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Phylogenetic Relationships Among Two Nigerian Goat Breeds and Kalahari Red Goat of South Africa

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ABSTRACT: Mitochondrial DNA polymorphism and phylogenetic relationships among two Nigerian goat breeds: West African Dwarf (WAD) (n=35) and Red Sokoto (RS) (n=37), and Kalahari Red (KR) goat (n=38) imported from South Africa were investigated due to dearth of published information on the breeds. First hypervariable region (HV1) of mtDNA (481bp) sequenced from 110 goats represented 68 haplotypes. Polymorphism of HV1 fragment was high, haplotype diversities were 0.985±0.011, 0.992±0.008 and 0.869±0.030 for WAD, RS and KR respectively. Corresponding nucleotide diversities were 0.0144±0.0068, 0.0166±0.0079 and 0.0299±0.0067. Maximum-likelihood tree constructed with 68 haplotypes and 22 reference haplotypes representing 6 haplogroups worldwide revealed that all 28 haplotypes of WAD, 32 of RS and 6 out of 11 haplotypes of KR belong to haplogroup A, while 5 haplotypes of KR belong to lineage B. Results of this investigation will be useful for planning improvement programme for the local populations.
Keywords: mtDNA; Polymorphism; Phylogenetic relationships; Goat

Introduction

West African Dwarf (WAD) and Red Sokoto (RS) goats constitute two of the indigenous goat breeds of Nigeria, majority (95%) of which is kept by resource poor farmers (Osinowo and Abatan (1990)). They are adapted within two different geographical regions, namely the Rainforest South (dominated by WAD) and the Northern Guinea Savanna/semi-arid north (dominated by RS). They vary in growth rates, body weights and milk production potentials (Bemji et al. (2008)). The Kalahari Red (KR) goat, an important-meat producing breed in South Africa (Kotze et al. (2004)) was imported into Nigeria in 2011. The breed is superior to both Nigerian local breeds in terms of growth rate, body weight and milk yield, besides its adaptation to a wider range of environmental conditions (http://www.indigenousbreeds.co.za/indigenousbreeds/goat/kalahari). The pressing need to improve the growth rate and body weight of the Nigerian local breeds, especially the WAD was the basis of their importation by the Federal University of Agriculture, Abeokuta, Nigeria. Evaluation of genetic diversity of the three populations will further give insight about the prospect of achieving genetic progress from selection programmes and/or crossbreeding. Genetic diversity studies based on different microsatellite loci have been reported for WAD and RS populations (Adebambo et al. (2011); Okpeku et al. (2011)), as well as KR breed (Kotze et al. (2004); Visser et al. (2004)). All microsatellite loci studied were polymorphic as indicated by moderate to high number of alleles per locus and high polymorphism information content values.

There is however paucity of information on analysis of mitochondrial DNA (mtDNA) marker-based genetic diversity of the breeds under consideration. Mitochondrial DNA is highly favoured as a marker of choice because it is maternally inherited, has high mutation rate and copy number and lacks recombination unlike autosomal or *X* chromosome specific loci (Pereira et al. (2010)). Many studies on different goat populations around the world have focused on analysis of the first hypervariable (HV1) region of mitochondrial D-loop (Luikart et al. (2001); Fan et al. (2007); Naderi et al. (2007); Naderi et al. (2008); Wang et al. (2008); Amills et al. (2009); Groeneveld et al. (2010); Han et al. (2010); Benjelloun et al. (2011); Cinar Kul and Okan (2011); Zhao et al. (2011); Martínez et al. (2012); Lin et al. (2013)). Apart from very high haplotype diversities reported by most of the authors, weak phylogeographic structure among different populations was also observed (Luikart et al. (2001); Fan et al. (2007); Naderi et al. (2007); Naderi et al. (2008); Amills et al. (2009); Benjelloun et al. (2011); Zhao et al. (2011); Okpeku et al. (unpublished)). Insignificant geographic structuring was mainly attributed to worldwide distribution of dominant A haplogroup which included more than 90% of individuals among other haplogroups (B, B1, B2, C, D, F and G) reported (Naderi et al. (2007)). In view of lack of published information on mtDNA-based genetic analysis of Nigerian goats and the Kalahari Red of South Africa, this study was designed to investigate genetic diversity and phylogenetic relationship among the three goat populations.

Materials and Methods

Blood sample collection and DNA extraction. A total of 110 blood samples were collected from three breeds of goats representing two Nigerian goat breeds (West African Dwarf, n = 35 and Red Sokoto (n = 37) and Kalahari Red goat (n = 38) from the stock imported from South Africa. For indigenous goats, individuals from each breed were sampled from remote villages within their geographical regions, while ensuring no two individuals were related. Samples from Kalahari Red were drawn from unrelated individuals of the parent stock which excluded offspring born at the Institute of Food Security, Environmental Resources and Agricultural Research Farm, Federal University of Agriculture, Abeokuta, Nigeria. The blood samples were

placed on Whatman FTA classic cards until DNA extraction was carried out using standard commercial kits according to manufacturer's instructions.

Polymerase chain reaction (PCR) amplification and sequencing. The HV1 region of mtDNA D-loop was amplified and sequenced in STABVIDA laboratory, Portugal. The primers CAP-F (5′-CGTGTATGCAAGTACATTAC-3′) and CAP-R (5′-CTGATTAGTCATTAGTCCATC-3′) were used to amplify a 579-bp DNA fragment. The PCR cycling protocol by Naderi et al. (2007) was adopted and a 481 bp segment of the PCR products sequenced.

Data analyses. The sequences were edited with ChromasPro v1.7.5 and aligned with Clustal W in MEGA v5.2.2 (Tamura et al. 2011)). Genetic diversities and phylogenetic analyses were carried out using a 453 bp region shared by 110 mitochondrial sequences in the current study and other reference sequences. Haplotype diversity (*h*) and nucleotide diversity (π) were estimated using DnaSP v5.10.01 software (Librado and Rozas, 2009)). To identify possible phylogenetic clades, maximum-likelihood tree was estimated with MEGA v5.2.2 following alignment of sequences with 22 reference sequences (Naderi et al. 2007)) representing diversity of six haplogroups (A, B, C, D, F and G) found worldwide. Reliability of the phylogenetic tree was assessed using bootstrap percentages computed after 1000 replications.

Results and Discussion

Sequence diversity. Polymorphism of HV1 fragment was high, haplotype diversities were 0.985±0.011, 0.992±0.008 and 0.869±0.030 for WAD, RS and KR respectively (Table 1). Corresponding nucleotide diversities were 0.01440±0.00679, 0.01662±0.00788 and 0.02994±0.00673. These results compare favourably with estimates reported for WAD and RS using a larger sample size (Okpeku et al. (unpublished)) and several studies around the world (Luikart et al. (2001); Naderi et al. (2007); Wang et al. (2008); Amills et al. (2009); Benjelloun et al. (2011); Cinar Kul and Okan (2011); Zhao et al. (2011)). High genetic diversity may partly result from high mutation rate of the control region, multiple maternal wild ancestor (Naderi et al. (2007)) and capture of large part of the wild diversity during domestication (Benjelloun et al. 2011)).

Table 1. Number of haplotypes, haplotype and nucleotide diversities

Breed	N	Nh	hd±SD	π±SD
WAD	35	28	0.985±0.011	0.0144±0.0068
RS	37	32	0.992±0.008	0.0166±0.0079
KR	38	11	0.869±0.030	0.0299±0.0067

N: Sample size, Nh: number of haplotypes, hd: haplotype diversity, π: nucleotide diversity, SD: standard deviation

Phylogenetic relationship. The first hypervariable region of mtDNA sequenced from 110 goats revealed a total of 68 different haplotypes. Tree topology (Figure 1) obtained with maximum-likelihood phylogenetic method for the observed haplotypes and 22 reference sequences (Naderi et al. (2007) grouped the three goat populations into two distinct mtDNA lineages, A and B. All 28 haplotypes of WAD, 32 haplotypes of RS and 6 out of 11 haplotypes of KR belong to haplogroup A while 5 haplotypes of KR clustered with haplogroup B. Lineage A is widely reported in different studies around the world as predominant (Luikart et al. (2001); Naderi et al. (2007); Wang et al. (2008); Han et al. (2010); Benjelloun et al. (2011); Zhao et al. (2011); (Martínez et al. (2012); Lin et al. (2013)). Naderi et al. (2007) further reported more than 90% worldwide distribution of lineage A from a large scale mtDNA analysis of domestic goats which is in agreement with the current observation that lineage A was predominant. While a neighbor-joining tree of breed relationship revealed that indigenous goats of Sub-Saharan Africa were grouped according to their geographic origins based on analysis of different microsatellite loci (Chenyanbuga et al. (2004)), recent fragmentation of local goat populations into discrete breeds is not detectable with mtDNA markers (Naderi et al. (2007)). It has been established that the geographical structure of goats is weaker than that for cattle and sheep (Luikart et al. (2001)). The B lineage, according to the authors is mostly found in whole Asia with few individuals from Sub-Saharan Africa and one European goat from Greece. Since KR goats were developed from two lines (red-head boar and "unimproved indigenous" goats) (Campell, 2003)), it is not surprising that haplogroups A and B were represented in the breed, thereby supporting the hypothesis of multiple maternal origins (Luikart et al. (2001)). The B lineage is likely to have arisen in Asia, according to the latter authors, while distribution to other locations has been attributed to human migration (Naderi et al. (2007)). Generally, haplogroups B, C, D, F and G are rare or absent (Luikart et al. (2001); Naderi et al. (2007); Amill et al. (2009)).

Conclusion

Mitochondrial DNA analysis of the three breeds of goats revealed that West African Dwarf and Red Sokoto goats of Nigeria and Kalahari Red goat of South Africa are highly diverse. The WAD and RS from Nigeria belong to lineage A, while lineages A and B were detected in Kalahari Red of South Africa, further supporting the possibility of multiple maternal origins. The mtDNA diversity coupled with large phenotypic variations already reported in the breeds can be exploited as raw materials for genetic improvement of economically important traits in the local goat populations.

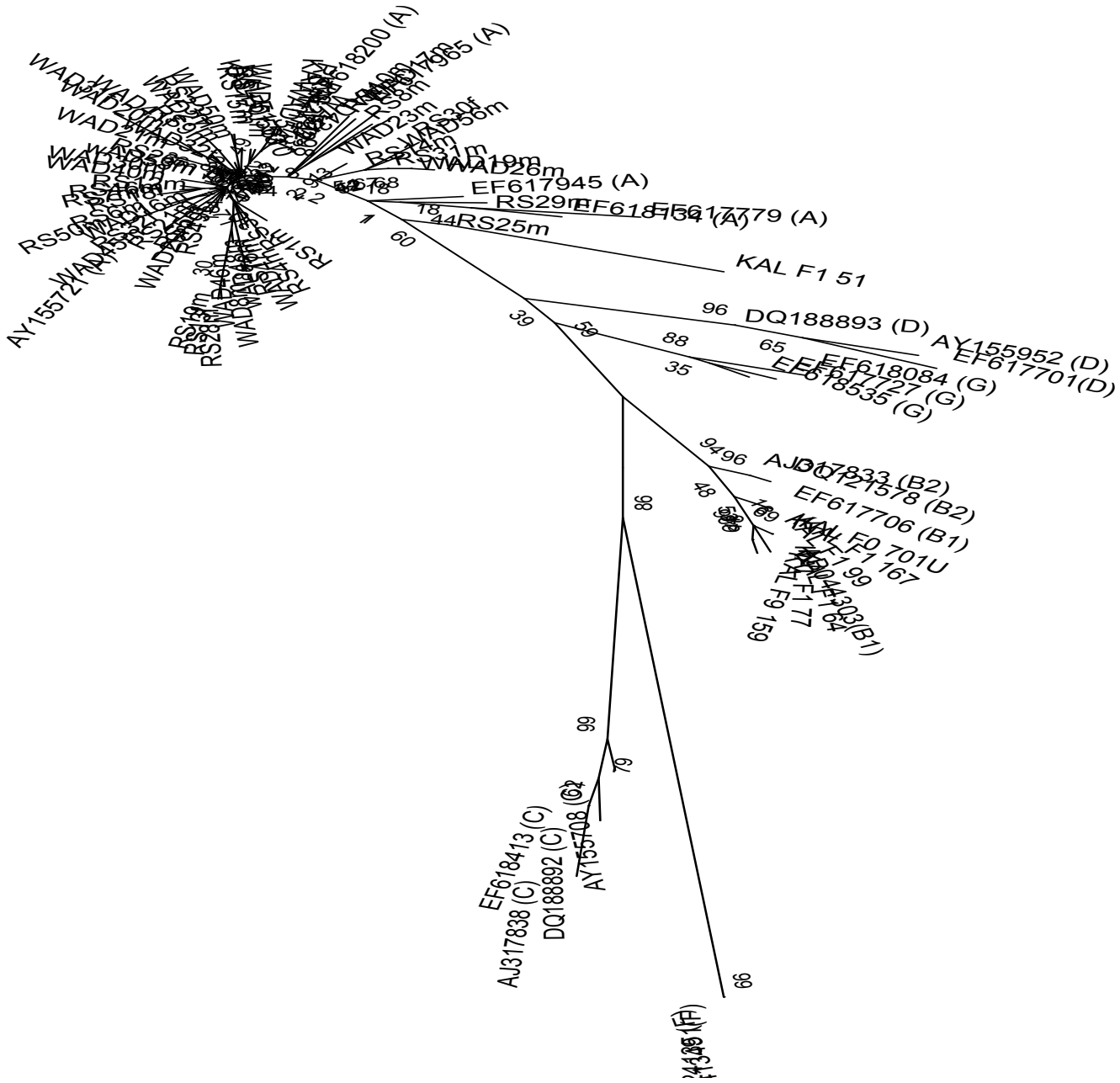

Figure 1. Tree topology obtained with maximum-likelihood phylogenetic method for 68 haplotypes and 22 reference haplotypes. Codes starting with two letters represent GeneBank Accession numbers of the 22 reference sequences. Other codes starting with WAD, RS and KAL correspond to haplotypes belonging to West African Dwarf, Red Sokoto and Kalahari Red goats respectively. Numbers at major clades represent bootstrap percentages computed after 1000 replications.

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