Analysis of Y Chromosome Diversity and Genetic Relationships of The African Domestic Sheep

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Abstract

Two male specific Y chromosome (*MSY*) markers, a single nucleotide polymorphism (SNP) A/G and a microsatellite marker *SRYM18* were used to study genetic relationships and Y chromosome diversity of 35 distinct sheep populations. Five microsatellite alleles of sizes 131 bp, 139 bp, 141 bp, 143 bp, and 145 bp were detected. The 143 bp allele was distributed in both the thin-tailed and fat-tailed sheep and across geographical regions. It was the most common on the continent with a frequency of 72.9%. The 141 bp allele was most common in the thin-tailed sheep compared to the fat-tailed sheep with a continent-wide frequency of 19.7%. The 145 bp allele was only found in the fat-tailed sheep at a frequency of 8.8%. The 131 bp and the 139 bp alleles were the least common on the continent with a respective frequency of less than 1%. Based on the distribution of these alleles in populations, the most diverse population was the thin-tailed Djallonké of Senegal and Maure of Mali. The lowest Y chromosome diversity was observed in the three fat-tailed populations, Gumuz, Sekota and Tukur of Ethiopia which had only one allele type, the 143 bp. Other than these five populations all the other populations had at least two different alleles. SNP screening was less informative as only the A allele was detected. Three haplotypes 141/A, 143/A and 145/A with a respective frequency of 16.7%, 72.5% and 10.8% were constructed based on the data from the two markers. Haplotype 143/A was most common in the fat-tailed sheep of East Africa (90.5%) and Southern Africa (60.0%) while haplotype 141/A was most common in the thin-tailed sheep of West Africa (87.5%) This study shows that genetic variations on the Y chromosome exists within African sheep and corroborate existing archaeological information suggesting distinct origin of the West African thin-tailed and the fat-tailed sheep of East and Southern Africa.

Key Words: Sheep, Y chromosome, *SRYM18*.

Introduction

The origin of the modern domestic sheep (*Ovis aries)* remains uncertain. Existing wild species includes urial (*Ovis viginei,* disputed scientific classification), mouflon (*Ovis musimon*), argali (*Ovis ammon*), bighorn (*Ovis canadensis*), thinhorn (*Ovis dalli*) and Barbary sheep (*Ammotragus lervia*). Of these, the only presumptive wild ancestors of the domestic sheep are urial sheep, mouflon sheep and argali sheep (Ryder, 1984). Sheep domestication is thought to have started in Western and Eastern Asia (MacDonald, 2000). Using mitochondrial DNA, Hiendleder *et al*. (1998), suggested two ancestral maternal sources for *Ovis aries*. The first ancestor is shared by the modern domestic sheep and European mouflon, while the second ancestor is yet to be identified. That study suggested that the European mouflon could have been derived from an early-domesticated sheep other than urial and argali. This view has received more support (Hiendleder *et al.* 2002), but in addition a third maternal lineage that suggest multiple independent domestication events has also been reported in the Turkish sheep (Pedrosa *et al.* 2005).

The sheep breeds of Africa are broadly classified into two main types, thin-tailed (TT) and fat-tailed (FT) (Mason & Maule, 1960). After domestication in Asia, these sheep entered into the African continent through two entry points, the Isthmus of Suez and the Horn of Africa (MacDonald, 2000). The thin-tailed sheep were likely the first type of sheep to enter the African continent possibly through the Isthmus of Suez into the present day Egypt or through the South extremity of the Sinai Peninsula and spread to West Africa, and the present day Sudan (Wilson, 1991; Muigai, 2003) and possibly Southern Africa. The fat-tailed sheep likely entered the continent first, through the Isthmus of Suez into the current Egypt and spread westward replacing some of the thin-tailed sheep of North Africa and later through the Horn of Africa before spreading in the whole of East Africa, Southern Africa and to some extent the Sahelian belt. This theory of sheep entry into the continent through separate points was initially based on archaeological and breed observation information, but has recently received further support from studies using molecular markers (Muigai, 2003).

Since their domestication around 7000 to 9000 Before Christ (BC), sheep have significantly influenced the economic, social and cultural foundation of human society. In sub-Saharan Africa, it is estimated that there are over 240 million sheep (FAO, 2004). About 80% of the African sheep are local breeds that are maintained under traditional farming systems (Lebbie *et al.* 1996). Consequently, these breeds have become adapted to a wide range of environments, showing high levels of phenotypic variability and increased fitness under natural conditions. These naturally evolved genetic characteristics provide sustainable options for disease resistance, survival and efficient production that has been ignored in the drive for technological and management solutions to individual problems of livestock production in the tropics. For example, in most livestock breeding programmes a small number of males with 'good characteristics' are normally used. If unchecked, this could lead to inbreeding and therefore reduce diversity in subsequent generations (Bell, 2003). According to Rege *et al.* (1996), 30% of the indigenous animal genetic resource of Africa is at high risk of loss. An essential step in any strategy for the conservation of animal genetic resources involves documentation of the available livestock and measurement of their diversity and genetic relationships.

With the advent of molecular techniques, an increasing number of studies have focused on genetic characterization of domestic breeds using molecular markers. Such studies have relied exclusively on autosomal and mitochondrial DNA (mtDNA) markers. A study by Muigai (2003) using autosomal microsatellite markers revealed that Africa is hosting a very large and diverse indigenous sheep genetic resource that needs to be conserved.

Unlike autosomal or X-specific loci, both mtDNA and the male-specific region of the Y chromosome do not recombine with autosomes or the X chromosome. Therefore, polymorphisms on a mitochondrial molecule or on a Y chromosome share the history of a single female or a single male lineage, respectively. The use of Y chromosome haplotyping in natural populations of species, other than humans is ,however, still hindered by the lack of Y chromosome markers and sequence information. In cattle, analysis of the Y chromosome has proven to be highly insightful for unraveling the processes of domestication and breed development (Bradley *et al.* 1996; Hanotte *et al.* 2000; Kikkawa *et al.* 2003). Recently in sheep, a novel bi-allelic SNP located in the *MSY* region (Meadows *et al.* 2004) and a polymorphic multi-allelic microsatellite *SRYM18* (Australian Sheep Gene Mapping Website, 2003) has

been described. In this study, the two Y specific DNA markers were used to define Y chromosome diversity and relationships of the African male sheep.

Materials and Methods

DNA Samples

The following African populations/breeds were studied: (1) Afar (FT, Ethiopia, n=32), (2) Arsi-bale (FT, Ethiopia, n=22), (3) Bali-bali (TT, Mali, n=7), (4) Balami (TT, Nigeria, n=12), (5) Black head Persian (FT, S. Africa, n=10), (6) Damara (FT, Namibia, n=18), (7) Djallonké (TT, Nigeria, n=19), (8) Djallonké (TT, Senegal, n=34), (9) Gumuz (FT, Ethiopia, n=19), (10) Horro (FT, Ethiopia, n=19), (11) Kabale (FT, Uganda, n=7), (12) Karakul (FT, Namibia, n=25), (13) Maure (TT, Mali, n=12), (14) Moroto (FT, Uganda, n=20), (15) North West Highland (FT, Ethiopia, n=25), (16) Pedi (FT, S. Africa), (17) Red Maasai (FT, Kenya, n=15), (18) Red Maasai (FT, Tanzania, n=22), (19) Rutana (TT, Ethiopia, n=21), (20) Sabi (FT, Zimbabwe, n=14), (21) Sekota (FT, Ethiopia, n=15), (22) Sukuma (FT, Tanzania, n=24), (23) Tukur (FT, Ethiopia, n=17), (24) Touareg (TT, Mali, n=19), (25) Tswana (FT, Botswana, n=11), (26) Ugogo (FT, Tanzania, n=18), (27) Wollo (FT, Ethiopia, n=19) and (28) Yankassa (TT, Nigeria, n=10). The following non-African breeds were also included in the study: Barbados black belly (TT, US Virgin Island, n=5), St Croix (TT, US Virgin Island, n=12), Lohi (FT, Pakistan, n=6), Portuguese white merino (TT, Portugal, n=12), Salt range (FT, Pakistan, n=4), Swaledale (TT, UK, n=7) and Texel (TT, UK, n=3). Sampling was based on the geographical locations as well as history and relatedness of breeds/populations. DNA and peripheral blood lymphocyte cells samples were obtained from ILRI sheep blood and DNA database. DNA was extracted using a modified phenol/chloroform protocol (Sambrook *et al.* 1989).

Microsatellite Analysis

DNA amplification was done using GeneAmp PCR system 9700 (Applied Biosystems, Warrington, UK). A 10 μl reaction for the microsatellite marker *SRYM18*, consisted of 40 ng template DNA, 0.1 μM of each primer (Forward = 5' fluorescent Tag 6FAM–GGCATCACAAACAGGATCAGCAAT–3'; Reverse = 5'–GTGATGGCAGTTCTCACAATCTCCT-3'), 1.5 mM $MgCl_2$, 0.125 mM dNTP, and 0.3 U *Taq* DNA polymerase (Promega, Madison, U.S.A). Initial denaturation was done for 5 minutes at 95 °C followed by 30

cycles of 30 seconds at 95 °C, 30 seconds annealing at 60 °C, 1 minute of primer extension at 72 °C and a final extension of 10 minutes at 72 °C. Female samples and water were used as negative controls.

Genotype analysis was carried out on the automated capillary DNA sequencer (ABI 3730) and the GeneMapper™ software V 3.7 (Applied Biosystems, Warrington, UK). Scored alleles were used to calculate allele frequencies. The allele data was used to calculate variance within breeds, breed types and groups using Arlequin software V 3.0 (Excoffier *et al.* 2005). Logistic regression was done to test the significant differences in the proportion of each allele using PROC LOGISTIC in SAS (PC-SAS V 9.1, SAS institute, Cary, NC, USA).

To confirm the scored genotypes and assess the level of homoplasy, the PCR product of selected alleles were purified using Qiagen kit and sequenced using BigDye® terminator cycle sequencing kit according to the manufacturer's recommendation (Applied Biosystems, 2001). Data of the DNA sequence was edited using Chromas lite software V 2.01 and aligned using ClustalX (1.81) (Thompson *et al.* 1997).

Single Nucleotide Polymorphism Analysis

A 20 µl reaction for the single nucleotide polymorphism marker *SRY3* consisted of 40 ng template DNA, 0.1 µM of each primer (Forward = 5' fluorescent Tag PET – TCAGTAGCTTAGGTACATTCA – 3'; Reverse = 5' - GTGCTACATAAATATGATCTGC - 3', 1.5 mM $MgCl_2$, 0.125 mM dNTP, and 0.6 U *Taq* DNA polymerase (Promega, Madison, U.S.A). Initial denaturation was done for 15 minutes at 95 °C followed by 35 cycles of 45 seconds at 95 °C, 45 seconds annealing at 60 °C, 45 seconds of primer extension at 72 °C and a final extension of 10 minutes at 72 °C. Female samples and water were used as negative controls.

Screening was based on RFLP using A/G SNP specific *Fnu4H*I restriction enzyme. A 27 µl digestion reaction consisted of 15 µl aliquot of the PCR product, 10 µl of triple distilled deionized water, 2 U *Fnu4H*I restriction enzyme and 1X NEB buffer 4 (50 mM potassium acetate, 20 mM Tris-acetate, 10 mM magnesium acetate, 1 mM DTT, pH 7.9) (New England BioLabs, Inc., Beverly, USA). The PCR plate containing the digestion mix was incubated for 2 hours at 37°C. Fragments were analyzed on a 2% agarose gel using 1X Tris-Borate EDTA (pH 8.0) gel and running buffer. Existence

of two bands (138 bp and 473 bp) is an indication of the A SNP while existence of 3 bands (49 bp, 89 bp and 473 bp) is an indication of the G SNP.

Results

The microsatellite marker *SRYM18* was optimized against both male and female individuals and was found to be male specific. Genotyping revealed the presence of five alleles of sizes 131, 139, 141, 143 and 145 bp. The allele frequencies for each breed were used to construct pie charts (Figure 1). In most breeds, two different alleles were detected with varying frequencies indicating Y chromosome diversity. Two African breeds, Djallonké from Senegal and Maure of Mali showed the highest Y chromosome diversity with three different alleles, the 139 bp, 141 bp and 143 bp. This level of diversity was not observed in any other breed from outside Africa. In another three African breeds, Gumuz, Sekota and Tukur which are fat-tailed sheep from Ethiopia, only one allele type (143 bp) was observed implying lack of Y chromosome diversity. A similar scenario was also observed in a majority of breeds from outside Africa with the exception of St Croix from the US Virgin Island and Swaledale from the UK that had two alleles each.

The sheep breeds from each region of Africa were segregated into two main breed types, fat-tailed sheep and thin-tailed sheep. The allele frequencies for either of the breed type were calculated and the results are presented in Table 1. The 143 bp allele is the most common in both the fat-tailed (80.6%) and thin-tailed (51.3%) sheep of Africa. The allele frequencies are significantly different between the two types of sheep ($P < 0.0001$). The 143 bp allele is also significantly more frequent in the fat-tailed sheep of East Africa (90.2%) compared to the fat-tailed sheep from Southern Africa (53.0%) and thin-tailed sheep of West Africa (51%) ($P < 0.0001$). In the Asian fat-tailed sheep, it has a frequency of 40.0% while in the thin-tailed sheep of Europe and Central America, it has a frequency of 63.0% and 13.0% respectively. The frequency of this allele in the Asian and European sheep is not significantly different from the frequency of this allele on the African continent ($P > 0.05$). But, the frequency in Central America is significantly lower than the frequency on the African continent ($P < 0.05$).

The 141 bp allele appears in both the thin-tailed (47.0%) and fat-tailed (10.0%) breed types of Africa. These allele frequencies are not significantly different between the two sheep types ($P > 0.05$). It has a significantly higher frequency (35.0%) in the Southern Africa fat-tailed sheep compared to 1.2%

in the East African fat-tailed sheep ($P < 0.0001$). Although the Southern Africa frequency (35%) for the fat-tailed sheep is quite close to that of the West African thin-tailed sheep (47%), it is significantly different ($P < 0.0001$). The 141 bp allele does not appear in any breed outside Africa.

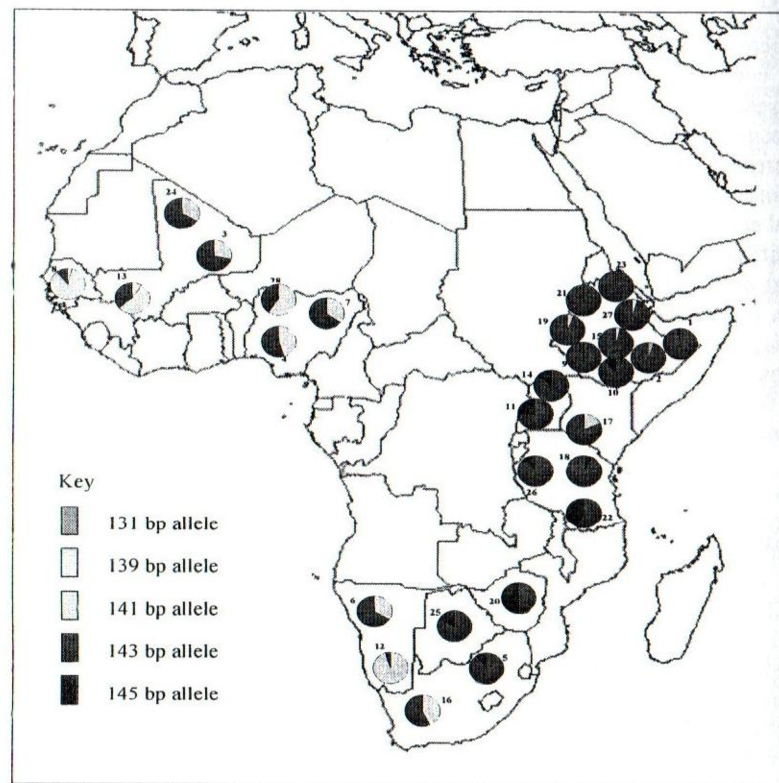

Figure 1. Pie charts of allele frequencies at microsatellite *SRYM18* for African breeds. Each breed is identified by a unique number provided in the section for DNA samples.

In the fat-tailed sheep, the 145 bp allele had a frequency of 8.8% and it was not observed in the thin-tailed sheep breeds of Africa. Regionally, its frequency is significantly higher within the Southern African fat-tailed sheep

breeds (12%) compared to 7.8% in the East African fat-tailed sheep breeds ($P < 0.05$). On the contrary, in non African breeds it had a significantly higher frequency of 87.0% in the thin-tailed breeds of Central America and 37.0% in the thin-tailed breeds of Europe compared to 6.5% for the African sheep ($P < 0.05$). The allele was however, absent in the fat-tailed breeds of Asia.

Table 1. Microsatellite allele frequencies of sheep breeds classified by type and region

Breed type	Number and % frequency					
Allele in bp	131	139	141	143	145	Total
E. African fat-tailed	1.0	1.0	3.0	221.0	19.0	245
% Frequency	0.4	0.4	1.2	90.2	7.8	100
Southern African fat-tailed	0.0	0	30.0	45.0	10.0	85
% Frequency	0.0	0.0	35.0	53.0	12.0	100
African fat-tailed	1.0	1.0	33.0	266.0	29.0	330
% Frequency	0.3	0.3	10.0	80.6	8.8	100
East African thin-tailed	0.0	0.0	1.0	15.0	0.0	16
% Frequency	0.0	0.0	6.3	93.7	0.0	100
West African thin-tailed	0.0	2.0	54.0	45.0	0.0	101
% Frequency	0.0	2.0	47.0	51.0	0.0	100
African thin-tailed	0.0	2.0	55.0	60.0	0.0	117
% Frequency	0.0	1.7	47.0	51.3	0.0	100
African fat-tailed and thin-tailed	1.0	3.0	88.0	326.0	29.0	447
% Frequency	0.2	0.7	19.7	72.9	6.5	100
Asian fat-tailed	0.0	6.0	0.0	4.0	0.0	10
% Frequency	0.0	60.0	0.0	40.0	0.0	100
European thin-tailed	0.0	0.0	0.0	12.0	7.0	19
% Frequency	0.0	0.0	0.0	63.0	37.0	100
Central American thin-tailed	0.0	0.0	0.0	2.0	13.0	15
% Frequency	0.0	0.0	0.0	13.0	87.0	100
Total pop. size						491

The 139 bp allele was detected in only one fat-tailed individual (0.4%) and two thin-tailed individuals (2.0%) from East and West Africa, respectively. In non-African breeds, it had a frequency of 60% in fat-tailed sheep from Pakistan. None was observed in breeds from Europe and Central America.

The 131 bp allele was observed in a single fat-tailed sheep (Arsi-bale 10) from East Africa and the calculated frequency was 0.4%. To rule out on artifacts, the DNA from this individual was re-amplified and re-genotyped and the same result was confirmed.
Analysis of molecular variance showed that the highest variation (55.3%) was within breeds. This diversity decreased to 16.9% when sheep in the same geographical area were compared to each other and; 27.9% when sheep in different regions of Africa (East, West and Southern Africa) were compared to each.

Sequence data showed that the pentanucleotide repeat is fixed at three units in the domestic sheep and European mouflon *Ovis musimon*. This pentanucleotide repeat unit was fixed at four in the bighorn *Ovis canadensis* and thinhorn *Ovis dalli* (Meadows *et al.* 2006).

Single Nucleotide Polymorphism Analysis

The SNP marker *SRY3* was optimized against both male and female individuals and was found to be male specific. Subsequently all the 491 individuals were screened for the SNP and scored accordingly. All individuals (n = 136) had the A allele, the rest of the samples did not amplify for unknown reasons. This allele was detected in both the thin-tailed and fat-tailed sheep of Africa as well as non African sheep.

Haplotypes

A combination of alleles at different loci leads to formation of different haplotypes. This provides more information compared to the use of one locus. Three haplotypes (H4, 145/A; H6, 143/A and H8, 141/A) were identified (data not shown) with frequencies of 10.8%, 72.5% and 16.7% respectively in the African sheep. Since only the A allele was observed for the SNP, all the haplotypes only differ in their microsatellite fragment size. Consequently, haplotype frequencies match their respective allele frequencies; the small variation is due to differences in success of amplification of the two markers.

Discussion

The microsatellite data shows that genetic variation on the Y chromosome exists within African sheep breeds. Selection and breeding based on a few individuals could lead to fixation of the selected allele within the breed (Henson, 1992), which in turn could reduce diversity. Given the culture of African pastoralists, selection is not strictly practiced. Animals are kept for multipurpose use rather than for a single commercial objective (McCorkle, 1999) and mating within and some times between herds is not strictly controlled. In three fat-tailed sheep (Gumuz, Sekota and Tukur) from Ethiopia only one allele type (143 bp) was observed, indicating that diversity in these breeds is limited. In contrast, Djallonké from Senegal and Maure from Mali, both of which are thin-tailed sheep from West Africa, had the highest level of diversity with three different alleles (139 bp, 141 bp and 145 bp). The higher diversity in the West African sheep compared to the Eastern and Southern Africa sheep could be an indication of different breeding structures in their centres of origin before entry into Africa. The West African sheep breeds could have originated from a centre of domestication with a higher genetic pool compared to that of the Eastern and Southern Africa sheep breeds, alternatively, this could also be attributed to higher levels of crossbreeding.

The distribution of diversity was highest within breeds, between breeds in different geographical regions and low between breeds in the same geographical location. Most likely this could be due to trade and crossbreeding (McCorkle, 1999), as well as low mutation rates on the mammalian Y chromosome (Hellborg & Ellegren, 2004; Meadows, *et al.* 2004). Our results agree with an earlier study using autosomal microsatellite markers (Muigai, 2003). In our study, the diversity of the Y chromosome microsatellite was found to be higher in the African sheep compared to breeds from outside the African continent. However, when these results were compared to the results obtained from Meadows *et al.* (2006), the African sheep Y chromosomes are as diverse as that of sheep from outside Africa. It is likely that the low number of non-African sheep used in our study may have contributed to the low diversity observed in non-African samples.

The sheep breeds in different geographical locations of Africa are phenotypically different. This study has shown that the 141 bp allele is common within the thin-tailed sheep. Similarly, the 143 bp and 145 bp alleles are common within the fat-tailed sheep. This supports the hypothesis that the

African fat-tailed and thin-tailed sheep entered into the continent separately. The 141 bp allele is likely to be the most ancient on the African continent followed by the 143 bp and 145 bp alleles, respectively. These findings are consistent with archaeological studies that suggest a different origin for the West Africa thin-tailed sheep and the fat-tailed sheep of East and Southern Africa. Muigai (2003) using autosomal microsatellite markers made a similar observation and further proposed that there could have been migration of sheep from West Africa to Southern Africa. This study provides further evidence for the migration of sheep from Eastern and Western Africa to Southern Africa. The presence of both the 141 bp and 143 bp alleles in all the three regions could be a result of introgressions. This shows that African stock-raisers may have used crossbreeding strategies to improve their sheep stock as proposed by McCorkle (1999).

Characterization of the A/G SNP described in sheep produced only the A allele. Comparative sequencing (Meadows *et al.* 2004; Meadows *et al.* 2006) has shown that an A/G allele exists on the ovine Y chromosome, though the G allele is very rare and so far has not been reported in any African sheep (Meadows *et al.* 2006). Most of our samples did not amplify for unknown reasons, possibly due to mutations at the primer site or at the *Fnu4HI* restriction site. No polymorphisms were observed at the SNP and therefore no new information was obtained through the study of this marker compared to the microsatellite marker. In other words, haplotypes combining the microsatellite locus and the SNP, had frequencies identical to the microsatellite allele frequencies. However, it should be noted that we failed to amplify the *SRY3* loci in most individuals. From these results, despite the fact that the A SNP allele was the only one observed, it is premature to conclude that it is the only one present on the African continent.

Eleven haplotypes have been reported in sheep (Meadows *et al.* 2006) based on the two loci. The five haplotypes reported in the African and European samples were: H4 (145 bp/A), H6 (143 bp/A), H8 (141 bp/A), H9 (131 bp/A) and H10 (110 bp/A). On the contrary, haplotype H5 (145 bp/G) and haplotype H7 (143/G) reported by Meadows *et al.* (2006), were not observed in the African breeds studied (Arsi-bale, Balami, Pedi, Sabi, Sukuma, Tswana and West African dwarf sheep). In our case, the haplotypes detected were, H4, H6 and H8. This study therefore, confirms the presence of haplotype, H4, H6 and H8 in African sheep. The H6 haplotype has been associated with the European mouflon (Meadows *et al.* 2006). Since this haplotype is also common in the sheep of Africa, it is therefore most likely

that the African sheep share a patriline with the European mouflon (*Ovis musimon*). The African and Asian domestic sheep share the 139 bp allele, which is lacking in sheep from Europe and Central America. This confirms that the African and Asian sheep share a patriline.

Conclusions and Recommendations

This study has contributed to the understanding of the origin, migration, diversity and breeding structure of the African sheep. Studies of the Y chromosome polymorphism in livestock are still rare. There is lack of informative markers at the Y chromosome. However, in the recent past, the interest in Y chromosome has been rekindled by the development of new markers (Australian Sheep Gene Mapping Website, 2003; Meadows, *et al.* 2004). The results presented in this study are an initial attempt at the use of Y chromosome markers in the genetic characterization of the African sheep. New information generated from future genetic and phenotypic studies, together with these and previous genetic and phenotypic findings if harmonized will be invaluable for breeding, conservation and utilization of the African sheep.

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