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MICROSATELLITE-BASED ANALYSIS OF TWO NIGERIAN INDIGENOUS GOAT BREEDS

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ABSTRACT

This study was conducted to determine the genetic variation between Sahel and Red Sokoto goat breeds of Nigeria .One hundred and twenty goats, 60 each of both Sahel and Red Sokoto breeds obtained from Maigatari market in Jigawa State and Animal Science Departmental Farm in Zaria, Kaduna State, respectively. Tissue samples were collected randomly from each breed using an Allflex ear punch tissue sample collector and aliquoted into plastic tubes containing the Allflex preservative. DNA extraction, amplification and sequencing were carried out at the International Livestock Research Institute (ILRI), Nairobi, Kenya. The DNA was extracted from the tissue cells using the Pure Link™ Genomic DNA minikit according to the manufacturer's specifications and protocol and were genotyped using 25 microsatellite markers as recommended by FAO/ISAG. Twenty three of the twenty five microsatellite markers used in this analysis had four or more alleles. The mean Shannon index, observed and expected heterozygosity and inbreeding coefficient (F_{is}) for Sahel goats were 1.642, 0.609, 0.700 and 0.143 respectively. For the Red Sokoto goats, the mean Shannon index, observed and expected heterozygosity and inbreeding coefficient (F_{is}) were 1.635, 0.644, 0.708 and 0.087, respectively. The genetic distance as well as the genetic differentiation (F_{st}) between the breeds was low, 0.04 and 0.026, respectively, however; the gene-flow (Nm) was high (5.78). This study has shown that Sahel and Red Sokoto goats have some genetic similarity owing to the fact that they have a common ancestry and are both Northern breeds. Measures to conserve the uniqueness/distinctiveness of these breeds should be sought through in-situ and ex-situ conservation. Gene flow between populations should be controlled by adopting effective breeding and management practices.

Key words: Sahel, Red Sokoto goats, Gene-flow, Genetic distance, Genetic differentiation

INTRODUCTION

Goats like other livestock species play a significant role in sustainable agriculture. They can survive poor quality diets as well as harsh environmental conditions (Hoste *et al.,* 1988). As such, goats are strategic in increasing livestock productivity in rural agricultural systems (Fitzugh *et al*., 1992). The population of goats in Nigeria is estimated at 53.8 million traditionally reared stock contributes 99.97%, while 0.03% of the stock is commercially managed (FAO Statistics 2011). Sahel, Red Sokoto (Maradi), Kano Brown and West African Dwarf goats are the most important goat populations found in the country. The Sahel and Red Sokoto /breeds are two important breeds in Northern Nigeria and possess certain unique features that enable them adapt to this geographical location. The Sahel (Brono White) is known for their

long legs, short fine coat and long droopy ears (Akpa *et al.*, 1998). However, the Red Sokoto has a uniformly dark red coat colour, valuable skin, with short curved ears and both sexes are horned. These two breeds have been observed to migrate over the years to different parts of the country in search of feed and water and are beginning to lose their distinctive characteristics due to interbreeding and indiscriminate mating with other breeds. Therefore, there is an urgent need to define strategies for conservation of these breeds in their natural habitat. The study of genetic variation plays an important role in developing rational breeding strategies for economical animal species. It is generally considered that the amount of genetic variability within an organism as ascertained by the number of alleles at each locus has an impact on the breed's differentiation (Hussain *et al*., 2013). Microsatellite analysis is one of the best tools to characterize and define a breed to support the traditional methods for goat breed differentiation (Martinez *et al.*, 2006). This study is therefore aimed at determining the genetic variation as well genetic distance between the Sahel and Red Sokoto goats of Northern Nigeria.

MATERIALS AND METHODS

Tissue samples from one hundred and twenty unrelated individual goats from the two breeds were used for this study. The Sahel goats were obtained from the Maigatari international market in Jigawa State, while the Red Sokoto goats were sampled from the Animal Science Departmental Farm, Ahmadu Bello University, Samaru, Zaria. For each breed, sixty (60) goats of both sexes were randomly sampled. Tissue samples were collected using an Allflex ear punch tissue sample collector and aliquoted into plastic tubes containing the Allflex preservative. DNA extraction, amplification and sequencing were carried out at the International Livestock Research Institute (ILRI), Nairobi, Kenya. The DNA was extracted from the tissue cells using the Pure Link™ Genomic DNA minikit according to the manufacturer's specifications and protocol. Twenty five microsatellite markers were used in this study as recommended by the International Society for Animal Genetics and Food and Agricultural Organization (FAO, 2011).

Polymerase Chain Reaction (PCR) Conditions

The PCR typing was carried out on 50ng of the genomic DNA in a 10µl reaction, comprising 10pM each of fluorescently labeled PCR primers, 1µl of PCR buffer (Amersham) 20µM each of dCTP, dGTP, dTTP and dATP, 0.5 units of Taq polymerase per reaction and 1.5-3.0 µl of MgCl2 depending on the primer. The mixture was cycled on the Hybaid Omnigene thermal cycler with cycling conditions of initial denaturation at 95°C (4 minutes), annealing at 50 -65°C for 45 seconds and final extension at 72°C (15 minutes) for 35 cycles.

Multiplexing was carried out following the recommendations of the ISAG/FAO panel. Genotyping was done using the ABI 3700XL automated capillary sequencer using Liz 500-350 internal lane size standard to size fractionate all amplified products. Allele size calling and binding was done with the aid of Gene Mapper 3.5 (Applied Biosystems).

Statistical analysis

The allele frequencies, observed and expected heterozygosities, genetic distance as well as genetic differentiation were computed using Gen AlEX 6.5 genetic analysis statistical package of (Peakall and Smouse 2012).

RESULTS AND DISCUSSION

Table 1 shows the number of effective alleles, observed (H_o) and expected heterozygosity (H_e), Shannon index (I) and fixation index (F_{is}) for Sahel goat population. Fixation index (F_{is}) is a measure of standardized variance in allele frequencies that detect departure from Hardy-Weinberg Equilibrium caused by inbreeding.

All the markers used in this population were highly informative, with allele numbers ranging from 3 to 19 as such exhibiting sufficient polymorphism. The number of effective alleles for this population ranged from 1.24 (ILSTS 005) to 7.90 (ILSTS 087). The values of H_o ranged from 0.140 (ILSTS 005) to 0.967 (ILSTS 087), while the expected heterozygosity ranged from 0.194 (ILSTS 005) to 0.879 (SRYM 18). The Shannon index (I) ranged from 0.408 to 2.455 and in most cases was above 1.0, implying high level of homogeneity amongst individuals in the population. Negative F_{is} values were obtained at five (5) loci (ILSTS029, ILSTS087, SPS113, SRCRSP 3, and MAF209) indicating out breeding at such loci. The different measures of genetic variation such as Na, Ne, and I, showed that all of the studied loci were highly informative, indicating high polymorphism across the loci. The negative F_{is} values located at five (5) loci indicated the presence of more heterozygotes at those loci, while the remaining twenty (20) loci with positive values suggest a heterozygote deficit, implying an inbred status of the population.

Allelic frequencies, observed and expected heterozygosity and fixation-index for the Red Sokoto goat population are shown in Table 2. All the primers/markers (microsatellite) used for this population were informative, displaying sufficient polymorphism with the number of alleles ranging from 3 to 16. It has been suggested that microsatellites ought to have at least 4 alleles to be useful for the evaluation of genetic diversity (Nassiry *et al.,* 2009).

The observed heterozygosity (Ho) ranged from 0.229 (INRABERN 185) to 0.983 (SRYM 18), while the expected heterozygosity (He) ranged from 0.251 (INRABERN 185) to 0.892 (ILSTS 087). The Shannon index for this population ranged from 0.482 to 2.140 and in most cases were higher than 1, implying the presence of heterozygotes within the population. The inbreeding index (F_{is}) for this population ranged from -0.018 to 0.666 and negative F_{is} values were obtained for seven loci (ILSTS 11, ILSTS029, OarFCB 304, SPS 113, SRCRSP 3, SRCSP 9, and SRYM18).

These negative values suggest out-breeding (mating of individuals who are less related than the average relationship of the population) at such loci and the presence of some heterozygotes. However, there were more positive F_{is} values at the remaining eighteen loci indicating high level of inbreeding in this population. The presence of negative F_{is} values in these populations suggests that homozygous deficiencies may have arisen from population sub-division owing to genetic drift, null alleles and selection against inbreeding. These negative values 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)

The Pair wise Population Matrix of gene flow, Genetic Distance and Genetic differentiation between the goat populations are presented in Tables 3 and 4.

High gene flow (5.78) was observed between Sahel and Red Sokoto goat populations, suggesting considerable exchange of genetic materials between the two breeds. According to (Okpeku *et al*., 2011); this high gene flow is as a result of indiscriminate mating, due to the normadic pastoral management system practiced by rearers of these breeds in Northern Nigeria.

The genetic distance between Red Sokoto and Sahel (0.04) was low, implying proximal interbreeding between the two populations. Similarly, low genetic differentiation (0.026) was observed between the breeds, this according to (Laval *et al*., 2000) may have resulted from migration, genetic drift and interbreeding. The results obtained for the genetic distance and differentiation were lower than those obtained by (Okpeku *et al*., 2011) and (Adebambo, 2003) for the same breeds, this may be due to differences in sample size used in its computation.

Table 1: Allelic Frequencies, Heterozygosity and F-statistics in Sahel goats

Locus	**N**	**Na**	**Ne**	**I**	**Ho**	**He**	**F_{is}**
OarFCB20	39	8.000	3.422	1.585	0.282	0.708	0.601
BM6444	58	12.000	4.778	1.858	0.665	0.791	0.171
CSRD247	48	8.000	5.505	1.848	0.729	0.818	0.109
ETH10	60	16.000	5.492	2.216	0.650	0.818	0.205
ILSTS005	57	3.000	1.240	0.408	0.140	0.194	0.276
ILSTS11	47	11.000	3.398	1.666	0.596	0.706	0.156
ILSTS029	60	8.000	1.980	0.974	0.583	0.495	-0.179
ILSTS087	60	17.000	7.895	2.302	0.967	0.873	-0.107
INRA023	48	12.000	5.878	2.046	0.708	0.830	0.146
INRABERNI185	42	4.000	1.477	0.627	0.310	0.323	0.042
INRABERN172	32	9.000	4.983	1.851	0.313	0.799	0.609
MAF65	45	10.000	1.590	0.949	0.200	0.371	0.461
MAF70	59	11.000	4.841	1.866	0.637	0.793	0.210
MCM527	59	6.000	1.902	0.994	0.203	0.473	0.571
OarFCB48	56	8.000	3.158	1.549	0.571	0.683	0.164
OarFCB304	53	15.000	5.739	2.133	0.811	0.826	0.017
SPS113	59	13.000	3.971	1.759	0.898	0.748	-0.201
SRCRSP3	52	5.000	3.414	1.349	0.712	0.707	-0.006
SRCSP9	59	11.000	6.662	2.103	0.778	0.850	0.083
SRYM18	60	19.000	8.295	2.455	0.850	0.879	0.033
TCRVB6	45	13.000	4.776	1.981	0.778	0.791	0.016
OarPC34	33	10.000	7.026	2.079	0.729	0.858	0.081
SRCSP5	55	12.000	5.566	1.975	0.727	0.820	0.113
MAF209	60	4.000	3.259	1.271	0.750	0.693	-0.082
INRA063	52	5.000	2.914	1.210	0.596	0.657	0.092
Mean	51.920	10.000	4.366	1.642	0.609	0.700	0.143

N=sample size, Na= no. of alleles, Ne=no. of effective alleles, I=Shannon index, Ho =observed heterozygosity, He= expected heterozygosity and F_{is} = fixation index/inbreeding coefficient.

Table 2: Allelic Frequencies, Heterozygosity and F-statistics in Red Sokoto goats

Locus	N	Na	Ne	I	Ho	He	F_{is}
OarFCB20	43	8.000	4.007	1.625	0.488	0.750	0.349
BM6444	57	16.000	5.891	2.178	0.807	0.830	0.028
CSRD247	56	8.000	6.131	1.907	0.750	0.837	0.104
ETH10	60	14.000	4.511	1.933	0.533	0.778	0.315
ILSTS005	60	5.000	1.343	0.557	0.250	0.255	0.021
ILSTS11	57	10.000	2.897	1.511	0.667	0.655	-0.018
ILSTS029	60	4.000	2.035	0.898	0.583	0.509	-0.147
ILSTS087	60	14.000	9.290	2.392	0.867	0.892	0.029
INRA023	56	11.000	5.412	1.955	0.750	0.815	0.080
INRABERNI185	35	3.000	1.336	0.482	0.229	0.251	0.091
INRABERN172	54	12.000	4.780	1.933	0.426	0.791	0.461
MAF65	31	17.000	4.3888	2.140	0.258	0.772	0.666
MAF70	56	10.000	3.831	1.639	0.714	0.739	0.033
MCM527	60	6.000	2.446	1.179	0.367	0.591	0.380
OarFCB48	60	12.000	4.330	1.753	0.733	0.769	0.046
OarFCB304	60	13.000	5.586	1.973	0.850	0.821	-0.046
SPS113	60	13.000	3.329	1.536	0.950	0.700	-0.035
SRCRSP3	59	6.000	2.596	1.175	0.627	0.615	-0.020
SRCSP9	57	11.000	4.570	1.770	0.912	0.781	-0.168
SRYM18	60	14.000	6.040	2.124	0.983	0.834	-0.178
TCRVB6	53	11.000	6.242	2.046	0.736	0.840	0.124
OarPC34	58	13.000	6.451	2.082	0.810	0.845	0.041
SRCSP5	60	9.000	5.169	1.809	0.767	0.807	0.049
MAF209	60	5.000	2.915	1.221	0.533	0.657	0.188
INRA063	60	4.000	2.266	1.059	0.500	0.559	0.105
Mean	55.680	9.960	4.312	1.635	0.644	0.708	0.087

N=sample size, Na= no. of alleles, Ne= no. of effective alleles, I=Shannon index, Ho =observed heterozygosity, He= expected heterozygosity and F_{is} = fixation index/inbreeding coefficient.

Table 3. Gene Flow between Populations.

Population 1	Population 2	Gene Flow (Nm)
Red Sokoto	Sahel	5.78

Table 4. Genetic distance (below diagonal) and Genetic differention (F_{st}) (Above diagonal) between Sahel and Maradi goat breeds.

	Sahel	Maradi
Sahel	*	0.026
	*	
Maradi	0.04	*

CONCLUSION

The microsatellites used in this study displayed sufficient polymorphism in Sahel and Red Sokoto goats and the genetic distance (0.04) and differentiation (0.026) between the breeds was low however, gene-flow was high (5.78). The information obtained in this study will aid rational development, utilization and conservation of these indigenous goat breeds in Nigeria.

RECOMMENDATIONS

In-situ and ex-situ conservation measures should be sought to ensure distinctiveness of these breeds.

Improved management and breeding practices should be put in place to control gene flow between populations.

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