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Estimation of Diversity in Sudanese Goats using Microsatellite Markers

Y. A. Hassan[1]*, J.Birungi [2] and M. Agaba[2]

[1]Animal Production Research Centre (APRC), Kuku, P.O.Box 89, Sudan.

[2]International Livestock Research Institute (ILRI), Biosciences for Eastern & Central Africa Hub, Nairobi, P.O.Box 30709, Kenya.

Corresponding Author (butana43@yahoo.com.)

Summary

The objective of this study was to determine the intra-and inter genetic diversities of four different goat populations in Sudan namely Desert and Tagger (indigenous) and South African Boer and Kalahari Red (introduced). A total 120 DNA samples were genotyped using five microsatellites markers (*BMS2508, BM143, OarAE101, OarAE129, OarHH55*) which are recommended by Food and Agriculture Organization (FAO) of the United Nations and International Society for Animal Genetics (ISAG). The pooled average range of the genetic variability parameters for the four populations studied were: Observed allele numbers (N_a), Effective number of alleles (N_e), Observed heterozygosity (H_o), Expected heterozygosity (H_e), Shannon's Index (I), were: 4.2 (Kalahari Red) to 8.4 (Tagger)., 2.2 (Kalahari Red) to 4.3 (Tagger)., 0.438 (Kalahari Red) to 0.592 (Tagger)., 0.538 (Kalahari Red) to 0.751 (Tagger)., 0.992 (Kalahari Red) to 1.533 (Tagger), respectively. The polymorphic information content (PIC) for all the markers used scored 100%, indicating that all the markers used were appropriate for mapping the diversity of the populations studied. Within populations differentiation was considerable as indicated by the F_{is}estimate of 0.031 for Boer goats and 0.311 for Desert goats. The results reflect that the populations studied contain a valuable and substantial genetic diversity and there is a good scope for bringing effective sustainable conservation and genetic improvement.

Keywords: Goat, Microsatellites, Indigenous, Introduced, Diversity

Introduction

The Goat is a multipurpose animal that was domesticated around 9000-7000 BC. Goats form an integral part of agricultural systems in many countries and

have been in human service earlier and longer than cattle and sheep. These animals are widely distributed across all agro-ecological zones in Sudan.

Goats play a crucial role in the subsistence economy of rural communities in Sudan, where they are generally raised by poor farmers. Goat population in Sudan is estimated 31 million heads (MoLFR, 2014). The major goats' populations are named as Sudan Nubian, Sudan Desert, and Sudan Mountain Goats (*Taggar*), (A.O.A.D, 2001). Some information is available regarding the phenotype and performance of Sudanese goats(Mudalal *et al*, 2014., Bushara *et al*, 2013., Elamin, 2012., Bushara, 2011., Ismail *et al*, 2011.,Elabid, 2008). Little had been done regarding genetic characterization of Sudanese goats using biochemical markers (Hassan *et al*, 2013 and Hassan *et al*, 2010) and DNA markers (Chessa *et al*, 2007). The South African Boer and Kalahari Red goat flocks were imported into Sudan in 2012 by the Federal Ministry of Agriculture Livestock, Fisheries and Rangeland. Estimating of genetic diversity in the two imported breeds will give further insight into the prospect of achieving genetic progress from selection programs and/or cross-breeding with other local Sudanese goat populations.

There are no previous studies on the diversity of Sudan's goats. Hence, it was considered essential to estimate the diversity of indigenous and recently introduced goats in Sudan in view of the socioeconomic importance of goats in Sudan. There is worldwide recognition of the need for the conservation of livestock diversity (FAO, 1995a) and for characterization and relationships within and between breeds. Among available markers, microsatellites are the markers of choice for biodiversity evaluation due to their unique characteristics and ease of applications. They are among the most useful markers as they are easily transferred across ungulate taxa, being widely and successfully applied in conservation and diversity analysis (Maudet *et al*., 2002). Fluorescent based automated fragment analysis with multiplexing is a cost effective way to increase the throughput for simultaneously typing of numerous microsatellite markers.

The unique merits of Sudan's goat are a result of evolutionary forces and their interaction over long periods of time. However, these merits might have been diluted due to intermixing, sub-structuring and/or consequent genetic drift in the population over time. Therefore estimating genetic diversity among and within population may help to evaluate these factors

and provide genetic information to be used in the conservation and sustainable improvement.

Materials and Methods

Ear tissue punches were randomly collected from 120 does from different locations representing two indigenous (Taggar n=74 and Desert n=13) and two introduced South African goats (Kalahari Red n=23 and Boer n=10). DNA was extracted from ear punch tissues following salting out method as described by (Sambrook *et al*, 1989). Genomic DNA was amplified by Polymerase Chain Reaction (PCR) using five single sequence repeats (SSR) or microsatellites (Table 1), located on chromosome 6 and selected from the list recommended by the United Nations Food and Agriculture Organization (FAO) and International Society of Animal Genetics (ISAG).

To establish high throughput SSRs analysis, the five SSRs markers were arranged by fluorescent dye label, into 3 multiplexed PCR panels (Multiplex Set-1: 3 SSR markers), Multiplex Set-II: 3 SSR markers) and Multiplex Set III: 2 markers). Each multiplex PCR was carried in 10 µl reaction volume containing 50 ng of DNA, 1x µl PCR Masterrmix (Bioneer) and 5 pmol of each primers (forward primer labeled at 5` with FAM, PET and VIC fluorescent dyes).

The PCR mix was subjected to an initial denaturation at 95°C for 3 min, followed by 35 cycles of 30 s at 94°C, 30 s at 58°C (for Set-I and II) or 60°C for (Set-III) and 30 s at 72°C with final extension of 20 min. 0.5 microliter of multiplex PCR products was mixed with 15 µl of GeneScan-500 LIZ Size Standard (Applied Biosystems) and 1 microliter of Hi Di formamide (Applied Biosystems) and subjected to electrophoresis (1.5 agarose, 2.5µl gel red, at 135 volts current for 35 min). The resulting PCR mixture was denaturated by incubation at 95°C for 3 min using Thermocycler (Gene Amp™ PCR System 9700, Applied Biosystems, USA).

Fragments were identified and eluted by capillary electrophoresis and collected data were analyzed by Gene Mapper version 4. The genotyped data were analyzed for statistical significance using GenAlex 6.5 (PeakallandSmouse, 2012).

Table 1. SSR Information used in this study

Markers	Marker sequences		Florescent Dye	Allele Size (bp)
	Forward (5`- 3`)	Reverse (3`-5`)		
BMS2508	Tttctgggtttacaaaatgctc	Tttcttaggggagtgttgattc	PET (Red)	103-153
BM143	Acctgggaagcctccatatc	Ctgcaggcagattctttatcg	FAM (Blue)	94-116
OarAE101	Ttcttatagatgcactcaagctagg	taagaaatatatttgaaaaaagtgtat	FAM (Blue)	99-137
OarAE129	aatccagtgtgtgaaafactaatccag	gtagatcaagatatagaatatttttcaacacc	VIC (Green)	141-175
OarHH55	gttattccatattctttcctccatcataagc	Ccacacagacaactaaaacccagc	PET (Red)	113-151

Results and Discussion

The selected Short Sequences Repeats(SSRs) were successfully amplified in three multiplex sets. Designed considering annealing temperature, product size and dye label. A similar throughput multiplex system for goats was demonstrated (Meutchieye et al, 2014) for Cameroonian goats using 12 SSRs and Hassen *et al* (2012) in Ethiopian goats using 15 SSRs. The four goat populations (Sudan Taggar, Sudan Desert, South African Boer and Kalahari) exhibited high genetic diversity (Table 2) of all markers studied, where BMS2508 marker displayed a maximum allele numbers of 33 while BM143 marker, displayed the minimum allele number of 21 alleles. Barker (1994) suggested that SSR loci should have more than four alleles for studies of genetic distances to reduce the standard error of distance estimates.

Table 2. Genetic Diversity Parameters for the loci pooled over the four goat populations studied

Marker	Min.AZ (bp)	Max.AZ (bp)	N_a	N_e	H_o	H_e	I	PIC (%)	F_{is}
BMS2508	103	153	33	17.45	0.673	0.712	1.60	100	0.082
BM143	94	116	21	14.47	0.103	0.701	1.38	100	0.854
OarHH55	113	151	22	10.76	0.537	0.589	1.19	100	0.066
OarAE101	99	137	25	10.45	0.663	0.606	1.19	100	-0.046
oarAE129	141	195	23	13.03	0.672	0.672	0.91	100	-0.013
Overall Mean			24.8	13.23	0.531	0.561	1.25	100	0.189

AZ = Allele Size

The overall pooled number of alleles (N_a), observed heterozygosity (H_o), expected heterozygosity (H_e) and polymorphic information content (PIC), for the four populations studied were presented in table(2). They were, 0.531, 0.561, 0.684 and 100, respectively. The values for these parameters are higher than those reported by Meutchieye *et al.* (2014) for H_O (0.219), H_e (0.298) and PIC of (0.39). In our study, the overall N_a value was 24.8 which is moderately less than the value of (26.1) that was reported by Meutchieye *et al* (2014) for Cameroonian native goat ecotypes, but is higher than the value of N_a = (14.6)reported for 8 Turkish goat populations by Bulut *et al.* (2016) . The differences between our findings and those reported by Meutchieye *et al.* (2014) and Bulut *et al,* (2016) might be due to differences in the number of markers used and their informativeness for genotyping.

Hassen *et al.* (2016) reported a value of 2.13 for Shannon's Index for diversity in3 Syrian goat populations which is higher than the estimate of (1.25)found for Sudan goat populations in the present study. The difference might be associated with the number of microsatellites used in these two studies, and the sample size. The inbreeding coefficient (F_{is}) in the present study amounted to 0.189 which is less than the value of 0.32 that was reported for Syrian goats (Hassen *et a*l., 2016).

Table 3. Genetic Diversity estimates in Sudan Tagger goat

Marker	**Min.AZ (bp)**	**Max.AZ (bp)**	**N_a**	**N_e**	**H_o**	**H_e**	**I**	**PIC (%)**	**F_{is}**
BMS2508	103	143	14	6.10	0.784	0.836	2.14	100	0.063
BM143	96	116	08	5.09	0.068	0.804	1.78	100	0.915
OarHH55	113	149	07	3.29	0.664	0.701	1.48	100	0.075
OarAE101	99	137	11	3.44	0.730	0.709	1.56	100	-0.029
oarAE129	141	175	09	3.40	0.712	0.706	0.71	100	-0.009
Overall Mean			9.8	4.3	0.592	0.754	1.53	100	0.203

Table 4: Genetic Diversity estimates in Sudan Desert goat

Marker	Min.AZ (bp)	Max.AZ (bp)	N_a	N_e	H_o	H_e	I	PIC (%)	F_{is}
BMS2508	103	153	10	5.93	0.085	0.831	2.01	100	-0.018
BM143	98	108	04	3.45	0.015	0.710	1.31	100	0.783
OarHH55	113	151	07	3.52	0.615	0.716	1.51	100	0.140
OarAE101	101	127	06	2.40	0.462	0.583	1.23	100	0.208
oarAE129	141	175	05	3.57	0.400	0.720	0.400	100	0.444
Overall Mean			6.4	3.77	0.315	0.712	1.29	100	0.311

Table 5. Genetic Diversity estimates in SA.Boer goats

Marker	Min.AZ (bp)	Max.AZ (bp)	N_a	N_e	H_o	H_e	I	PIC (%)	F_{is}
BMS2508	103	143	5	3.56	0.750	0.719	1.42	100	-0.043
BM143	94	102	5	3.66	0.125	0.727	1.42	100	0.828
OarHH55	113	135	3	1.56	0.429	0.357	0.66	100	-0.200
OarAE101	105	127	4	2.39	0.714	0.582	1.06	100	-0.228
oarAE129	141	169	5	3.66	0.875	0.727	1.42	100	-0.204
Mean			4.4	2.97	0.579	0.622	1.20	100	0.030

Table 6. Genetic Diversity estimates in SA. Kalahari goats

Marker	Min.AZ (bp)	Max.AZ (bp)	N_a	N_e	H_o	H_e	I	PIC (%)	F_{is}
BMS2508	103	131	4	1.86	0.313	0.463	0.82	100	0.325
BM143	94	100	4	2.28	0.063	0.561	1.01	100	0.889
OarHH55	113	145	5	2.39	0.438	0.582	1.12	100	0.248
OarAE101	99	113	4	2.23	0.625	0.551	0.92	100	-0.135
oarAE129	141	163	4	2.40	0.750	0.534	1.09	100	-0.284
Mean			4.6	2.23	0.438	0.538	0.99	100	0.209

The polymorphic Information Content (PIC) value of 1was found for all the Sudan goat populations (indigenous and introduced) under study (table, 3, 4, 5 and 6).This estimate is higher than those reported for 10 Indian goat populations (0.08 – 0.90) by Romamoorthi *et al.* (2009), Aggarwal *et al.* (2007) and Widmer *et al.*(2001). Also, Mahmoudi *et al.* (2011) and Mahmoudi *et al.* (2010) reported PIC range in 6 Iranian goat populations of 0.71 – 0.81 which is less the estimate reported here. The findings in our study are also higher than those reported by Qi *et al.* (2009) and Li *et al.*(2002) in 22 goat populations in China. The present results confirm that the five microsatellites used are appropriate markers to be used for molecular diversity studies in goats.

A relatively high rate of inbreeding (F_{is}= 0.311, 0.209, 0.203 and 0.03) was shown in (Table, 3,4,5 and 6) was found in Sudan Dessert, SA-Kalahari, Sudan Taggar and SA-Boer, respectively. Among the four populations the F_{is} values were highest for Sudan Desert, possibly because of the small population size from which the sample was taken.

Hassen *et al*, (2016) reported F_{is} value of 0.33 from Syrian Jabali goat data, which was a higher estimate than those found in the present study in Sudan Tagger, SA- Kalahar and SA-Boer. The inbreeding coefficient of Sudan Desert goats is close to that of Syrian Jabali goat.

The F_{is} estimates obtained for Sudan Desert, SA-Kalahari and Sudan Tagger showed high rates of inbreeding within the populations when compared with the 45 rare breeds of 15 European and Middle Eastern countries reported by Canon *et al.* (2006) who reported an estimate of F_{is} of 0.10.

The Boer goats sampled from Kuku Seed Stock farm, showed rather low inbreeding .This could be associated with the large number of foundation animals used as bucks (245), while this is not true for the Kalahari flock (95 buck) that showed a higher inbreeding coefficient.

Conclusion

To conclude, the present study showed that all the five SSR markers used were appropriate for molecular characterization of Sudan Desert, Sudan Taggar and SA-Boer and Kalahari Red goats bred in Sudan. They amplified successfully and exhibited high PIC and allelic polymorphism. Based on allele numbers, observed and expected heterozygosities, the four goat population's exhibited good amount of genetic diversity. The results reflect that the populations studied contain valuable and substantial amounts of genetic diversity within flocks studied and there is a good scope for bringing effective genetic improvement, sustainable conservation and designing future policies for those goats in Sudan.

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Authors:

Yassir Ahmed Hassan

Josephine Birungi

Nielson Mandela Agaba