic and Heterozygosity for all Loci in WAD and RS goats.

Marker	Parameters	WAD (n=45)	RS (n=50)
HSP-90	Ne	1.5857	1.6756
	I	0.5561	0.5930
	Na	2	2
	Ho	0.4889	0.5600
	He	0.3735	0.4073
	Ne1	0.3694	0.4032

WAD = West African Dwarf goat; RS = Red Sokoto goat; Ne= Effective number of alleles; l = Shannon information index; Na= Observed number of alleles; Ho= Observed heterozygosity; He= Expected heterozygosity; Ne1= Expected heterozygosity.

Table 3 provides F-statistics and gene flow for each site, whereas Table 4 shows the genetic identity and distance between WAD and RS goats. Fis, Fit, Fst, and Nm values were, respectively, -0.3576, -0.3554, 0.0016, and 152.7813. The mean genetic identity (Nei's) and genetic distance (Nm) between Red Sokoto and WAD goats were 0.9996 and 0.0004, respectively. This implied that there was little genetic divergence and a high degree of genetic similarity among the groups. Ideal genetic variation in WAD and RS goats should be moderate within populations and somewhat high between populations.

Table 3. 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

* Nm = Gene flow estimated from Fst = 0.25(1 - Fst)/Fst.

Table 4. Genetic Identity and Genetic distance between WAD and 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

The genotype distributions for AA, AC, and CC in WAD were 0.511, 0.489, and 0.00, respectively; the comparable values in Red Sokoto goats were 0.44, 0.56, and 0.00. (Table 1). The findings of the Chi square analysis indicate that there is no substantial divergence ($p < 0.05$) between the genotype frequencies observed and predicted. This suggests that the flocks under consideration do not adhere to the Hardy-Weinberg equilibrium for the gene locus. On the other hand, allele C had a higher frequency (0.28) in Red Sokoto and a lower frequency (0.2444) in WAD goats. In those Red Sokoto goats, the A allele frequency was 0.72, but in WAD it was 0.75. Red Sokoto goats and WAD goats have a genetic gap of 0.0004 between them. This is smaller than the range of 0.003 to 0.097 recorded by Al-Barzinji and Hamad (2017) for 14 Spanish sheep breed, and 0.16 observed by Oladepo et al. (2020) for Yankassa and WAD sheep. Although Spanish sheep belong to the same breed, WAD and Yankassa sheep are fundamentally of separate breeds within the same species. This suggests that there is substantial allele commonality at the gene locus within the boundaries of the tiny populations under study. This implies that the populations that were the subject of the research were composites of smaller groups. Nevertheless, the highly substantial ($p < 0.001$) variations seen between Yankassa and WAD sheep imply that a variety of subpopulations with various gene frequencies were present in the samples.This explained why there was a brief rise in homozygotes and a drop in heterozygotes in both groups.

The results of the study show that the native goat breed populations of Nigeria have low gene percentages, low genetic distance, and great genetic closeness. Overall, the results are quite consistent and fall into the ranges of genetic similarity reported by Liu et al. (2020) and Zou et al. (2020), which are 0.74 and 0.90. It also corroborated Molotsi's et al. (2020) findings, according to which RAPD markers indicated a genetic similarity of 0.97 and 0.96%, respectively, between local goat breeds in Iraq. Less than the results of the investigation, Edelman and Mallet (2021) found 90% (0.9) genetic similarity between the Egyptian sheep breeds Barki and Ossimi. On the other hand, Aboul-Nega et al. (2022) found that four Egyptian sheep breeds had 95% genetic similarity, which is quite close to the results of this study. A common predecessor between the groups may have contributed to the study's remarkable 99.96% genetic similarity. Conversely, it could have something to do with the uncontrolled inbreeding and mating practices that characterize Nigeria's traditional livestock farming. This analysis revealed a very little genetic distance (0.0004).Six Iranian goat breeds were examined for genetic diversity by Kondratyeva et al. (2019), who discovered that genetic distances across populations varied from 0.081 to 0.227. This is higher than the genetic separations between the groups.Genetic distances across black goat populations were found to range from 0.1051 to 0.2978 by Liu et al. (2022), which was also bigger than the value recorded in this study. The genetic distance between Sokoto Red and WAD goats in this study was 0.0004, which was lower than the 0.39 between RS and WAD goats previously reported by Adigun et al. (2021) from a smaller sample gathered from several Nigerian states and the 0.268 reported by Akintunde et al. (2024). This demonstrated that in southern Nigeria, there was more cross-breeding between goats and humans and less inbreeding within goat groups, leading to a higher density of raised goats in the region. The genetic distance (0.0004) between WAD and SR goats indicates the closest ancestry between the breeds.With a GST score of 0.5117 (51.17%), this result was significantly lower than that of Onzimo et al. (2018), who used the RAPD-PCR approach to reach the goal of Eight Turkish breeds.Microsatellite DNA markers were utilized by Rotimi et al. (2017) to determine the Gst value of 0.157 (15.7%) in native goats residing in Sub-Saharan Africa. Marshall et al. (2019) also obtained a Gst value of 0.1922 (19.22%) using Tunisian ovine breeds. The coefficient of differentiation among the population genes (Gst) that they evaluated, which was 0.2766 (27.66%), indicating that there was not much variety among the black goat populations. According to the authors of (Şen et al. 2021), population genetic divergence only happens when groups are isolated from one another, either totally or partially. Taylor and Larson (2019) used neutral molecular markers to find that breed associations were more closely connected with the geographical locations of the breeds than with their physical distinctions. This implies that genetic drift is the primary driver of genetic divergence across populations. The significant genetic variety observed within population groupings may be explained by natural selection favoring heterozygosity or subdivision along with genetic drift, according to Toro and Maki-Tanila's 2007 proposal. This might be brought on by overlapping generations and demographic blends from various geographic locations. According to Omotoso et al. (2019), these characteristics have a more significant impact on the effective population's size.This is demonstrated by the current situation of Nigeria's inadequate breeding program and inadequate facilities for raising cattle.The study's low percentage of gene differentiation (0.0004) suggested that there was no gene drift, and it could have something to do with the haphazard mating that occurs when these breeds travel the country under close observation for business purposes. This supported the theory of Hoffmann et al. (2021) that migration significantly reduces the amount of genetic diversity across populations. The main outcome of gene flow is the consistency of allele frequencies among populations. After adjusting for all three loci, the estimated amount of gene flow between the two populations from Gst (Nm) is 152.7813. This was higher than the range acquired by Eydivandi et al. (2021), who reported ranges of 0.46–6.21 in seven West African goat breeds using microsatellite markers and almost similar to the value obtained by Tella (2023) who used microsatellite markers to investigate the genetic diversity of two native goats in Nigeria. Guo et al. (2019) discovered that there was 1.149 and 0.509 times more gene flow between the two groups, respectively, in their analysis of three Chinese cow populations. The study's greater gene flow value indicates that populations may be growing more homogenous. Wright (1931) asserted that when the gene flow value is greater than one, populations become homogenized. Based on the study's gene flow estimates, a significant quantity of genetic material was transferred and these goats moved about. This may be due to the fact that some of these animals are indigenous to northern Nigeria, where most households and communities maintain their cattle primarily through pastoralist nomadic lifestyle. They could also have something to do with the comprehensive management systems that allow animals to wander freely and subsist on their own in the great majority of rural South West homes and communities. This facilitates and strengthens the ability of neighbors to trade animals for breeding or conservation, or for related species

to gather on grasslands to breed. Hoffmann et al. (2021) suggest that migration may have a more significant role in explaining the decline in genetic variety among populations than either drift or mutation.

The RS and WAD goats in this investigation had observed heterozygosity scores of 0.56 and 0.4889, respectively. For goats with RS and WAD, the expected heterozygozity was 0.4073 and 0.3735, respectively. Both population structure factors and breed variants in goats might be the origin of these variations or disparities in expected heterozygozity. The merging of two previously isolated populations may have the effect of breaking isolation (Bayrem, 2018). A number of reasons, including overlapping generations, population mixing from various climatic locations, natural selection with heterozygosity in mind, subdivision with genetic drift, and other causes, could account for the observed heterozygosity in this study, according to Dapas and Dunaif (2022).For the HSP 90 gene, the number of discovered alleles remained consistent, while the number of functional alleles fluctuated. In RS goats and WAD goats, the effective number of alleles was 1.6756 and 1.5857, respectively. It was feasible to determine that RS had more effective alleles (1.6756) than WAD. This proved that the two breeds' genetic diversity was different.

Both goat breeds' inbreeding coefficients (Fis) indicated low levels of heterozygosity, suggesting that high levels of homozygosity would have been the outcome. This might be the cause of the two goat breeds examined in this study's lack of projected heterozygozity. The fixation index (Fst) value of 0.0016 and Fis value of -0.3576 for the two breeds in this study indicate that inbreeding is taking place. Fis dimensions confirm how dissimilar to Hardy Weinberg it is. Genetic equilibrium, when Fis is positive, the locus is in heterozygozity deficiency (HWE); otherwise, it is 0.

A negative Fis score, according to Belay et al. (2024), suggested that the heterozygote level was larger than what HWE had projected. The results of the study demonstrate that inbreeding happens often in small populations. Nonetheless, these outcomes might be explained by population and selection against inbred individuals. Higher Fis levels in sheep may result from smaller populations, stronger selection pressure, or improper measuring techniques, according to Belay et al. (2024).The Fis value in our investigation aligned with the findings of Mukhina et al. (2022), who reported that it varied between -0.02 in hybrid and -0.017 in Cashmere.

The low Fst value indicates that there was little diversity in the population within and between the experimental animals (breeds) and this was in line with this study,s findings.. There appears to be an excess of heterozygosity in goat populations, as indicated by the individual's inbreeding coefficient of -0.3576 when compared to the subpopulation (Fis); more research is needed to validate this. Omoteso et al. (2019) suggest that the low genetic differentiation and high degree of gene flow (Nm) might have led to population mixing. The results of the study showed that groups who were closer together geographically had stronger genetic ties, most likely as a result of founder effects and interbreeding, particularly near borders. The results showed that the Sokoto Red and WAD goats have the closest genetic kinship (0.9996). This may be explained by the adaptability of these breeds to their respective regions, Sokoto Red goats fared better in the country's dry, semi-arid areas than WAD goats, which flourished in the humid southern region.

Conclusion

The study's findings showed that groups with close geographic proximity shared a greater number of genetic linkages, perhaps as a result of interbreeding and founder effects, particularly near borders. The results showed that the genetic link between Red Sokoto and West African Dwarf goats was the closest (0.9996). This may be explained by these breeds' adaption to their particular regions: The country's semi-arid, arid areas were more adapted for Red Sokoto goats than for West African Dwarf goats, which flourished in the country's humid south. Better genetic research and marker-assisted choices in goat improvement initiatives might be accomplished with increased collection of animals from various goat breeds.

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