



## Analysis of genetic structure of West African Dwarf goats by allozyme markers



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### ABSTRACT

Genetic variation at three allozyme (Haemoglobin, Carbonic Anhydrase and Transferrin) loci and population structure hypothesis were examined for West African Dwarf goat populations in four Southwestern state of Nigeria. One hundred and forty animals, twenty from each sampling area comprising Ijebu-Ode and Ado-Odo, (Ogun state), Ondo, (Ondo state), Ile-Ife, Osogbo and Iwo, (Osun state), and Ibadan, (Oyo state), were randomly selected. Estimates of genetic variability such as effective number of alleles and gene diversities revealed substantial genetic variation frequently displayed by allozyme markers. Numbers of alleles observed across the allozyme loci varied from 246 to 250 with an overall mean of  $247.33 \pm 2.31$ . Average polymorphism across the studied loci and expected gene diversity in the population were  $0.63 \pm 0.09$  and  $0.44 \pm 0.09$ , respectively. Population was observed to be significantly differentiated into different groups, and showed fairly high level of outbreeding ( $f = -0.16 \pm 0.44$ ) and excess heterozygosity.

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### 1. Introduction

In humid and hot climate of West and Central Africa, the present day goats of this type tend to be dwarf, which is believed to be due to natural selection on thermoregulation under the unfavourable humid and hot climate. It is possible that this was deliberately selected for by owners just for its oddness. The distribution of this goat type extends southwards through Central Africa as far as Democratic Republic of Congo, Angola and the north of Namibia (Mason, 1984). The true type of this goat is considered to be confined to fifteen countries in West and Central Africa, all of which except the Central African Republic have an Atlantic coastline (Guinea Bissau, Guinea, Liberia, Sierra Leone, Cote d'Ivoire, Ghana, Togo, Benin, Nigeria, Cameroon, Congo, Equatorial Guinea, Gabon, Democratic Republic of Congo, and Central African Republic). It is also found in Senegal (Wilson, 1991). Goats constitute the largest group of small ruminant livestock in Nigeria totaling about 53.8 million and also constituting 6.2% of the World's goat population (FAOSTAT, 2011). Surveys have shown that up to 85% of rural households, poor farmers and small-time business people of all

age groups and sexes keep them (FDLPCS, 2007). There are three main breeds of goat in Nigeria, the West African Dwarf, the Sokoto Red and the Sahel. Goats are renowned for their hardiness and can survive in most environments: West African Dwarf (WAD) goats are kept in the forest zones and in the Middle belt; Sokoto Red are kept throughout the north; and Sahel goats are restricted to a strip along the frontier with the Republic of Niger, (Bourn et al., 1994). Goats located in northern part of the country were found to be markedly more productive than WAD goats, with lower ages at first kidding and shorter kidding intervals, though they produced fewer kids per kidding (Bourn et al., 1994). In addition the WAD goat often has short bowed legs attributed to achondroplasia (Wilson, 1991). They are essentially confined to the humid forest zones with more than 240 growing days and in excess of 1500 mm rainfall/annum; most of this zone are infected with tsetse fly making trypanosome infections prevalent, the production system are agricultural, peri-urban and to a lesser extent agro-pastoral with ethnic group (Wilson, 1991). Livestock plays a significant role in the livelihood of rural populations and the agricultural development of Sub-Saharan Africa. In this region, livestock production is characterized by diverse and complex production systems (Udo and Cornelissen, 1998). Livestock production contributes significantly to improved family nutrition and health, and the sale of animals and their products helps to improve and stabilize household income.

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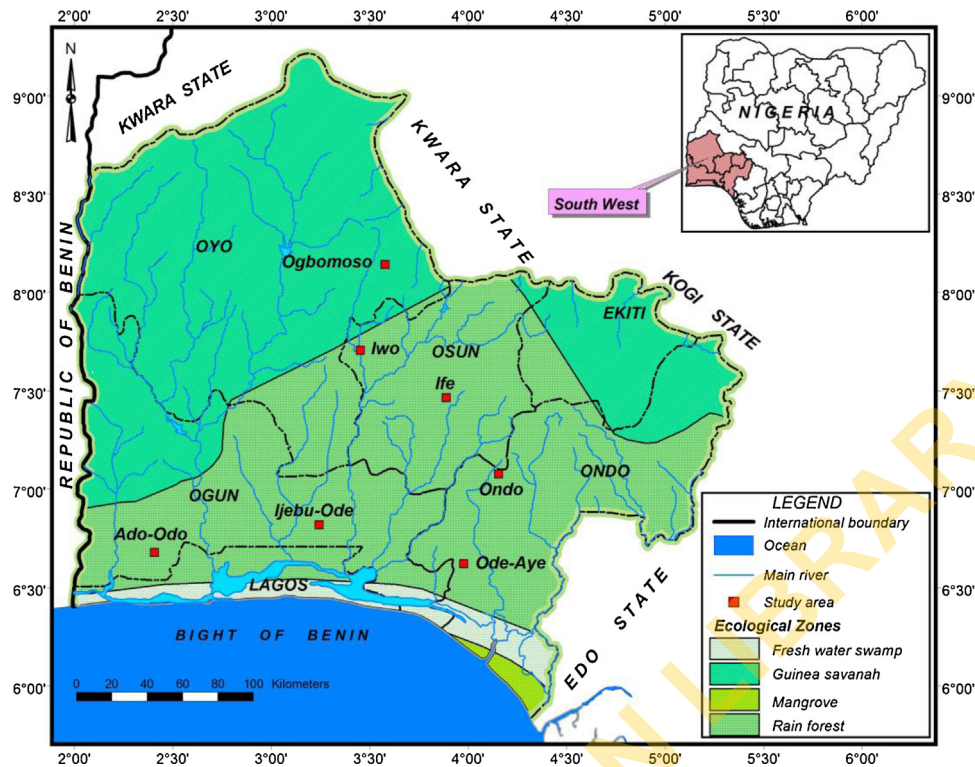


Fig. 1. Map of Southwestern Nigeria indicating sample area.

The intangible products obtained from animals are important in areas lacking formal insurance and developed financial markets (Udo and Cornelissen, 1998). Goat, sheep and poultry are considered as forms of security and sources of independent income especially for poor women (Paris, 2002). Furthermore, animals are used in social cultural functions e.g., in religious ceremonies (Jahnke et al., 1988; Jabbar et al., 1995). The ability of goats to tolerate harsh climates, the presence of trypanotolerance in some breeds (Salako, 2004), suitability to traditional systems on account of small size, short generation interval (Abdul-Aziz, 2010) and ability to thrive on poor quality diets provided by scarce grazing on marginal lands (Adedeji et al., 2011) all combine to make small ruminants strategic to increasing livestock productivity in rural agricultural systems (Adebambo et al., 2004; Adedeji et al., 2011). Despite these advantages, little attention had been paid to the genetic characterization and possible improvement of small ruminants in Nigeria.

The future development of livestock production in Sub-Saharan Africa is hindered by limited knowledge of the genetic potential of the local genetic resources and ways to best utilize these resources in a sustainable manner. The genetic makeup of the indigenous animal is often associated with low production potential in indigenous animals (Bosso, 2006). However, according to him, indigenous animals have not been adequately characterized for their production potential and this have led to the growing recognition of the need to characterize, utilize and conserve indigenous Animal Genetic Resources (AnGR). Because of this lack of information, decisions on genetic improvement of indigenous animals are often made which have negative consequences on indigenous AnGR (Rege et al., 2002). It is important to know the diversity and relationship between the African breeds and strains of livestock. Moreover, it is important because, quite often, the same breed may be known by different names or two breeds may be known by the same name, based on geographical locations of such populations (Gwakisa et al., 1994). The need to curb the threats of dilution and extinction of the African animal genetic resources, by strategic development and

conservation has never been more justifiable and is now, with the available technology, timely. Efficient strategies for conservation require sets of genetic markers, which characterize distinct populations (Kemp and Teale, 1994). Genetic improvement of indigenous breeds of livestock is very valuable because of high adaptability to harsh environmental conditions of nutrition, climate and disease compared with exotic breeds (Fitzhugh et al., 1992). According to Groeneveld et al. (2010) many breeds of livestock may become lost germplasm in many third world countries due to crossing with exotics, which in addition to uncontrolled breeding in extensive management systems pose a great risk for the loss of valuable genes. To understand natural genetic variation in native goats as well as formulate conservation policies, better genetic characterization is required to balance the competing needs of genetic improvement and conservation of native germplasm to preserve the age-long relationship between native livestock and dwellers in rural agricultural systems (Groeneveld et al., 2010). The primary aim of studying genetic diversity is to understand the extent of differentiation of populations within species. According to Hanotte and Jianlin (2005) population genetics is about microevolution and it is the study of genetic variation in populations. This variation involves the change of allele frequencies, genotype frequencies and phenotype frequencies. Population genetics predicts diversity that is determined by a number of factors including selection, mutation rate, recombination, genetic drift and effective population size. The amount and nature of genetic variation in a population allows estimates of effective population size, population history (migration, bottleneck, recent expansion), population structure, how selection acts on genes and location of diseases genes (Qualitative Trace Loci (QTL) mapping). Primary indicators of animal genetic diversity should address both between-breed and within-breed components. Priority breeds for conservation should be the ones with the largest within breed diversity and should maximize the conservation of between breed diversity. Both within and between breed diversity parameters are classically measured using molecular

**Table 1**  
Measures of genetic variation at studied blood protein loci in West African Dwarf goat.

SN	Loci	Sample size	Observed no of alleles	Effective number of alleles	Shannon's information index	Heterozygosity			Heterozygote deficiency
						Observed	Expected	Nei's	
1	Hb	123	246	1.52	0.53	0.34	0.34	0.34	0.28
2	CA	123	246	1.94	0.68	0.46	0.49	0.48	−0.70
3	Tf	125	250	2.00	0.69	0.84	0.50	0.50	−0.13
Mean		123.67	247.33	1.82	0.63	0.55	0.44	0.44	−0.18
St. dev			2.31	0.26	0.09	0.26	0.09	0.09	0.50

Hb—Haemoglobin, CA—Carbonic anhydrase, Tf—Transferrin.

genetic markers. In both cases soundly-based priority decisions for conservation at the global level will require the availability of large datasets. The mean number of alleles (MNA) observed ( $H_O$ ) and expected ( $H_E$ ) heterozygosity are the most commonly calculated population genetic parameters for assessing within breed diversity (Hanotte and Jianlin, 2005).

Traditional methods used to study genetic variability of animals and populations employed polymorphism in protein markers and genetic variation of haemoglobin (Hb) (Buvanendra et al., 1981; Imumori et al., 1999) and transferrin (Tf) types in goats (Moruppa, 1985; Kitalyi, 1998; Yakubu et al., 2010a,b; Mourad et al., 2001). Although DNA-based technologies are now the methods of choice for genetic characterization of livestock (Arora et al., 2011), several alternative assays, such as protein/allozyme polymorphisms, remain tremendously useful, especially in developing countries, because of their utility, ease, cost and amount of genetic information accessed or simplicity of data interpretation (Rege and Okeyo, 2006). Mwacharo et al. (2002) reported that for populations whose genetic status is unknown, protein polymorphism may be used first to verify the degree of genetic relationship. Various electrophoresis techniques have made rapid progress since Smithies successful segregation in 1955 of serum protein using starch gel electrophoresis. Protein polymorphism indicates that the analogous protein has two or more genetic variations. It is caused by nucleotide alternation in the DNA chain that results in the substitution of amino acid of polypeptide chain (Lu et al., 2006). Analysis of genetic markers based on protein variants detected by electrophoretic method has been a tool for studying genetic differentiation among breeds and in phylogenetic studies (Imumori et al., 1999; Menrad et al., 2002; Nyamsamba et al., 2003; Ibeagha-Awemu and Erhardt, 2004; Camoglu and Elmaci, 2005). However, biochemical variants of different proteins may present higher accuracy procedures for a better measurement of genetic variation in WAD goat breeds because of their polymorphism and simple mode of inheritance.

However, there is very little baseline information on the extent of natural genetic variation in WAD goats in Nigeria. Therefore, the present study aimed at using the allozyme analysis in investigating and estimating genetic diversity among different populations of WAD goat breeds in Southwestern Nigeria. The results of this study will provide useful genetic information essential for developing more effective extensive molecular characterization of WAD goat in Nigeria and understand the genetic diversity of WAD goat to implement steps so as to ensure their conservation and rational utilization for improvement of these genetic resources and productivity for the benefit of the farmers. The main objective of the study is to investigate and estimate the genetic diversity among WAD goat population within Southwestern state in Nigeria.

## 2. Material and methods

### 2.1. Experimental procedure

One hundred and forty animals, twenty from each sampling area comprising Ijebu–Ode and Ado–Odo, (Ogun state), Ondo, (Ondo state), Ile–Ife, Osogbo and Iwo, (Osun state), and Ibadan, (Oyo state)

**Table 2**  
Wright's  $F$ -statistics analyses for 3 protein loci in WAD goats.

Locus	$f(F_{IS})$	$\theta(F_{ST})$	$F(F_{IT})$	Nm <sup>a</sup>
Hb	0.13	0.06	0.18	3.68
CA	0.05	0.03	0.08	8.15
Tf	−0.67	0.01	−0.66	32.40
Mean	−0.16	0.03	−0.17	13.51
S.E. <sup>a</sup>	0.44	0.03	0.46	16.85

<sup>a</sup> Nm—Gene flow estimated from  $F_{ST} = 0.25(1 - F_{ST})/F_{ST}$ ; Hb—Haemoglobin; CA—Carbonic anhydrase; Tf—Transferrin.

as shown in Fig. 1, were randomly selected. 5 mL of blood was collected from each animal by jugular venipuncture and placed in heparinized tubes to prevent coagulation. Procedure describe by RIKEN (2006) was followed in rest of the processes carried out. Red blood cell was prepared from the erythrocyte fraction of heparinised blood by centrifuging at 2500–3000 rpm for 10 min at 4 °C. The Red Blood Cell (RBC) was washed in saline (0.155 M NaCl) three times and centrifuged at 2500–3000 rpm for 5 min at 4 °C. The RBCs were lysed with a fourthfold volume of distilled H<sub>2</sub>O to release Hb. The plasma fraction is separated from the erythrocyte fraction of heparinized blood by centrifuging at 2500–3000 rpm and the supernatant was used. Cellulose acetate plates were soaked in the same buffer as the electrode buffer. Samples were applied on to the plate using the applicator and once loaded; plates are rested on the wicks in the tank. The loaded zone on the plate was positioned at the cathodal end of the tank for the majority of the enzyme systems which migrate anodally. For those systems which migrate cathodally e.g carbonic anhydrase (CA), the loading zone was placed on the anodal end. The plates were removed from the tank and placed in an empty Petri dish after the completion of the gel run. They were stained with Ponceau stain before they dry out. After 20 min the plate was sufficiently stained, it was destained several times until clear and sharp bands appear, the bands were scored visually based on their migratory pattern as described by RIKEN (2006) and direct counting was used for calculating gene frequencies.

### 2.2. Statistical analysis

Tools for Population Genetic Analyses (TFPGA) (Miller, 1997) software was used to generate the genetic distance according to Nei's (1972), the allele frequency, observed and expected heterozygosity, Hardy-Weinberg Equilibrium, the inbreeding coefficients i.e. Wright's  $F_{IS}$  and  $F_{IT}$ ,  $F_{ST}$  estimates, and drawing UnPaired Group Method of Algorithm (UPGMA) dendrograms.

## 3. Results and discussion

Various measures of genetic variation are presented in Table 1. The  $F$ -statistics estimates of population structure are presented in Table 2. The number of alleles observed across the allozyme loci varied from 246 (Hb and CA) and 250 (Tf) with the mean of  $247.33 \pm 2.31$  (Table 1). The observed number of alleles across the loci was more than the effective number of alleles (1.52–2.00) as expected. The Shannon information index showed that most of the

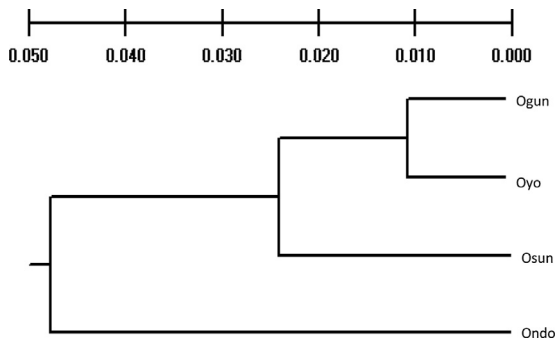


Fig. 2. Dendrogram showing similarity among four populations of WAD goat in Southwestern Nigeria based on blood protein analysis.

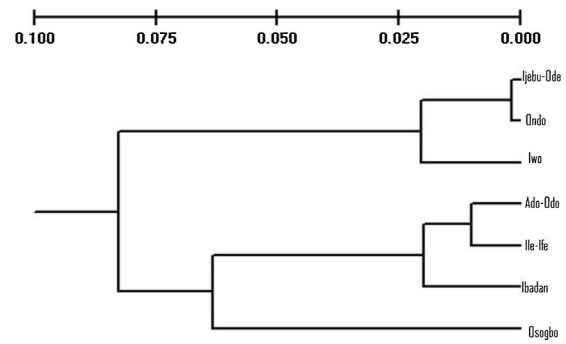


Fig. 3. Dendrogram of WAD goat populations showing similarity among seven sampled areas in Southwestern Nigeria based on blood protein analysis.

loci were highly informative indicating the polymorphism across the loci with an overall mean of  $0.63 \pm 0.09$ . The average  $H_O$  was more than the  $H_E$  (Table 1). The average expected gene diversity (Nei, 1973) within the population ranged from 0.34 (Hb) to 0.50 (Tf) with an overall mean of  $0.44 \pm 0.09$ . Only Tf showed significant deviations from Hardy–Weinberg equilibrium out of the three loci. All the loci except Tf showed significant heterozygote deficiency in the WAD goat population. The overall means for the Wright's  $F$ -statistics for population subdivision were significantly different from zero (Table 2). The average genetic variation (0.55) observed in this study was lower than the values reported for Indian breeds of goat, Black Bengal (0.69), Chegu (0.66), (Behl et al., 2003; Ganai and Yadav, 2001; Kumar et al., 2005) and West African Dwarf goat (0.75) (Okpeku et al., 2011), though they were all microsatellite based which are highly polymorphic than allozymes. However, it was higher than what was reported for Marwari (0.45), Barbari (0.50), Asian goat breeds of Sri Lanka South (0.48), Sri Lanka N-Central (0.49), Hat Yai (0.43); Australia (0.45) (Barker et al., 2001) and Swiss goats 0.51–0.58 (Saitbekova et al., 1999). Despite the fact that they were microsatellite based, it was higher than magnitude of genetic variation reported among populations of Asian breeds of goat, Chiang Mai (0.39), Sabah (0.31), Sarawak (0.33), Ujung-Pandang (0.38), (Barker et al., 2001) and Korean goats (0.36) (Kim et al., 2002) and Sub-Saharan African goat breeds of Kigezi (0.38), Mubende (0.36), Ndebele (0.34), West African Dwarf (0.37), Northwest Highland (0.41) and Pafuri (0.38) (Chenyambuga et al., 2004). All  $f$  ( $F_{IS}$ ) estimates (Table 2) across the loci except Tf were significantly positive (significant heterozygote deficit) based on table wide randomizations ( $P < 0.05$ ). The  $f$  estimates ranged from 0.05 to 0.13 with an average of  $-0.16 \pm 0.44$  indicating an outbred population or excess heterozygosity. The lower genetic variations observed in this study as compared to what was reported by Okpeku et al. (2011) for WAD goat may be due to a higher rate of inbreeding in this goat population and also due to high level of polymorphism in the microsatellites marker compare to allozymes. The  $F_{ST}$  values ranged from 0.01 for Tf to 0.06 for Hb. Low  $F_{ST}$  indicates some measure of gene flow between the sampled populations, with Hb locus recording the highest gene flow of 0.06. Mujibi (2005) reported a low  $F_{ST}$  of 5.8% for WAD goats in Kenya; therefore, gene flow estimates in this study suggest mobility and considerable exchange of genetic material among these WAD goat populations (Figs. 2 and 3).

#### 4. The dendrogram

The dendrogram showed a measure of close relationship among the WAD goat populations until it branched into two main groups with populations from Ogun, Oyo and Osun state clustering together and population from Ondo state forming another branch then later the second branch that late emerged in which population from Osun branched away leaving population from Ogun

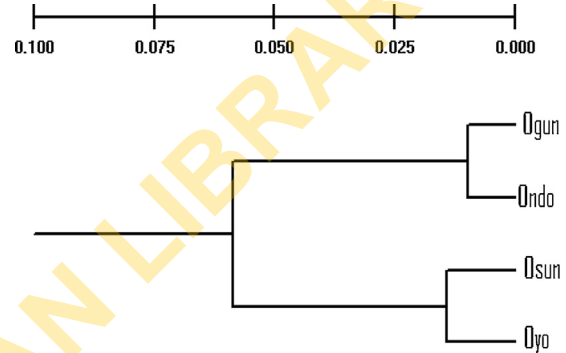


Fig. 4. Dendrogram showing similarity among four populations of WAD goat in Southwestern Nigeria at the haemoglobin locus.

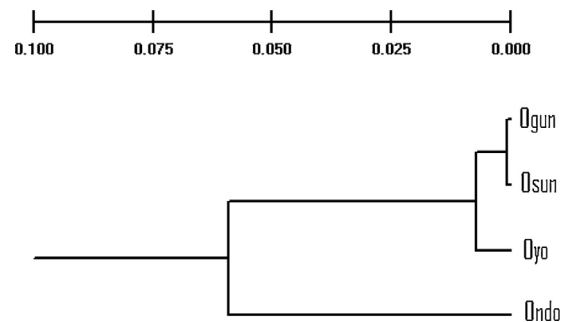
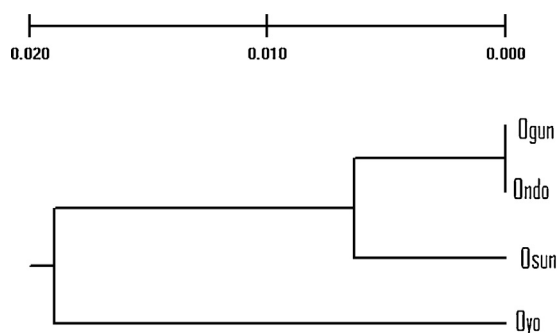


Fig. 5. Dendrogram showing similarity among four populations of WAD goat in Southwestern Nigeria at the Carbonic anhydrase.

and Oyo to clustered together (Figs. 4), Toro and Maki-Tanila (2007) suggested that the high genetic diversity observed within population groups could arise from overlapping generations and population mixtures from different geographical locations, with natural selection favouring heterozygosity or subdivision accompanied by genetic drift. The effect of these factors according to Agha et al., (2008) is more pronounced when the effective population size is very large, which is supported by the poor infrastructure on ground presently for livestock improvement and lack of proper breeding policy in Nigeria (Figs. 5 and 6).

#### 5. Conclusion

The present study reveals that the WAD goat has substantial genetic variation but there is fairly high degree of outbreeding as indicated by  $f$  value ( $-0.16$ ) indicating excess heterozygosity. The influx of other germplasm is affecting the founder alleles in the gene pool of WAD and breed purity is at stake. It becomes more pertinent especially in a country where breed wise census is not



**Fig. 6.** Dendrogram showing similarity among four populations of WAD goat in Southwestern Nigeria at the Transferrin locus.

available. This is the high time but still safe enough to strengthen the conservation programme. For conservation, efforts should be made to provide elite breeding bucks to the farmer as male: female sex ratio is drastically low. Breeding strategies should therefore be designed under field conditions for conservation and improvement of this breed of Southwestern Nigeria having unique attributes like body size, trypanosotolerant adaptability to the rain forest zone of Nigeria with high fecundity and productivity under harsh conditions and minimal/zero input system.

### Conflict of interest

The authors declare no conflict of interest regarding this article.

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