Genetics & Biodiversity Journal

Journal homepage: https://journals.univ tlemcen.dz/GABJ/index.php/GABJ

ISSN: 2588-185X

Original Research Paper

Genetic diversity and phylogenetic structure of Balami and West African Dwarf sheep populations of Nigeria using mitochondrial DNA D-loop sequences

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Article history: Received: September 3rd 2022; Revised: November 25th 2022; Accepted: December 14th 2022

Abstract

Information on the geographic distribution of livestock species is an important tool for their utilization, genetic improvement, and conservation. It is therefore necessary to explore the genetic relationships between livestock breeds and their diversities. This study assessed the genetic diversity and phylogenetic structure of two Nigerian indigenous sheep populations that are distinctly different in geographical distribution and phenotypic attributes. Sixty hair samples were collected from Balami and West African Dwarf (WAD) sheep. Twenty- three haplotypes were observed with no haplotype shared between Balami and WAD sheep which is an indication of clear separation between the two populations. Haplotype diversity was higher in Balami than in WAD but the nucleotide diversity was higher in WAD than in Balami. The number of polymorphic sites varied between the populations with WAD having higher polymorphic sites (48) than Balami (46). Analysis of the mitochondrial lineage showed that these breeds originated from a common source but with a divergence between the two sheep populations considering their particular traits. In conclusion, overall haplotype and nucleotide diversities for these sheep populations were high, most of the variations were found within the population than between populations. These sheep breeds were genetically different as a result of their geographical distributions. The study contributes to the knowledge of the existing genetic status of Nigerian indigenous sheep populations and the wide genetic variation between these two breeds that could be exploited in genetic improvement programs.

Keywords: Breeds, conservation, genetic diversity, geographic distribution, sheep.

الملخص

تعد المعلومات المتعلقة بالتوزيع الجغرافي لأنواع الماشية أداة مهمة لاستخدامها وتحسينها الجيني وحفظها. لذلك من الضروري استكشاف العلاقات الوراثية بين سلالات الماشية وتنوعها. قيمت هذه الدراسة التنوع الجيني والبنية الوراثية لاثنين من مجموعات الأغنام النيجيرية الأصلية التي تختلف بشكل واضح في التوزيع الجغرافي والسمات المظهرية. تم جمع ستين عينة شعر من Balami وWest African Dwarf (WAD) sheep . ولوحظت ثلاثة وعشرون نمطًا فرعيًا مع عدم وجود نمط haplotype مشترك بين Balami وأغنام WADوهو مؤشر على الفصل الواضح بين السكان. كان تنوع Haplotype أعلى في Balami منه في WAD ولكن تنوع النيوكليوتيد كان أعلى في WAD منه في Balami. اختلف عدد المواقع متعددة الأشكال بين السكان مع WAD التي تحتوي على مواقع متعددة الأشكال أعلى (48) من Balami (46). أظهر تحليل سلالة الميتوكوندريا أن هذه السلالات نشأت من مصدر مشترك ولكن مع اختلاف بين مجموعتي الأغنام بالنظر إلى سماتها الخاصة. في الختام، كانت تنوع haplotype وnucleotide بشكل عام لهذه الأغنام عالية، تم العثور على معظم الاختلافات بين السكان من السكان. كانت سلالات الأغنام مختلفة وراثيا نتيجة لتوزيعها الجغرافي. تساهم الدراسة في معرفة الوضع الجيني الحالي لسكان الأغنام النيجيريين الأصليين ويمكن استغلال التباين الجيني الواسع بين هذين السلالتين في برامج التحسين الجيني

الكلمات الرئيسية: السلالات، الحفظ، التنوع الجيني، التوزيع الجغرافي، الأغنام.

Introduction

Domestic animal diversity is essential as a strategy for food security due to the unpredictable future population growth, climate change, and more virulent pathogens of disease (Crepaldi *et al*., 2001; Yunusa *et al*., 2013). To understand and develop endogenous economic traits of breeds, optimize breeding strategies, and regulate germplasm conservation, knowledge of genetic

variation within and among different animal breeds is very important (Yeo *et al*., 2000). Farm animal genetic diversity is required to meet current production needs in various environments to allow sustained genetic improvement and facilitate rapid adaptation to changing breeding objectives (Glowatzki-Mullis *et al*., 2008; Kevorkian *et al*., 2010).

Globally, sheep have the highest number of recorded breeds contributing 25% to total mammalian breeds adapted to a broad range of environments (Gizaw, 2008). Sheep have essential socio-economic and socio-cultural roles in local communities, particularly in developing countries (Popoola and Oseni, 2018). Production of types of sheep breeds depends on environmental conditions, the desired management intensity of sheep farmers, and personal preference (Fasae *et al*., 2012). Nigerian indigenous sheep breeds are still genetically unimproved and the pressure of modern genetic improvement has increased the need to understand their natural genetic variation better and formulate policies for germplasm conservation (Agaviezor *et al*., 2013). Thus, the need to understand the diversity of these sheep breeds considering their economic importance in regions where they are found to develop strategies for improvement, sustainable use, and biodiversity conservation.

In Nigeria, there are four well-defined sheep breeds; these are Yankasa, West African Dwarf (WAD), Balami, and Uda. These sheep differ considerably in attributes such as body size, coat color, body weight, body length, and other features. According to Adu and Ngere (1979), WAD sheep are the most numerous in the humid Southwestern part of Nigeria, a large proportion of which are under extensive management systems and are primarily reared for their meat. They can utilize fodder resources high in crude fiber and possess high productive performance and small body size, making it feasible to adapt to extreme environmental conditions (Oyedipe *et al*., 1986). Balami is a white hair-type breed that is widely distributed across the North-Eastern territory of Nigeria, Cameroun, and Chad Republic (Ferguson, 1964). Balami is the largest-bodied indigenous sheep in Nigeria. It is confined to the semi-arid North of Nigeria.

Mitochondrial DNA (mtDNA) has reportedly been used to gather the history and domestication of livestock species (Pariset *et al*., 2011). The mtDNA is the DNA located in the mitochondria; it is inherited from the mother or maternally inherited, enabling the possibility to trace maternal lineage far back in time (Meadows *et al*., 2007). Analyses of the control region (the displacement-loop) of mitochondrial DNA (mtDNA) and nuclear DNA are the most useful examinations and are informative genomic elements for explicating the origin, diversity, genetic relationship, and diversification of livestock (Dorji *et al*., 2010). Mitochondrial DNA (mtDNA) diversity is a useful molecular tool in establishing phylogenetic relationships among breeds and at the species level (Zhao *et al*., 2011). This study aimed to evaluate the genetic diversity of the Balami and WAD sheep populations of Nigeria using the D-loop sequence of the ovine.

Materials and Methods

Study area and study population

The sheep populations (WAD and Balami) were randomly sampled in Ibadan, the capital city of Oyo State, Nigeria. Ibadan is the largest city in Nigeria with several cattle ranches, a dairy farm, and a commercial abattoir in the city; it is a strategic location in the country where an appreciable number of livestock species such as cattle, pig, chicken, sheep, and goat can be found.

Sixty animals were sampled from each breed for reliable estimation of allele frequencies and allowed for possible losses, mistyping missing values, and genetic subdivisions within breeds (FAO, 2011). One hundred and twenty hair samples were randomly collected from individual animals depending between 2015 and 2016 on the owners' pedigree information. The hair was pulled from the animals to ensure that follicles were obtained along with the hair, which was stored in hair sample cards and taken to the laboratory (International Livestock Research Institute (ILRI), Nairobi, Kenya) for genomic DNA extraction.

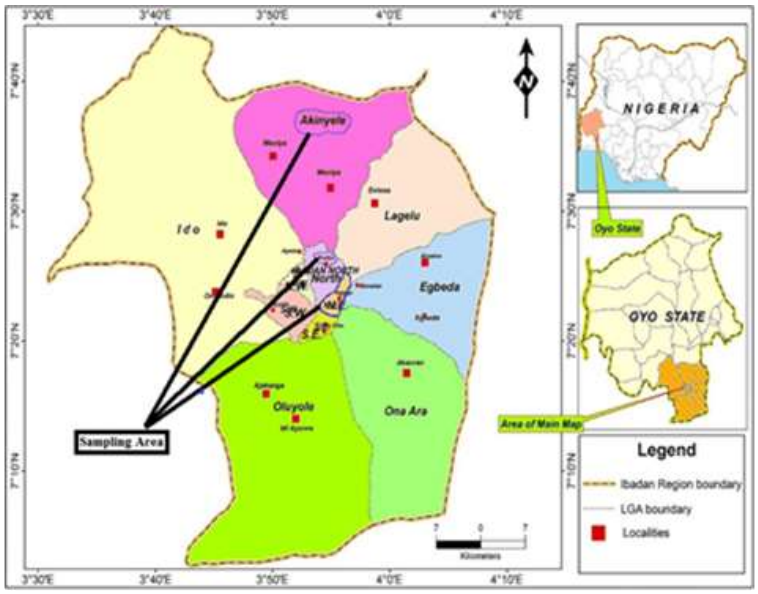

Figure. 1. Map of Ibadan showing locations where the animals were sampled

DNA Extraction, Polymerase Chain Reaction Amplification and Sequencing

Total DNA was extracted from hair samples (at least 10 strands) which were cut off from the base to include follicles (0.5 – 1cm piece) using Qiagen DNeasy® Blood and Tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The DNA was eluted from silica columns in 25 µL of DNase-free water. The DNA integrity was checked on a 0.8% (w/v) agarose gel electrophoresis at 4 V/cm2. Quantification of DNA yield and quality assessment was determined on a NanoDrop™ 2000c Spectrophotometer (Thermo Scientific, Wilmington, DE, USA), considering the 260/280 ratio close to 2.0 as highly pure DNA.

Figure 2. West African Dwarf (WAD) sheep

Figure 3. Balami sheep

Polymerase chain reaction amplification and sequencing

The extracted DNA was amplified via polymerase chain reaction (PCR) in a Mastercycler Nexus GS using published primers (Hiendleder *et al*., 1998): tRNA-proline (5-CAGTGCCTT GCTTTGGTTAAGC-3) and tRNA-phenylalanine (5-CACCATCAACCC CAAAGCTGAAG-3). A total volume of 20µl amplification reactions contained 10 -50 ng template DNA, 10.9 µL nuclease-free water, 4µl 5X Phusion HF Buffer, 0.4 µL dNTPs (10mM), 0.5µl Phusion polymerase and 0.6µl dimethyl sulfoxide (DMSO) in PCR strip of 8 thin-walled 0.2ml tubes (TreffLab , Teff AG, Degersheim, Switzerland). The PCR cycling profile involved an initial denaturation at 98°C for the 30s, 35 cycles of amplification at 98°C for 30s, annealing at 62°C for 30s, extension at 72°C for 45s, final extension at 72°C for 5 min and held at 20°C until analysis. Five microliters of the PCR products (pre-stained with 3µl of loading dye) were separated in a 1.5 % agarose gel pre-stained with 0.5µg/ml

gel red. Electrophoresis was carried out for 90 minutes at 50 V using Mupid®- One submarine Electrophoresis System. The resulting amplified bands were visualized and photographed using GelDoc–It®2 310 Imager (Ultra-violent products (UVP) Bioimaging System Ltd, Cambridge, UK). Amplified PCR products were sequenced with the Applied Biosystems Automated 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA) using the Big Dye Terminator chemistry and AmpliTaq-FS DNA polymerase. Sequencing reactions used a tRNA-proline primer (5-CAGTGCCTTGCTTTGGTTAAGC-3).

Statistical analyses

Haplogroup status analyses of the sheep

All chromatogsheep were generated and visualized with BioEdit Software (http://www.mybiosoftware.com/alignment/1013). Published mtDNA control region sequences from GenBank of sheep from 17 sheep populations were included in the analysis. Sequences were aligned by the ClustalW W algorithm (Thompson *et al*., 1994), a component of the program MEGA 6 (Tamura et al., 2013), and saved as a MEGA alignment file. To facilitate the recognition of the haplogroup status of each individual, 17 sheep mtDNA control region reference sequences belonging to five known haplogroups/lineages recommended by Teasdale *et al*., (2015) were also included in the analysis.

Genetic diversity analyses

The level of genetic diversity was represented as haplotype diversity and its standard deviation; nucleotide diversity and its standard deviation; the average of nucleotide differences; the average number of nucleotide substitutes per site between populations; polymorphic sites and haplotype number for each sheep population were estimated using the DnaSP package v5 10.01 (Librados and Rozas, 2009).

Construction of phylogenetic tree

The phylogenetic tree was constructed for all the haplotypes using the Neighbour-Joining (NJ) algorithm (reference) as implemented in MEGA6 to visualize the genetic relationship between individuals and populations. To evaluate the level of confidence that can be associated with each bifurcation, the NJ tree was reconstructed following 1000 bootstrap replications. To complement the NJ tree while obtaining further insights, and in greater detail into the genetic relationships between the haplotypes, the median-joining (MJ) network of haplotypes was constructed using the Network v4.6 software. A sequence from the GenBank (Accession No.: AF039578) was used to align the whole D-loop region. Molecular variance (AMOVA) was performed using Arlequin v3.5 (Excoffier and Lischer, 2010) to partition genetic diversity and variation amongst populations and groups of populations, raggedness index ‘r’ (Harpending 1994) and Fu’s Fs (Fu, 1997) tests were also conducted to determine whether patterns of mitochondria sequence variation obtained were consistent with predictions of the neutral model.

Results

Genetic diversity indices of mtDNA D-loop of Balami and West African Dwarf sheep are presented in Table 1. Sixty eight (68) polymorphic sites were observed, with WAD having a higher number of polymorphic sites (48) than Balami (46). A total number of 23 haplotypes were observed between the two populations. Mismatched means and variances were obtained with Balami having higher values of 2.476 and 5.540, respectively. There was a high haplotype diversity index between the two populations. Balami had a higher haplotype diversity index (0.96±0.04) than WAD (0.91 ± 0.06). Similarly, the nucleotide diversity index between the two populations differed with WAD recording a higher value of nucleotide diversity (0.01115 ± 0.0023) than Balami (0.00788 ± 0.00015). The average nucleotide difference K between the two populations was 12.574 and 8.895 for WAD and Balami respectively.

Results of the population demographic and neutrality test in Balami and West African Dwarf sheep are shown in Table 2. Fu’s Fs tests were performed to infer the demographic history of the sheep populations. Results showed that the sum of square deviation (SSD) was statistically significant.

Raggedness index (r) values for the two populations were positive and significant for WAD but not significant for Balami. The mismatch distributions for estimation of population size change for WAD and Balami using pairwise comparison shows a sharp variation for both observed and expected pair-wise differences, the fluctuation was higher in Balami (Figure 3 A, B, and C).

Table 1: Genetic diversity of mtDNA D-loop of Balami and West African Dwarf ram

Indices	**Balami**	**WAD**	**Overall**
Number of polymorphic sites (S)	46	48	68
Number of haplotypes (H)	12	11	23
Mismatched observed mean	2.476	1.002	
Mismatched observed variance	5.540	2.430	
Hd ± SD	0.962 ± 0.040	0.912 ± 0.056	0.968 ± 0.019
π ± SD	0.00788 ± 0.00015	0.01115 ± 0.0023	0.01040 ± 0.0022
Average Nucleotide difference (K)	8.895	12.574	11.736
		Dxy = 0.01105	

Hd – Haplotype diversity, SD – Standard deviation, π – Nucleotide diversity, K – Average of nucleotide differences, Dxy – Average number of nucleotide substitute per site between populations.

Table 2. Population demographic and neutrality test in Balami and West African Dwarf sheep

Tests	**Balami**	**WAD**	**Overall**
Sum of square deviation (SSD)	0.200*	0.100*	0.142*
Raggedness index r	0.395 ^{ns}	0.197*	0.279*
Fu's Fs	-2.391**	0.500**	-5.315**

Table 3 Distribution of haplotypes between Balami and West African Dwarf sheep

Haplotype	**Balami**	**WAD**	**Total**
1	3	0	3
2	2	0	2
3	1	0	1
4	1	0	1
5	1	0	1
6	1	0	1
7	1	0	1
8	1	0	1
9	1	0	1
10	0	1	1
11	0	1	1
12	0	5	5
13	0	1	1
14	0	2	2
15	1	0	1
16	1	0	1
17	1	0	1
18	1	0	1
19	1	0	1
20	0	1	1
21	0	1	1
22	0	2	2
23	0	1	1

** - significant at p<0.05, ** - significant at p<0.01, ns – not significant*

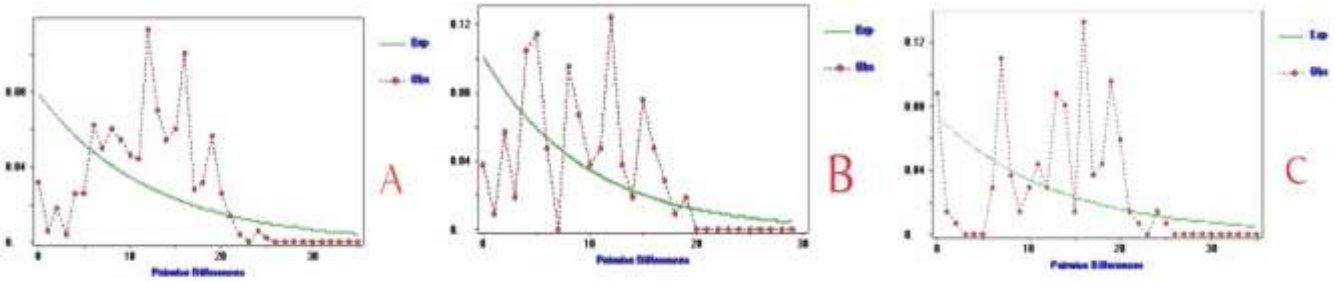

Figure 4. A- Population size changes (Mismatch distribution) for Balami and WAD populations; B- Population size changes (Mismatch distribution) for Balami; C-Population size changes (Mismatch distribution) for WAD

A total number of 23 haplotypes were observed between the two populations, with 12 haplotypes observed for Balami and 11 haplotypes observed for WAD. The two populations did not share any Haplotype (Table 3). However, Haplotype 12 found in WAD had the highest distribution (21.7%), followed by Haplotype 1 (13%) found in Balami.

Results of parameters of spatial expansion of Balami and WAD sheep assuming constant deme size are presented in Table 4. Values obtained for Tau, Theta, and M varied with Balami having higher values of Tau (5.269), Theta (2.697), and M (1.390) compared to values of Tau (2.961), Theta (1.360), and M (0.695) obtained for WAD

Table 4. Parameters of the spatial expansion of Balami and WAD sheep assuming constant deme size

Parameter	Balami	WAD
Tau	5.269	2.961
Theta	2.697	1.360
M	1.390	0.695

The analysis of molecular variation (AMOVA) for the two populations indicated that 79% of the total genetic variation presented in Balami and WAD was explained by genetic differences between individuals within populations. Only 20.9% of the variation was found among populations compared to 79% of the variation obtained within populations. The fixation index obtained was 0.20897 (Table 5).

Table 5. Analysis of molecular variance (AMOVA) based on the sheep populations

Source of variation	df	Sum of squares	Variance components	% variation
Among population	1	3.010	0.15264	20.90
Within population	63	17.333	0.57778	79.10
Total	64	20.344	0.73041	
FST = 0.20897				

FST-Fixation index

Figure 5 shows the Neighbour-joining tree of Balami and WAD populations with five reference haplogroups and Figure 6 shows the Median-joining network of haplotypes observed in Balami and WAD populations are represented by green circles. The frequency of the area of the circle is proportional to Haplotype frequency. The Neighbour-joining (NJ) and Median-joining (MJ) trees revealed that Balami and WAD belong to Haplogroup B, which implies that the two populations originated from a common source. The NJ tree measured the differences within and between the observed distinct Haplotype groups.

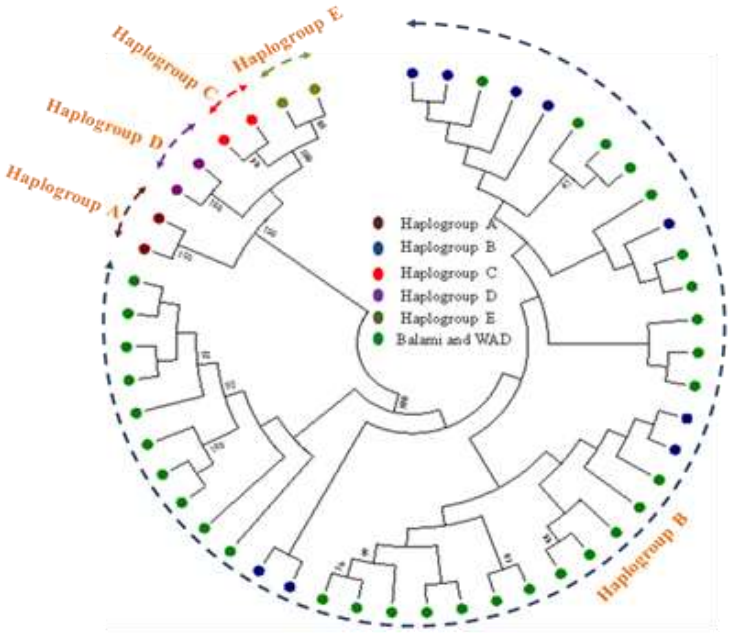

Figure 5. Neighbour-joining tree of Balami and WAD populations with five reference haplogroups

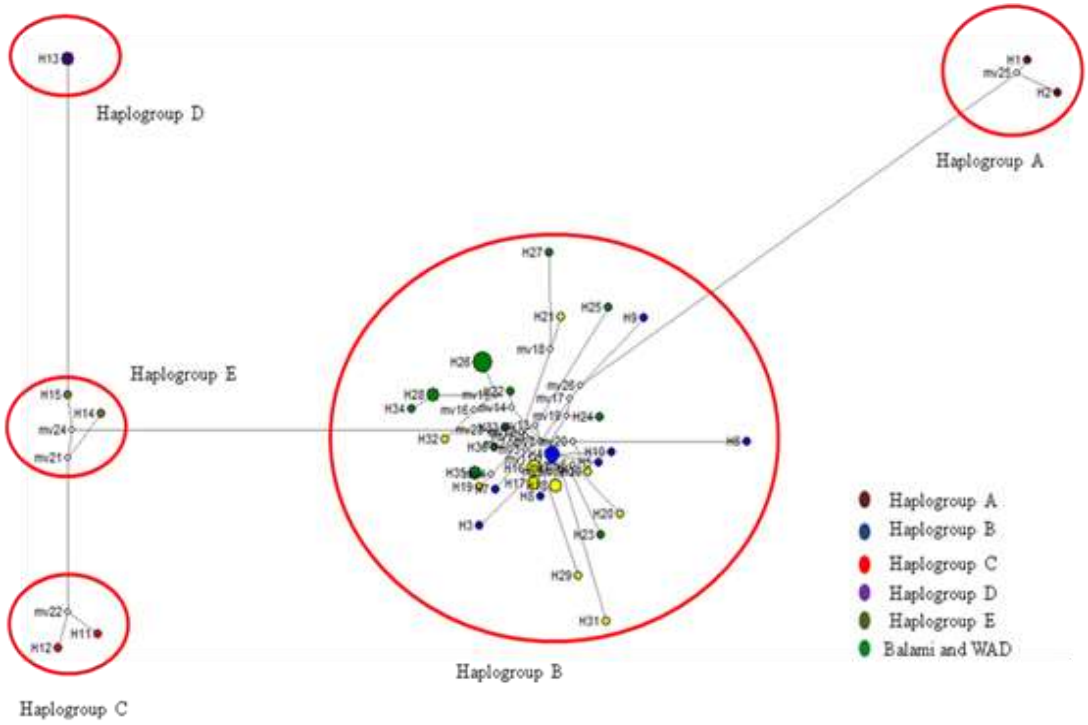

Figure 6: Median-joining network of haplotypes observed in Balami and WAD populations are represented by green circles. The frequency of area of circle is proportional to Haplotype frequency

Discussion

Mitochondrial DNA has been very widely used to It has also been used to investigate the genetic variation of species. It has also been used to assess phylogenetic relationships at inter- or intra- species levels in various livestock species such as cattle (Sasazaki *et al*., 2006), swine (Sultana *et al*., 2003), goats (Giuffra *et al*., 2000) and other species ((Tanaka *et al*., 1996). In this study, the genetic diversity and phylogenetic structure of Balami and WAD sheep populations were evaluated. The results of this study revealed variations in all the diversity indices between the two Nigerian sheep breeds. Different indices revealed the existence of genetic diversity between WAD and Balami sheep populations. There were variations in the polymorphic sites of the animals with WAD sheep having a higher number of polymorphic sites than Balami. This could be due to the fact that WAD sheep are predominant in the

area of this study. Results obtained for genetic diversity and mismatch distribution imply that WAD and Balami sheep must have undergone relative population expansion as reported by Qing *et al*., (2009). Results of variations between values obtained for polymorphic sites, haplotype numbers, mismatched observed variance, and mismatched means reported for these sheep populations were lower than to result obtained by Agaviezor *et al*., (2012).

High haplotype and nucleotide diversity indices were obtained in this study for WAD and Balami sheep populations. The small size of individual populations and their unique breeding histories may account for the high variability of genetic diversity (Joshi *et al*., 2013). High haplotype and nucleotide diversity values have been reported for the Nigerian sheep population (Agaviezor *et al*., 2012); Nepalese sheep breeds (Gorkhali *et al*., 2015); Racka and Turcana (Kusza et al., 2015); Albania, Greece and Italian sheep populations (Pariset *et al*., 2011), Portuguese sheep populations (Pereira *et al*., 2005), Balkan sheep (Cinkulov *et al*., 2008). The high level of intra-population diversity could be due to the independent geographical domestication and distribution of these sheep populations (Gorkhali *et al*., 2015). The average of nucleotide differences was higher in WAD than in Balami. The average number of nucleotide differences and the average number of nucleotide substitutions per site were used to estimate the genetic distance between the breeds (Pariset *et al*., 2011). However, haplotype and the average number of nucleotide differences obtained in this study for both Balami and Uda sheep were lower to result reported for Chinese native sheep breeds (Wang *et al*., 2007; Lei *et al*., 2006); Mongolian and Chinese sheep (Luo *et al*., 2005) and Cameroon Western Highlands' Djallonke sheep (Ntsoli *et al*., 2022).

Statistically, significant values obtained for the Sum of Square of Deviation for both Balami and WAD sheep populations imply the deviation from the estimated sudden demographic model of expansion expected under the model of population expansion. Non-significant Raggedness index value obtained for Balami indicated population expansion for the sheep, unlike WAD sheep with a significant Raggedness index which implies that WAD showed more than one independent subpopulation with high homozygosity and less low heterozygosity (population subdivision). These results revealed a signal of demographic or spatial expansion in these indigenous Nigerian sheep breeds that may have occurred prior to or after being introduced into the country. The non-significant Raggedness index indicated a relatively good fit to a model of population expansion (Harpending, 1994).

The neutrality test used for the study was Fu's Fs (Fu, 1997). The result of Fu's Fs tests was significant and negative for Balami but significant and positive for WAD, indicating a sudden expansion in the population size of Balami; high homozygosity, and low heterozygosity in WAD. A similar result was reported by Joshi *et al*., 2013; Kusza *et al*., (2015). However, negative and statistically significant values of Fu's Fs observed in both populations (overall) reinstated evidence of past population expansions, which may be due to genetic hitch-hiking, background selection, and evolutionary force producing the observed population expansion pattern (Mariotti *et al*., 2013; Joshi *et al*., 2013; Okello *et al.* 2005; Fu and Li, 1993).

In the present study, based on Fu's Fs statistics and mismatch distribution results, the probability of observing a random neutral sample with a number of alleles was similar to the observed value. The mismatch distributions for estimation of population size change for WAD and Balami using pairwise comparison showed a sharp variation for both observed and expected pair-wise differences. The fluctuation was higher in Balami than in WAD. Kusza *et al*., (2015) reported a unimodal, bell-shaped and smooth mismatch distribution for Racka sheep, suggesting that the sheep population had undergone a demographic expansion and inconsistent demographic results for the Gyimesi sheep breed.

A total of 23 haplotypes were observed between the two populations out of which 12 haplotypes were observed in Balami while 11 haplotypes were observed in WAD. Further results showed that the two populations did not share any haplotype. The observation of specific haplotypes for WAD and Balami populations is an indication of clear separation between the populations. Othman et al., (2015) reported specific haplotypes for Italian and Egyptian sheep breeds. According to these authors, no haplotype was shared between the breeds, suggesting that a high level of genetic diversity was present

between these breeds. This pattern of haplotype distribution may be attributed to geographical distribution and husbandry practices (Gorkhali *et al.,* 2006).

The analysis of molecular variation (AMOVA) for the two populations indicated that 79.1% of genetic variation was observed within the population variance component while 20.9% of the genetic variation was included among the populations. This is an indication of a weak genetic structure (Kusza *et al*., 2015), which could result from hybridization, which occurred among closely related species as reported by Tubaro and Lijtmaer (2002). Similarly, Agaviezor *et al*., a (2012) reported a 0.23 % variation among Nigerian sheep populations compared with 99.77 % variation found within populations. The degree of population differentiation is a fixation of gene flow which was estimated by the genetic differentiation fixation index (FST). A very high level of gene flow is reflected for FST values less than 0.025 while the value of FST greater than 0.15 reflects essentially no gene flow (Wehausen 2016). In this study, the FST observed among the sheep population was 0.20897, the observed FST value indicated that there is no gene flow between Balami and WAD. This may be due to the geographical separation between Balami and WAD sheep which are from different regions. This has probably led to a moderate level of differentiation between these sheep breeds since genetic drift and natural selection are two main factors that give rise to genetic differentiation among populations (Qing *et al.,* 2009).

In order to assess the phylogenetic relationships, the neighbor-joining (NJ) and median-joining (MJ) dendrograms were generated. In the Neighbour-Joining tree, mtDNA sample sequences were distributed throughout the tree with generally short branches with a robust structure, which implies a recent origin of the diversity and suggests a pattern of a relatively recent population expansion. These findings suggest a common origin and maternal evolutionary history due to human-mediated breeding (Lavery *et al*., 1996). In order to assess the phylogenetic relationships between Balami and WAD sheep, the neighbor-joining (NJ) and median-joining (MJ) dendrograms were generated. In the Neighbour-Joining tree, mtDNA sample sequences were distributed throughout the tree with generally short branches with a robust structure, which implies a recent origin of the diversity and suggests a pattern of a relatively recent population expansion. The trees (NJ and MJ) revealed that Balami and WAD belong to Haplogroup B, this implies that the two populations originated from the same maternal origin. The results obtained in this study agreed with the report of Ntsoli *et al.,* (2022); Mariotti *et al*., (2013), where the affiliation of Djallonke sheep breed in Cameroon and Italian sheep breeds to the maternal lineage of haplogroup B was established based on the analysis of mtDNA respectively. The MJ network of WAD and Balami sheep confirms that these sheep populations belong to haplogroup B, whose ancestral base is located in Central Asia. A similar result was reported for Djallonke sheep breed in Cameroon (Ntsoli *et al*., 2022) who reported that Djallonke sheep breed in Cameroon belongs to haplogroup B with their ancestral base from Central Asia.

Conclusion

The results of this study contribute to the knowledge of the existing genetic status of Nigerian indigenous sheep populations. The overall haplotype and nucleotide diversities for these sheep populations were high. Most of the variations were found within the population than between populations. These sheep breeds were genetically different due to their geographical distributions, with Balami located in the country's Northern regions while WAD is regarded as a Southern Nigerian sheep breed. The finding suggests a probable origin from the very first domestic sheep ancestors in Asia which could have reached the country through various routes. Thus, successive migration and relative selection could explain the current mitochondrial DNA variability in the sheep populations. Thus, this possible indication of wide genetic variation between these two breeds could be exploited in genetic improvement programs for both breeds.

Acknowledgement

This project was supported by the BecA- ILRI Hub through the Africa Biosciences Challenge Fund (ABCF) program. The ABCF program is funded by the Australian Department for Foreign Affairs and Trade (DFAT) through the BecA- CSIRO partnership; the Syngenta Foundation for Sustainable Agriculture (SFSA); the Bill & Melinda Gates Foundation (BMGF); the UK Department for International Development (DFID); and the Swedish International Development Cooperation Agency

(Sida). Authors greatly appreciate the owners of the animals used in this study, small ruminant marketers and Ram Lover Association for supporting the sampling process.

Funding Information

This project was funded by the BecA- ILRI Hub through the Africa Biosciences Challenge Fund (ABCF) program. The ABCF program is funded by the Australian Department for Foreign Affairs and Trade (DFAT) through the BecA- CSIRO partnership; the Syngenta Foundation for Sustainable Agriculture (SFSA); the Bill & Melinda Gates Foundation (BMGF); the UK Department for International Development (DFID); and the Swedish International Development Cooperation Agency (Sida).

Data availability

Availability of data will be based on request

Author's Contributions

Popoola M.A. and Oseni S.O. designed the study, Popoola M.A conduted both the field and laboratory works, Machuka E helped in the laboratory work as Research Assistant, Githae D and Tarekegn G.M. were the biostatistians who helped with data analyses and Stomeo F guided in the laboratory work. The draft of the manuscript was proofread by Oseni, Machuka and Stomeo. The final draft was approved by all authors.

Ethics

Author declare that there are no ethical issues that may arise after the publication of this manuscript

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