# GENETIC DIVERSITY AND EVOLUTIONARY ANALYSIS OF GOAT mtDNA HVR1

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**Abstract**
*Seventy six sequences comprising of samples from Nigeria, India, China, South Korea and Kenya were used in the diversity and evolutionary studies of the mtDNA HVR1 region in goats. Genetic relatedness based on neighbor joining (NJ) tree and median joining network revealed that the Kenyan native goat as an intermediate breed between Nigerian and Asian goat breeds and a complex gene flow among Nigerian goat breeds. Significant positive Taijima's D value (2.335, $p<0.05$) for this tests discounted neutral hypothesis. Evolutionary analysis suggests both adaptive and purifying selection pressures that suggest a decrease in population size and or balancing selection in the mtDNA HVR1 region in goats.*

**Key words:** diversity, evolution, goat, mtDNA

## Introduction

Mitochondrial DNA (mtDNA) polymorphisms have been largely applied not only to better understand phylogenetic relationships in many animal species (Kim *et al.*, 2010; Taberlet *et al.*, 2011) but also in understanding maternal origin and migration of livestock(Akis *et al.*, 2014; Zhao *et al.*, 2014). The hypervariable region I (HVR1) of mitochondrial DNA (mtDNA) contains highly informative polymorphic sites useful for diversity studies in many organisms and species (Akis *et al.*, 2014; Zhao *et al.*, 2014).

Although a few studies examining the nuclear microsatellite DNA of Nigerian goats have been reported (Okpeku *et al.*, 2011; Muema *et al.*, 2009; Adebambo, 2003), there is no published report on mtDNA diversity in Nigerian goats. Therefore, we have investigated the hypervariable region 1 (HVR1) of the caprine mitochondrial genome in Nigerian goat breeds sequences along with archived sequences NCBI to gain a better understanding of matrilineal origin of Nigerian goats and evolutionary force at work. This will be important in facilitating the development of new approaches to improve selection and mating systems for animal breeding and conservation programmes.

## Materials and Methods

Sample collection and DNA isolation

Whole blood was collected from unrelated Nigerian goats belonging to the 3 major goat breeds [Red Sokoto (RSO); Sahel (SAH) and West African Dwarf (WAD)]. Samples were collected from different locations. Blood samples were collected by jugular venipuncture and DNA was isolated using ZymoBead™ Genomic DNA kit following the manufacturer's instructions.

PCR amplification and DNA sequencing

To amplify the HVRI of goat mtDNA, a pair of primers was designed using the known goat mtDNA sequence (AB044304, (FAOSTAT, 2013) in the GENETOOL package and synthesized in an ABI 392 Oligosynthesizer (Perkin Elmer, Foster City, CA). Primer sequences were as follows: forward '5-CATCCATATAACGCGGACAT-3' and reverse '5-GTGTGAGCATGGGCTGATTA-3'. PCR amplification was carried out in a 10 µl reaction volume containing 5 ng of DNA, 10 pM of each primer, 200 pM of dNTPs, 1X PCR buffer containing 2 mM $MgCl_2$, and 2U of AmpliTaq Gold (Perkin Elmer, Foster City, CA). Amplification was performed in a Veriti thermal cycler (Applied Biosystems, Carlsbad, CA) employing the following conditions: 94°C for 5 min; 35cycles at 94°C for 30sec, 54°C for 45sec, and 72°C for 2 min; and final extension at 72 °C for 5 min. The amplicons were sequenced using BigDye™ (Applied Biosystems, Foster City, USA) Terminator cycle sequencing kit in ABI Prism 3730 DNA Analyzer (Applied Biosystems, Foster City, USA) following manufacturer's protocol.

Data analysis

A total of Seventy six sequences comprising of samples from Nigeria, India, China, South Korea and Kenya were used in the analysis with one sequence from Ovis aries as an out group (accession numbers in additional file). All sequences were aligned MEGA 6.0 (Tamura et al. 2013). Haplotype and nucleotide diversity were estimated in DNASP 5.10.01 (Librado and Rozas, 2009). Median joining networks (Bandelt *et al.*, 1999) was drawn using the Network 3.1.1.1 software (www.fluxus-engineering.com).

Tests neutrality and natural selection acting on mtDNA HVR1 codons in goat

To test the hypothesis of neutrality, DnaSP v5.10.01 (Rozas *et al.* 2003) was used to estimates *Tajima's D* (Tajima, 1989). To determine the type of natural selection acting on *mtDNA HVR1* sequence were submitted to the Selection Server, version 2.2 (**Stern *et al.*** 2007). (http://selecton.tau.ac.il/results/1451327227/colors.html).

## Results

### Phylogenetic relationship of mtDNA Sequences in goats

We aligned all 76 sequences in MEGA 6 (Tamura et al. 2013) and representative samples were used in constructing Neighbor joining (NJ) tree. NJ tree revealed a close relatedness between Nigerian goats and Kenyan goats while Asian goats (Figure 1). Further study of genetic relatedness done in network (Figure 2) revealed Kenyan goat breed as an intermediate breed between Nigerian goat breeds.

### Neutrality test and prediction of selection pressure on mtDNA codon

In order to understand selection forces that might be operational in the process of diversification of mtDNA in goats; we conducted *Taijama's D*statistics with DnaSP v5.10.01 (Rozas *et al.* 2003) to examine whether the number of segregating sites departs from the neutral expectation (Table 1). Calculation was based on the total number of segregating sites. Significant positive *Taijima's D* value (2.335, $p<0.05$) for this tests discounted neutral hypothesis.

The MEC model of the Selection Server was used to evaluate prevailing selection pressure acting on the mtDNA codons. Projected positive or negative selection on amino acid sites for the MEC model is shown in Fig. 3. As can be seen, both purifying and adaptive selection pressures are actively at work suggesting both the influence of purifying and adaptive selection.

## Discussion

Nigerian goat mtDNA sequences showed some degree of diversity in the mtDNA HVR1 region. The total number of haplotypes generated in this study for supports a high degree of diversity in goat populations (Zhong *et al.*, 2013; Akis *et al.*, 2014). This result also compare well with a recent study in Chinese goats which observed a total of 192 haplotypes with 141 variable sites with haplotype and nucleotide diversities based on 481-bp segment of the mtDNA D-loop region, indicating a relatively high genetic diversity in Chinese black goats (Zhong *et al.*, 2013). NJ tree and Median joining network constructed revealed considerable diversity and interrelatedness among the different Nigerian goat breeds, this result supports Okpeku *et al.* (2011) microsatellites diversity in Nigerian goat breeds which was attributed to uncontrolled breeding among Nigerian goat breeds.

Evolutionary analysis conducted with MEC showed that both positive adaptive and negative purifying evolutionary process are at work in this region to maintain the integrity and functionality of the HVR1 region.

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Table 1. Population Statistics aneutrality test

| Population Statistics | | | | Neutrality test | |
|---|---|---|---|---|---|
| M | S | Pi | θ | Tajima's D | NS |
| 76 | 286 | 0.285 | 0.171 | 2. 335 | * |

*Significant at P<0.05

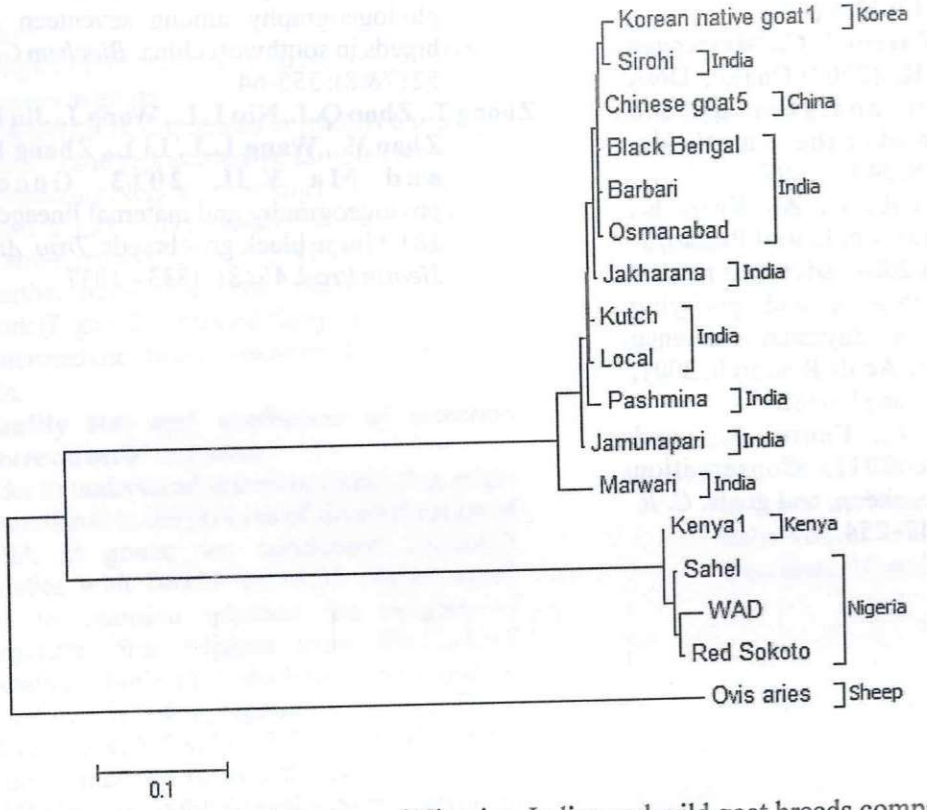



Figure 1 Neighbor=joining tree of Nigerian, Indian and wild goat breeds compared to wild goat mtDNA sequences

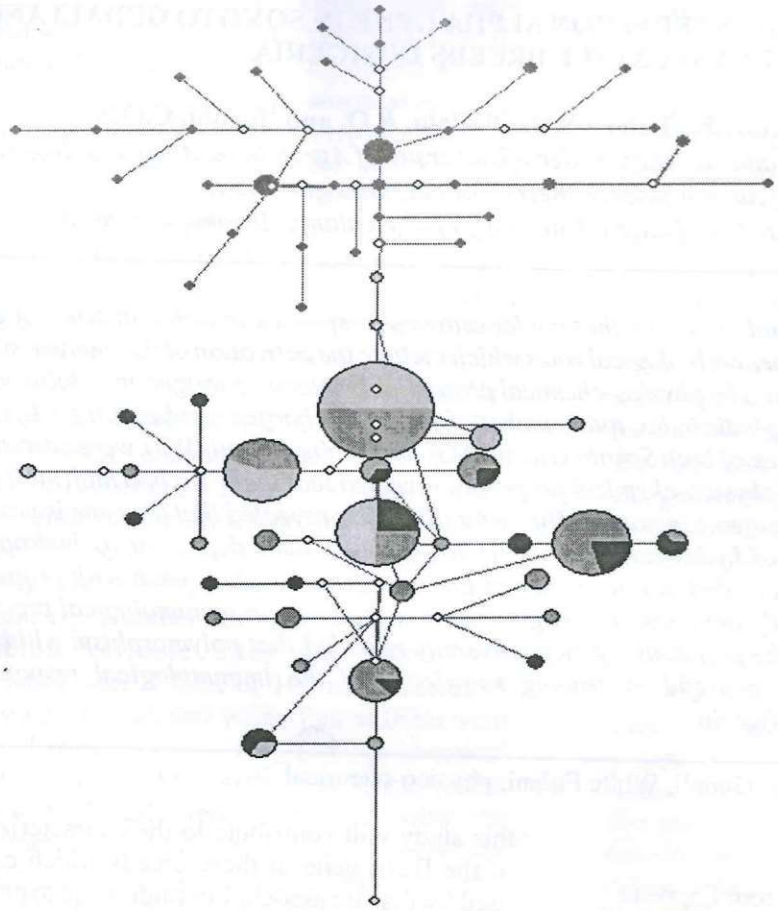
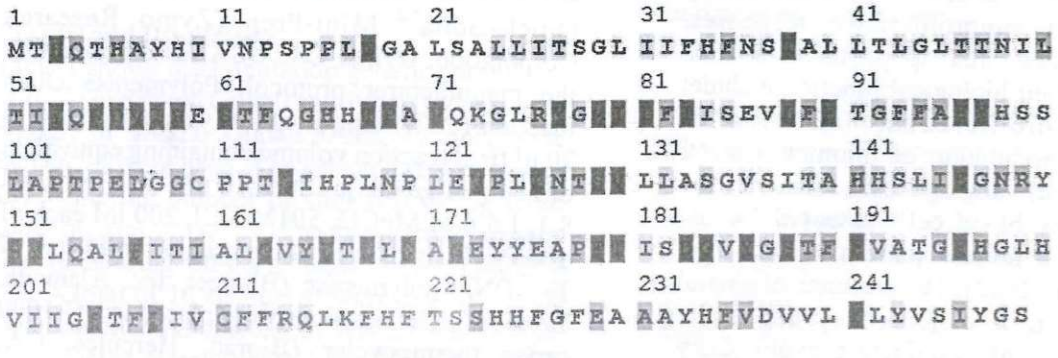


**Figure 2 Median Joining network showing relationship among goat mtDNA sequences**
**Color Legend**
**Black = Indian goats**
**Red = Chinese goats**
**Blue = Korean goats**
**Pink = Kenyan goats**
**Green = WAD goats**
**Brown = Red Sokoto Goats**
**Purple = Sahel goats**




Legend:

The selection scale:

1 2 3 4 5 6

Positive selection Purifying selection

Figure 3 Selecton MEC showing positive and negative selection in mtDNA HVR1 sequences of goats