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Genetic Analysis of Nigerian Indigenous Goat Populations Using Microsattelite Markers

Research Article

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ABSTRACT

This study was conducted to determine the genetic relationships among and population history of four Nigerian indigenous goat populations. A total of 200 goats from three breeds namely Sahel (60), Red Sokoto (60), West-African dwarf (60) and Kano Brown, a strain (20), were used for the study. Tissue samples were collected from the ear of animals using an Allflex® ear punch tissue sample collector, and aliquoted into plastic tubes containing the Allflex® tissue preservative. DNA extraction, amplification and sequencing were carried out at the International Livestock Research Institute (ILRI), Nairobi, Kenya. Genetic analysis of the DNA was carried out using 25 microsatellite markers proposed by Food and Agricultural Organization and International Society for Animal Genetics (FAO-ISAG). From the results of the study, the highest heterozygosity was observed in Kano Brown goats (0.68±0.04), which was followed by that of Red Sokoto goats (0.64±0.04) and that of West African dwarf goat (0.58±0.05) as the least. In the entire goat populations, low inbreeding was observed; mean F_{is} was 0.105 and F_{it} was 0.129. As expected, populations of Red Sokoto and Kano Brown showed higher genetic similarity as was seen in the genetic distance (0.022), confirming the notion that the Kano Brown is a strain from the Red Sokoto breed. Gene flow (Nm) played an important role in the genetic uniformity in populations of narrow geographical vicinity (14.868). The dendogram displayed a remarkable degree of consistency with the geographical origin of goats in this country. The information obtained in this study will aid in rational development, utilization and conservation of Nigerian indigenous goats.

KEY WORDS dendogram, genetic distance, microsatellite marker, Nigerian indigenous goat.

INTRODUCTION

Small ruminants play a very significant but often underestimated role in the livelihoods of rural farmers in Nigeria (Ajala *et al.* 2008). They are also an important source of animal protein and immediate cash, and have a variety of socio-cultural values (Odoi *et al.* 2000). Sahel (S), Red Sokoto (RS), Kano Brown (KB) and West African dwarf (WAD) goats are the most important goat populations found in Nigeria. The Sahel goats (Borno White or Kyalla) are common in the arid or Sahel region of the country. It is medium to large in size, long legged, and covered with coat of short fine hair and ears are long and droppy (Akpa *et al.* 1998). The Red Sokoto (Maradi) goat is the most numerous goat breed in Nigeria. It was originally confined to the Niger Republic and Sokoto province, but has now spread

throughout the Savannah belt. The skin of Maradi goat is among the most valuable of all goat skins. It is used in the leather industry locally and internationally (Akpa *et al.* 1998). The Kano brown goat is an ecotype of Red Sokoto goat. It is light brown in colour with similar body weight as the Red Sokoto goat. While the West African dwarf (WAD) goat, which is trypanotolerant, is widely distributed across the rain-forest belt of Southern Nigeria. They are short-legged and small-bodied animals, weighing between 22 to 26 kg (Mourad *et al.* 2000).

An assessment of the genetic variability in domestic goats is required to meet current production needs in various environments, to allow sustained genetic improvement, and to facilitate rapid adaptation to adverse weather conditions.

Microsatellites are ideal molecular markers for characterization. Microsatellites are widely used as genetic markers for the analysis of genetic variability within and between breeds due to their high number and distribution throughout the genome and the efficacy of genotyping (Okpeku *et al.* 2011a). Microsatellite markers have been identified and used in several livestock species; in cattle, Sharma *et al.* (2015) studied the genetic diversity and relationship of Indian cattle inferred from microsatellite and mitochondrial DNA markers, similarly Karacaoren and Kadarmideen (2008) evaluated the principal component and clustering analysis of functional trait in Swiss dairy cattle while Sraphet *et al.* (2008) carried out a research on the use of cattle microsatellite markers to assess genetic diversity of Thai swamp Buffallo. Ibrahim *et al.* (2010) studied the genetic diversity in Balkhi, Hashtnagri and Michni sheep populations using SSR markers; Laval *et al.* (2000) studied the genetic diversity of eleven European pig breeds. Also, Mtileni *et al.* (2012) studied the genetic diversity and conservation of South African indigenous chicken populations.

Several studies have been documented on the genetic diversity of goats based on microsatellite markers, such as Swiss breeds (Saitbekova *et al.* 1999), Chinese indigenous populations (Li *et al.* 2002; Li *et al.* 2004), Indian domestic goats (Rout *et al.* 2008), goats from Europe and Middle East (Canon *et al.* 2006). Scanty studies have been carried out on the genetic diversity of Nigerian goat (Adebambo *et al.* 2011; Okpeku *et al.* 2011a; Ojo, 2014; Yakubu *et al.* 2014).

The diversity in livestock is rapidly changing due to changes in market demand, as farmers have preference for more prolific species which are more productive. It is therefore important to conserve the genetic diversity among indigenous livestock species.

A Comprehensive knowledge of the existing genetic variability is the first step for conservation and exploitation of domestic animal biodiversity (Li *et al.* 2002).

The four goat populations of Nigeria (Sahel, Red Sokoto, Kano Brown and West-African dwarf goats) have been phenotypically characterized but their genetic characterization has not been fully exploited. Hence, the present study, characterization of Nigerian goat populations at molecular level using microsatellite markers, is aimed at estimating allelic frequency of different gene loci and the genetic relationships between the Nigerian goat populations, as well as determining the time of divergence for all the four goats populations.

MATERIALS AND METHODS

A total of 200 randomly sampled indigenous goats from three (3) breeds namely: Sahel, Red-Sokoto and West African dwarf goat and Kano-Brown were used for the study. For each of the first 3 breeds, a maximum of sixty (60) animals were sampled, and only twenty (20) were sampled from the Kano Brown.

Sample collection and DNA extraction

Microsatellite markers and genotyping

Twenty five microsatellite markers were used for the genotyping. They were randomly chosen from FAO recommended list (FAO, 2011) (Table 1). The annealing temperature of the primers ranged between 50 ˚C to 65 ˚C. A master mix was prepared according to the manufacturers specifications containing water, buffer, dNTPs, primers and TaqDNA Polymerase in a single tube and latter aliquoted into individual tubes. Magnesium chloride ($MgCl_{2)}$ and template DNA solutions were then added.

The sample was gently vortexed and briefly centrifuged to collect all drops from the walls of the tube and the samples were placed in a thermo cycler using the following setup: an initial denaturation at 95 ˚C (4 minutes), annealing at 50 ˚C (45 seconds) and extension at 72 ˚C (15 minutes) for 35 cycles (Ojo, 2014).

After amplification, the PCR products were ran on a 1.5% agarose gel and stained with PET dye and visualized by ultra-violet light exposure. Bands of the correct size were excised from the gel, documented and sequenced.

The study was conducted in accordance with institutional guidelines on the care and use of animals for scientific research, and in compliance with generally accepted rules of best practice worldwide.

Data analysis

The alleles and genotypic frequencies were directly identified from the gel, while genetic distance and polymorphic information content were computed using GenAlEX 6.5 genetic analysis statistical package of Peakall and Smouse (2012).

Table 1 Characteristics of microsatellite markers used for the study

N	Marker	Sequences (F /R: 5'→3')	Annealing temperature ˚C	Range (bp)		Dye	Gene bank access
1	SRCRSP03	CGGGGATCTGTTCTATGAAC TGATTAGCTGGCTGAATGTCC	55	98	122	NED	L22195
2	ILSTS005	GGAAGCAATTGAAATCTATAGCC TGTTCTGTGAGTTTGTAAGC	55	172	218	VIC	L23481
3	SPS113	CCTCCACACAGGCTTCTCTGACTT CCTAACTTGCTTGAGTTATTGCCC	58	134	158	6FAM	/
4	CSRD247	GGACTTGCCAGAACTCTGCAAT CACTGTGGTTTGTATTAGTCAGG	58	220	247	PET	/
5	MAF209	GATCACAAAAAGTTGGATACAACCGTG TCATGCACTTAAGTATGTAGGATGCTG	55	100	104	VIC	M80358
6	McM527	GTCCATTGCCTCAAATCAATTC AAACCACTTGACTACTCCCCAA	58	165	187	VIC	L34277
7	SRCRSP5	GGACTCTACCAACTGAGCTACAAG TGAAATGAAGCTAAAGCAATGC	55	156	178	PET	L22197
8	ILSTS087	AGCAGACATGATGACTCAGC CTGCCTCTTTTCTTGAGAG	58	135	178	NED	L37279
9	SRCRSP9	AGAGGATCTGGAAATGGAATC GCACTCTTTTCAGCCCTAATG	58	99	135	PET	L22200
10	OarFCB 304	CCCTAGGAGCTTTCAATAAAGAATCG GCGCTGCTGTCAACTGGGTCAGGG	56	150	188	/	L01535
11	ILSTS11	GCTTGCTACATGGAAAGTGC CTAAAATGCAGAGCCCTACC	58	250	300	6FAM	L23481
12	ETH10	GTTCAGGACTGGCCCTGCTAACA CCTCCAGCCCACTTTCTCTTCTC	55	200	210	PET	Z22739
13	MAF065	AAAGGCCAGAGTATGCAATTAGGAG CCACTCCTCCTGAGAATATAACATG	58	116	158	VIC	M67437
14	OarCP34	GCTGAACAATGTGATATGTTCAGG GGGACAATACTGTCTTAGATGCTGC	50	112	130	/	U15699
15	ILSTS029	TGTTTTGATGGAACACAG TGGATTTAGACCAGGGTTGG	55	148	170	NED	L37252
16	INRA023	GAGTAGAGCTACAAGATAAACTTC TAACTACAGGGTGTTAGATGAACT	58	196	215	NED	X80215
17	MAF70	CACGGAGTCACAAAGAGTCAGACC GCAGGACTCTACGGGGCCTTTGC	65	134	168	6FAM	M77199
18	INRA063	GACCACAAAGGGATTTGCACAAGC AAACCACAGAAATGCTTGGAAG	58	164	186	VIC	X71507
19	BM6444	CTCTGGGTACAACACTGAGTCC TAGAGAGTTTCCCTGTCCATCC	65	118	200	6FAM	G18444
20	OarFCB48	GAGTTAGTACAAGGATGACAAGAGGCAC GACTCTAGAGGATCGCAAAGAACCAG	58	149	173	PET	M82875
21	INRABERN172	CCACTTCCCTGTATCCTCCT GGTGCTCCCATTGTGTAGAC	58	234	256	PET	/
22	INRABERN185	CAATCTTGCTCCCACTATGC CTCCTAAAACACTCCCACACTA	55	261	289	PET	X73937
23	TCRVB6	GAGTCCTCAGCAAGCAGGTC CCAGGAATTGGATCACACCT	55	217	255	PET	L18953
24	SRYM18	GGCATCACAAACAGGATCAGCAAT GTGATGGCAGTTCTCACAATCTCCT	58	80	170	6Fam	Y chromosome
25	OarFCB20	GGAAAACCCCCATATATACCTATAC AAATGTGTTTAAGATTCCATACATGTG	58	93	112	VIC	L20004

The deviations of the locus/population combinations from Hardy Weinberg equilibrium (HWE) were determined using the statistical program of GenAlex 6.5 (Peakall and Smouse, 2012).

Phylogenetic trees were constructed from Nei's standard genetic distance (DS) and Cavalli- Sforza and Edwards genetic distance measure (DA) generated using the Neighbour Joining (NJ) and the Unpaired Group with

Arithmetic Mean (UPGMA) methods in the TREEVIEW program, GENEPOP version 3.4 (Raymond and Rousset, 1995).

RESULTS AND DISCUSSION

The means and standard errors of the effective alleles, observed and expected heterozygosities for each population are shown in Table 2. The mean number of different alleles, effective alleles, observed and expected heterozygosities as well as fixation index for the Sahel goat population were 10.00 ± 0.85, 4.37 ± 0.40, 0.61 ± 0.05, 0.70 ± 0.04 and 0.14 ± 0.044 respectively. Whereas for the Red Sokoto population, the mean number of different alleles, effective alleles, observed and expected heterozygosities, and the fixation index was 9.96 ± 0.80, 4.31± 0.38, 0.64 ± 0.04, 0.71 ± 0.03 and 0.09 ± 0.044, respectively.

In Kano Brown population, the mean number of different alleles was 7.60 ± 0.61, while that of the effective alleles was 4.67 ± 0.43. The mean observed and expected heterozygosities for this population were 0.68 ± 0.04 and 0.72 ± 0.04, respectively and the fixation index was 0.04 ± 0.04.

Also, the mean number of alleles for the West African dwarf goats was 9.52 ± 0.80, while the mean effective allele was 4.18 ± 0.42. The values obtained for the observed and expected heterozygosities for this population were 0.58 ± 0.05 and 0.68 ± 0.04, and the fixation index was 0.17 ± 0.06.

The overall means of the different alleles and effective alleles were 9.27 ± 0.39 and 4.38 ± 0.20, respectively. The overall means for the observed and expected heterozygosities were 0.63 ± 0.02 and 0.70 ± 0.02, respectively, while the fixation index was 0.11 ± 0.024. In all cases, the average observed heterozygosities were lower than the expected heterozygosities. The standard genetic distances calculated for the four goat populations (Table 3) showed that the shortest distance (0.022) was observed between Red Sokoto and Kano Brown, followed by the distance between Red Sokoto and Sahel goats (0.035). However, the farthest distance was obtained between West-African dwarf and Kano Brown (0.121).

The average observed and expected heterozygosities of the different goat populations as well as their Hardy-Weinberg equilibrium values are presented in Table 4. The average expected heterozygosity was lowest in West African dwarf goats (0.679), while the Kano Brown goats had the highest expected heterozygosity (0.721). Similarly, Kano Brown goats had the highest observed heterozygosity than all the other three goat breeds. In all cases, the average observed heterozygosities were lower than the expected heterozygosities and all populations showed significant deviations (P<0.01) from Hardy-Weinberg expectations.

The F-statistics ($F_{is,}$ F_{it} and F_{st}) and estimates of gene-flow across the goat populations are shown in Table 5. The F_{is} value for the entire population using the 25 markers ranged from -0.043 to 0.592. The F_{is} values were negative for five (5) loci (ILSTS029, ILSTS087, INRABERN 185, SPS 113 and SRYM 18) while the remaining loci revealed positive F_{is} values ranging from 0.012 at ILSTS11 to 0.592 at INRABERN172.

The mean F_{is} value was 0.105 ± 0.040.

The F_{it} value ranged from -0.016 to 0.601 and a mean of 0.129 ± 0.041, whereas the F_{st} value across the population ranged from 0.007 at INRA023 to 0.169 at MAF65 with a mean value of 0.03 ± 0.07. Gene flow (Nm) ranged from 1.226 for MAF65 to 35.598 for INRA023 with a mean value of 14.868 ± 1.799.

The dendogram or phylogenetic tree of the Nigerian goat mitochondria DNA (mtDNA) sequences is shown in Figure 1.

The tree shows that all the breeds originated from a common source or ancestry. However, West African dwarf goat diverged first and is more genetically distinct. This was followed by Sahel and lastly Red Sokoto and Kano Brown goats. Although separated, they are more genetically closely related.

In the present study twenty five micro-satellite loci were used to evaluate the genetic relationships within and between the Nigerian goat populations. The twenty five micro-satellite markers were all polymorphic in the goat populations with the exception of ILST005. The different measures of genetic variation such as Na, Ne and I, were highly polymorphic across loci. Comparatively, allelic frequency in Nigerian goats of 7.6 to 10.0 was higher than those of Iranian goats (7.3) and Canary Island goats (5.9) as reported by Mahmoudi *et al.* (2010) and Martinez *et al.* (2006) respectively, but lower than Spanish Guadarrama goats with allelic frequency of 9-36 (Serrano *et al.* 2009). The mean observed heterozygosity was lower than those of the expected as such, suggesting a deficit in heterozygosity as observed in the F values as well. The relative low heterozygosity suggests the presence of inbreeding, probably resulting from minimal reproductive isolation and larger effects of migration and genetic drift across the entire Nigerian goat population.

The close relationship observed between Red Sokoto and Kano Brown may have resulted from gene flow as these animals are in the same geographical location. The genetic distance between Red Sokoto and West African dwarf goats (0.29) was lower than the 0.39 reported by Adebambo (2003) but higher than 0.27 reported by Okpeku *et al.* (2011a) for the same breeds. This may have resulted from wider geographical locations from which these animals were sampled.

Table 2 Mean and standard errors for Shannon index, observed and expected heterozygosities and fixation index for each population

Population		Parameters[1]						
		N	Na	Ne	I	Ho	He	F_{it}
Sahel	**Mean**	51.920	10.000	4.366	1.642	0.609	0.700	0.143
	SE	1.726	0.845	0.397	0.108	0.047	0.037	0.044
Red Sokoto	**Mean**	55.680	9.960	4.312	1.635	0.644	0.708	0.087
	SE	1.556	0.799	0.375	0.102	0.044	0.034	0.044
Kano Brown	**Mean**	17.520	7.600	4.663	1.607	0.678	0.721	0.041
	SE	0.638	0.611	0.425	0.104	0.038	0.035	0.041
West African dwarf	**Mean**	52.400	9.520	4.177	1.568	0.584	0.679	0.167
	SE	2.149	0.796	0.417	0.110	0.054	0.041	0.059
Grand mean and SE over loci and populations								
		N	**Na**	**Ne**	**I**	**Ho**	**He**	**F_{it}**
Total	**Mean**	44.380	9.270	4.380	1.613	0.613	0.702	0.110
	SE	1.756	0.391	0.200	0.052	0.023	0.018	0.024

N: sample size; Na: no. of alleles; Ne: no. of effective alleles; I: Shannon index; Ho: observed heterozygosity; He: expected heterozygosity and F_{it}: fixation index.
SE: standard error.

Table 3 Pair wise population matrix of Nei unbiased genetic distance

Population	Red Sokoto	Kano Brown	Sahel
Kano Brown	0.02	-	-
Sahel	0.04	0.06	-
West African dwarf	0.29	0.12	0.05

Table 4 Hardy-Weinberg equilibrium for the Nigerian goat populations

Populations	Ho	He	HWE	α (0.01)
Red Sokoto	0.644	0.708	0.0058	**
Kano Brown	0.678	0.721	0.0026	**
Sahel	0.609	0.700	0.0118	**
West African dwarf	0.568	0.679	0.0133	**

Ho: observed heterozygosity; He: expected heterozygosity and HWE: Hardy Weinberg equilibrium.
** (P<0.01).

The significant deviations from the Hardy-Weinberg Equilibrium for the Nigerian goat populations, implies that there was a deficit of heterozygotes resulting from the non-random union of gametes in the population i.e. the mating among individuals in the population which are more related than the average relationship (Ojo, 2014). Similarly, migration, mutation and selection might have caused these deviations. Deviations from Hardy-Weinberg equilibrium at microsatellite level have been reported in various studies (Barker *et al*. 2001; Hassan *et al*. 2003; Laval *et al*. 2000; Luikart *et al*. 1999).

The presence of negative F_{is} values at five (5) loci (ILSTS029, ILSTS087, INRABERN 185, SPS 113 and SRYM 18) suggests heterozygote deficiencies which have also been reported in other studies on goats (Barker *et al*. 1997; Luikart *et al*. 1999; Agha *et al*. 2008; Rout *et al*. 2008; Dixit *et al*. 2009).

This heterozygote deficiency may have arisen due to population sub-structure from pooling together different populations (admixture) in the analysis (Cerda-Flores *et al*. 2002; Muema *et al*. 2009). Additional factors include population subdivision owing to genetic drift, null alleles and inbreeding (Hoarau *et al*. 2005).

The average F_{is} inbreeding coefficient for the entire goat population (0.11±0.04) was higher (0.04±0.13) than that obtained by Okpeku *et al*. (2011b) and could be a consequence of management systems (like tethering of the animals in the case of small flock size during crop season, herding of the flock by children and / or women, where the flock size is larger and some localized transhumance during the dry season). Tethering minimizes the number of different bucks that any particular female (doe) can mate, leading to service by only males preferred by the owner. While women and children herders usually avoid the mixing of different flocks or exchange of bucks from different areas even at the watering points. Localized transhumance also leads to minimal encounters between different flocks. All these practices and events tend to increase the rate of inbreeding (Ojo, 2014).

The relatively low total inbreeding (F_{it}=0.129) observed for the entire goat population may merely be an indication of gene flow and migration between the Nigerian goat populations. This implies a gradual erosion of desirable traits within the Nigerian goat population and if not controlled, deleterious genes would begin to express themselves with time.

Table 5 F-statistics and estimates of gene flow (Nm) over all populations

Locus	Genetic parameter			
	F_{is}	F_{it}	F_{st}	Nm
OarFCB20	0.398	0.438	0.066	3.562
BM6444	0.041	0.085	0.047	5.115
CSRD247	0.094	0.102	0.009	26.605
ETH10	0.267	0.279	0.016	15.229
ILSTS005	0.123	0.135	0.014	17.639
ILSTS11	0.012	0.022	0.010	24.477
ILSTS029	-0.124	-0.111	0.012	21.108
ILSTS087	-0.0.43	-0.035	0.008	31.383
ILSTS023	0.124	0.130	0.007	35.598
INRABERN185	-0.047	-0.016	0.030	8.036
INRABERN172	0.592	0.601	0.023	10.823
MAF65	0.437	0.533	0.169	1.226
MAF70	0.103	0.113	0.011	21.863
MCM527	0.401	0.410	0.014	17.220
OarFCB48	0.123	0.161	0.043	5.518
OarFCB304	0.024	0.038	0.015	16.862
SPS113	-0.369	-0.353	0.012	20.424
SRCRSP3	0.070	0.086	0.017	14.688
SRCSP9	0.026	0.056	0.031	7.875
SRYM18	-0.127	-0.104	0.021	11.709
TCRVB6	0.075	0.124	0.053	4.479
OarPC34	0.074	0.089	0.016	15.249
SRCSP5	0.121	0.133	0.014	17.938
MAF209	0.072	0.089	0.018	13.848
INRA063	0.151	0.212	0.072	3.230
Means	0.105	0.129	0.030	14.868
SE	0.040	0.041	0.07	1.799

F_{is}: within population inbreeding; F_{it}: total inbreeding estimate; F_{st}: measure of population differentiation and Nm: gene flow.
SE: standard error.

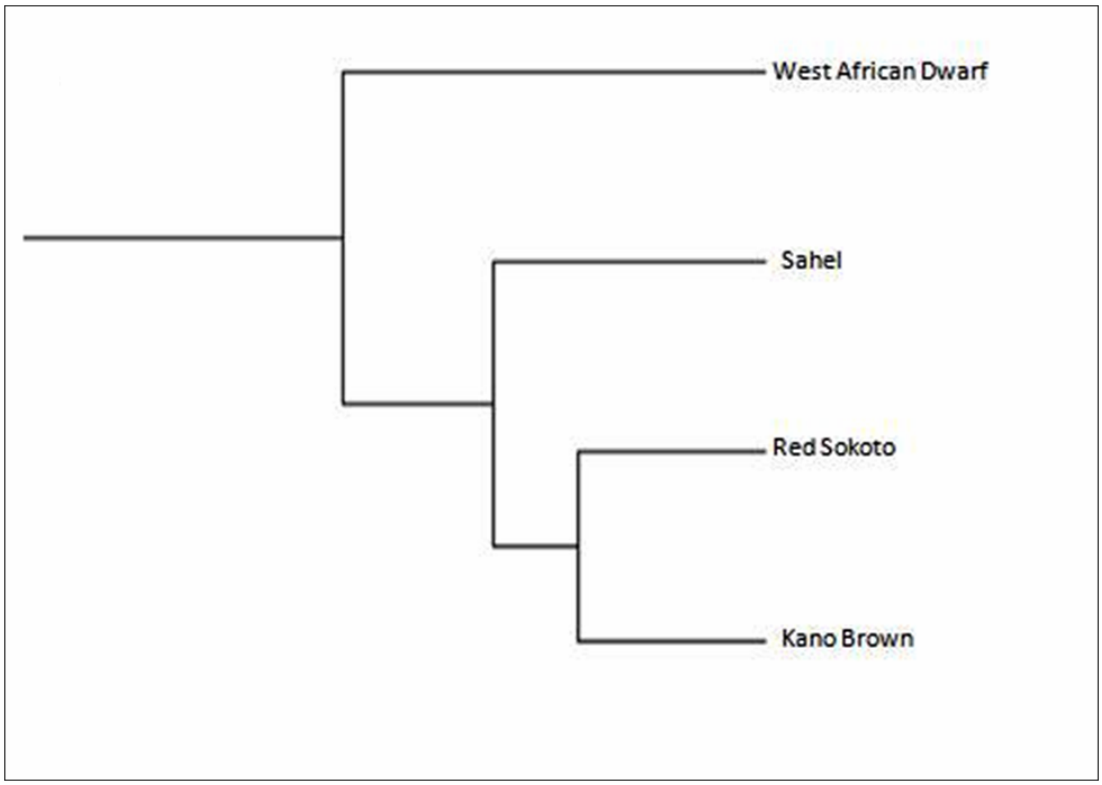

Figure 1 Dendogram representing the genetic relationship amongst four Nigerian populations

The F_{st} estimate which is commonly called the coefficient of gene differentiation obtained across all the loci implies that genotypic frequencies among the populations were not randomly distributed, meaning that the Nigerian goat populations have some genetic similarity amongst the sampled populations. The mean F_{st} value across all loci was low (0.03) compared to previous results of Nigerian goats (F_{st}=0.11, Okpeku *et al*. 2011b), Asian goats (F_{st}=0.14, Barker *et al.* 2001) and Chinese goats (F_{st}= 0.105, Li *et al.* 2002). However, Mujibi (2005) reported a moderate F_{st} of 0.058 for West African dwarf goats in Kenya. This variation may have resulted from population and breed differences. According to Laval *et al.* (2000), migration, interbreeding and genetic drift may exert a greater effect on the reduction in genetic differentiation between populations.

The high mean gene flow (14.868) across the populations suggests mobility and considerable exchange of genetic material among these goats which may have resulted from minimal reproductive isolation and larger effects of migration resulting in low genetic differentiation and inbreeding.

These could be attributed to the fact that most of these animals originated from Northern Nigeria where normadic pastoralism is the dominant livestock management system and to the extensive system of management, allowing the animals to roam freely and fend for themselves in most rural households and communities in the south. This enables and reinforces the ability of related animals to meet on pasture to breed or for neighbours to exchange related animals for upkeep or breeding (Okpeku *et al*. 2011b).

The dendogram separated the four Nigerian goat breeds according to their geographical location in the country. Evidenced from the dendogram, supports first divergence of West African dwarf goat, followed by Sahel and latter Red Sokoto and Kano Brown goat. From the dendogram, geographically adjacent populations were observed to be more genetically related as observed in Red Sokoto, Kano Brown and Sahel goats which are Northern breeds, though Sahel goat is located at the extreme North. However, West African dwarf is a Southern breed and was observed to be more genetically distinct.

CONCLUSION

The close kinship between Red Sokoto and Kano Brown goats suggest some past crossing between these two populations. The result of the dendogram was consistent with the background of the origin, history and geographical location of these goat populations. The dendogram showed that West African dwarf goat population is more distinct from the other goat populations. The information obtained in this study will aid rational development, utilization and conservation of the Nigerian indigenous goats (Ojo, 2014).

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