Full Length Research Paper

Genetic structure of indigenous sheep breeds in Nigeria based on electrophoretic polymorphous systems of transferrin and haemoglobin

Osaiyuwu Henry Osamede* and Salako Emmanuel Adebowale

Animal breeding and Genetics Unit, Department of Animal Science, University of Ibadan, Ibadan, Oyo State, Nigeria.

Received 21 April, 2015; Accepted 10 May, 2016

The study used 100 indigenous sheep comprising 25 Balami, 25 Uda, 25 Yankassa and 25 West African Dwarf breeds reared extensively. The blood samples were taken from Vena Jugularis, processed according to standard procedure and transferrin and haemoglobin examined using cellulose acetate electrophoresis. The observed allele frequencies and genotypes (%) were tested with Hardy-Weinberg’s Equilibrium ($\chi^2$). Seven alleles $Tf^A$, $Tf^B$, $Tf^C$, $Tf^D$, $Tf^E$, $Tf^G$ and $Tf^P$ controlling 23 genotypes were observed at the transferrin locus while two haemoglobin alleles ($Hb^A$ and $Hb^B$) controlling three phenotypes- $Hb^{AA}$, $Hb^{AB}$ and $Hb^{BB}$ were detected. Except for the West African Dwarf sheep, all the samples indicated genetic equilibrium revealed by significant difference between observed and expected genotypes at both loci. The observed significant difference between the frequencies of alleles and genotypes at the two studied loci in the West African Dwarf sheep can be used as a source of genetic diversity during selection for improvement. The phylogenetic analysis as viewed by the tree topology suggests that the Balami and Uda may have had the same migration route or may have been the same breed which had only just recently branched off through adaptive. Also, the West African Dwarf sheep may have been the first to branch off the path of migration and thus had more clearly defined migration route or origin.

Key words: Transferrin, haemoglobin, genetic structure, genetic diversity, Balami.

INTRODUCTION

Indigenous animal breeds in developing countries are constantly being replaced by their high-producing exotic counterparts in spite of their excellent adaptation to prevailing environmental conditions. This poses the danger of losing valuable genes for adaptation to extreme environments and disease which are of value in developing countries. Therefore, there is the need to understand the diversity of these indigenous breeds in order to develop strategies for improvement, sustainable use and conservation of the domestic animal biodiversity.

Blood protein and enzyme system have been found to exhibit heterogeneity in different farm animals (Elmaci, 2001). Polymorphic blood traits are useful in studies of relationship between populations, genetic structure of

*Corresponding author. E-mail: cosamede@yahoo.com. Tel: +2348064666074.
breeds and their evolution. Information on genetic variations of blood proteins and enzymes has also been used as an aid to parentage determination and indirect selection (Elmaci, 2001). Information on inherent genetic diversity is important in the design of breeding programmes for improvement, rational decision making on sustainable utilization and conservation of animal genetic resources. The genetically determined polymorphic systems in sheep blood and the opportunity for using them as genetic markers make it possible to conduct studies that are related to breed structure as well as changes that may have occurred in them in the process of introduction and selection (Slavov et al., 2004).

Polymorphism of blood proteins first offered the possibilities to study genetic differentiation before the advent of molecular markers. Consequently, several livestock breeds including the domestic sheep have been characterized for variations in blood proteins (Di Stasio, 1995; Mwancharo et al., 2002; Ibeagha-Awemu and Erhardt, 2004). This study therefore aims to quantify genetic diversity at the transferrin and haemoglobin loci in Balami, Uda, Yankassa and West African Dwarf (WAD) sheep breeds and to estimate the genetic distance between them.

MATERIALS AND METHODS

The four sheep populations indigenous to Nigeria, namely, Balami, Uda, Yankassa and West African Dwarf were sampled from small holder flocks and markets in Ibadan, Okene, Zaria, Iwo and Lokoja. A total of 100 blood samples comprising 25 healthy individual sheep of both sexes per breed were collected. Samples were collected from the jugular vein and emptied into 5 ml heparinised tubes using disposable needles and syringes and then preserved in ice boxes and transported to the Animal breeding and genetics laboratory for analysis.

The blood samples were centrifuged at 3000 rpm for 5 min. The plasma supernatant was decanted into clean plain tubes and stored until needed for transferrin analysis. The erythrocyte fraction was washed three times with physiological saline. After each centrifugation, the washing solution was removed by decanting. After the third washing, three parts distilled water was added to the erythrocyte fraction to release haemoglobin through lysis and the lysed samples were stored in a refrigerator until needed for further analysis. The cellulose acetate electrophoresis conditions were as described by RIKEN (2006) with minor modifications to suit the samples used in this study.

Red blood cells were lysed in 8 part dH2O 0.3 μl, for haemoglobin analysis with Tris EDTA as buffer while undiluted plasma was used to run transferrin with Tris glycine as running buffer. Electrophoresis lasted for 25 to 35 min and staining was done using ponceau S while destaining was with 5% acetic acid. The bands were scored visually based on their migratory pattern as described by RIKEN (2006).

Statistical analysis

Allelic variants for haemoglobin and transferrin locus were scored in order of increasing mobility, “A” being the allele with the slower of the two. Allele and genotype frequencies for each locus were computed by direct gene counting method and tested for fit to Hardy-Weinberg’s Equilibrium (HWE) using χ² goodness-of-fit-test. The observed and expected heterozygosities were calculated according to Nei (1973). The genetic differentiation among populations and fixation indices (Fis, Fit and Fst) were calculated according to the method of Wright’s (1978). The genetic distance (D) and genetic identity (I) were calculated according to Nei (1978). The Unweighted Pair Group Method of Algorithm (UPGMA; Sneath and Sokal, 1973) was used to view the tree topology of the dendrogram showing the relationship between populations. All computations were performed using Pogope (Yeh and Yong., 1999) and Tools for Population Genetic Analysis (TFPGA; Miller, 1997).

RESULTS

Gene frequencies

The two studied loci were polymorphic for all the breeds with nine allelic variants. Allele frequencies are given in Table 1. The highest number of alleles occurred at the transferrin (Tf, seven alleles) locus while Hb had two alleles. Except for the Tf which was very polymorphic, Hb alleles were present in the four breed populations studied at varying frequencies. The most frequent allele was HbA for Balami (0.72), Uda (0.72) and Yankassa (0.60), while HbB was most frequent in WAD (0.86).

Only seven alleles were found in this present study out of the twelve Transferrin alleles known in sheep breeds (Erhardt, 1986). Six alleles were detected in each of the populations at the Tf locus. The TfB allele was most frequent in the Balami while the TfA was the most frequent in the Uda population. The TfA and TfC alleles

Table 1. Allele frequencies of transferrin (Tf) and haemoglobin (Hb) for the breeds.

<table>
<thead>
<tr>
<th>Breeds</th>
<th>Transferrin (Tf) Alleles</th>
<th>Haemoglobin (Hb) Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nTf</td>
<td>TfA</td>
</tr>
<tr>
<td>Balami</td>
<td>25</td>
<td>0.12</td>
</tr>
<tr>
<td>Uda</td>
<td>25</td>
<td>0.30</td>
</tr>
<tr>
<td>Yankassa</td>
<td>25</td>
<td>0.30</td>
</tr>
<tr>
<td>WAD</td>
<td>25</td>
<td>0.12</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>0.21</td>
</tr>
</tbody>
</table>

WAD, West African Dwarf Sheep, HB, haemoglobin, TF, transferrin; A, B, C, D, E, F, G, P and S – alleles.
The Tf locus was the most polymorphic with twenty-three genotypes controlled by seven codominant alleles. In Balami, the most common genotypes were TfBB and TfBC with a value of 0.12; the most common genotype in the Uda population was TfNC with a frequency of 0.24 (highest genotype frequency); in the Yankassa populations, the TfAD, TfCG and TfDE were the most frequent genotypes with the same value of 0.12 while in the WAD population, the genotypes TfBG, TfCG and TfEG had equal frequencies which was the highest value in the Yankassa population while the TfG was the most frequent in the WAD population. TfG was absent in the Balami population but present in all other breeds while TfP was present only in the Balami and absent in all other breeds.

**Genotype frequencies**

The distribution of the genotypes and their frequencies are presented in Tables 2 and 3.

### Table 2. Genotype frequencies at the transferrin locus of the sheep populations and goodness of fit test of Hardy-Weinberg's equilibrium.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Balami</th>
<th>Uda</th>
<th>Yankassa</th>
<th>WAD</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>0</td>
<td>0.04</td>
<td>0</td>
<td>0.04</td>
<td>0.02</td>
</tr>
<tr>
<td>AB</td>
<td>0.04</td>
<td>0.08</td>
<td>0.4</td>
<td>0</td>
<td>0.18</td>
</tr>
<tr>
<td>AC</td>
<td>0.08</td>
<td>0.24</td>
<td>0.4</td>
<td>0.12</td>
<td>0.16</td>
</tr>
<tr>
<td>AD</td>
<td>0</td>
<td>0.08</td>
<td>0.12</td>
<td>0.04</td>
<td>0.06</td>
</tr>
<tr>
<td>AE</td>
<td>0.08</td>
<td>0.12</td>
<td>0.04</td>
<td>0.04</td>
<td>0.07</td>
</tr>
<tr>
<td>AG</td>
<td>0</td>
<td>0.04</td>
<td>0.04</td>
<td>0.08</td>
<td>0.03</td>
</tr>
<tr>
<td>AP</td>
<td>0.04</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.01</td>
</tr>
<tr>
<td>BB</td>
<td>0.12</td>
<td>0.04</td>
<td>0.04</td>
<td>0</td>
<td>0.05</td>
</tr>
<tr>
<td>BC</td>
<td>0.12</td>
<td>0.04</td>
<td>0</td>
<td>0</td>
<td>0.04</td>
</tr>
<tr>
<td>BD</td>
<td>0.04</td>
<td>0</td>
<td>0.04</td>
<td>0.04</td>
<td>0.03</td>
</tr>
<tr>
<td>BE</td>
<td>0.2</td>
<td>0.2</td>
<td>0</td>
<td>0.04</td>
<td>0.11</td>
</tr>
<tr>
<td>BG</td>
<td>0</td>
<td>0.04</td>
<td>0</td>
<td>0.12</td>
<td>0.04</td>
</tr>
<tr>
<td>CC</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.04</td>
<td>0.01</td>
</tr>
<tr>
<td>CD</td>
<td>0.04</td>
<td>0</td>
<td>0.12</td>
<td>0.08</td>
<td>0.06</td>
</tr>
<tr>
<td>CE</td>
<td>0.2</td>
<td>0</td>
<td>0.08</td>
<td>0</td>
<td>0.07</td>
</tr>
<tr>
<td>CF</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.12</td>
<td>0.03</td>
</tr>
<tr>
<td>CG</td>
<td>0.04</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.01</td>
</tr>
<tr>
<td>DD</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.08</td>
<td>0.02</td>
</tr>
<tr>
<td>DE</td>
<td>0</td>
<td>0</td>
<td>0.12</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>DF</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.04</td>
<td>0.01</td>
</tr>
<tr>
<td>EE</td>
<td>0</td>
<td>0.12</td>
<td>0</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>EF</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.12</td>
<td>0.03</td>
</tr>
<tr>
<td>FF</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.04</td>
<td>0.01</td>
</tr>
<tr>
<td>Total</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

$\chi^2$ | 11.28$^{ns}$ | 12.02$^{ns}$ | 21.34$^{ns}$ | 29.61$^*$ |

Ns = Not Significant (P>0.05); * = Significant (P<0.05).

### Table 3. Genotype frequencies and chi squared ($\chi^2$) test of Hardy-Weinberg’s equilibrium at the haemoglobin locus.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Balami</th>
<th>Uda</th>
<th>Yankassa</th>
<th>WAD</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>0</td>
<td>0.04</td>
<td>0.04</td>
<td>0.72</td>
<td>0.19</td>
</tr>
<tr>
<td>AB</td>
<td>0.56</td>
<td>0.56</td>
<td>0.72</td>
<td>0.28</td>
<td>0.53</td>
</tr>
<tr>
<td>BB</td>
<td>0.44</td>
<td>0.44</td>
<td>0.24</td>
<td>0</td>
<td>0.28</td>
</tr>
<tr>
<td>Total</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

$\chi^2$ | 0.66$^{ns}$ | 3.78$^{ns}$ | 3.78$^{ns}$ | 6.25$^*$ |

Ns = Not Significant (P>0.05); * = Significant (P<0.05).
Table 4. F-Statistics and gene flow based on haemoglobin and transferrin.

| Loci   | F_{is} | F_{it} | F_{st} | Nm  *
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb</td>
<td>-0.388</td>
<td>-0.069</td>
<td>0.230</td>
<td>0.836</td>
</tr>
<tr>
<td>Tf</td>
<td>-0.097</td>
<td>-0.034</td>
<td>0.058</td>
<td>4.092</td>
</tr>
<tr>
<td>Mean</td>
<td>-0.193</td>
<td>-0.047</td>
<td>0.123</td>
<td>1.791</td>
</tr>
</tbody>
</table>

*Nm = Gene flow estimated from F_{st} = 0.25(1 - F_{st})/ F_{st}. Hb = haemoglobin locus; Tf = transferrin locus.

were most frequent with a constant value of 0.12. Among all the breeds studied Tf^{AP}, Tf^{CC}, Tf^{CP}, Tf^{DG}, and Tf^{GG} were rare. All others were observed with varying frequencies.

Haemoglobin

Three genotypes of Hb (Hb^{AA}, Hb^{AB} and Hb^{BB}) determined by two codominant alleles were observed in Yankassa, whereas only Hb^{AB} and Hb^{BB} were observed in Balami and Uda, while the WAD had only Hb^{AA} and Hb^{AB}. The frequencies of Hb^{AB} and Hb^{BB} in Balami and Uda population were the same (0.56 and 0.44, respectively) with the frequency of Hb^{BB} being the highest. The frequency of Hb^{AA} was the highest (0.72) in the WAD while Hb^{AB} was 0.28. The frequency of Hb^{AA} was the lowest (0.04) in Yankassa while Hb^{AB} was the highest (0.72) and 0.24 for Hb^{BB}.

Chi square (χ²) test of Hardy-Weinberg Equilibrium

Hardy-Weinberg equilibrium (HWE) test for single locus was conducted for the populations within the two protein loci. Results shown in Tables 2 and 3 revealed that all the breeds had no significant deviation from HWE except WAD which deviated from the HWE at both protein loci.

F-Statistics and gene flow

Population differentiation revealed by fixation indices F_{is}, F_{it} and F_{st} for each of the loci studied across four Nigerian sheep population are shown in Table 4. The global heterozygosity deficit (F_{is}) was estimated at -0.047 and the within-breed deficit in heterozygote evaluated by F_{is} was -0.388 for Hb and -0.097 for Tf with a total mean of -0.193 for both loci. Global breed differentiation evaluated by F_{st}, was estimated at 0.123. The gene flow values for each of the loci studied was 0.836 for Hb and 4.092 for Tf. The mean gene flow over all loci was 1.791.

Genetic distance and genetic identity

The distance between populations ranged from 0.034 to 0.640. The smallest genetic distance was observed between Uda and Balami populations while the farthest distance was observed between WAD and Balami populations. Results of genetic identity are presented in Table 5. It showed that the Uda and Balami populations are more genetically alike (0.966) while the Balami and WAD populations were the least genetically identical (0.527).

Dendrogram

The genetic distance estimates were used to construct dendrogram based on individual locus and the pooled distances for the four loci. When the Dendrogram of genetic distance was viewed at the haemoglobin locus, the topology differentiated two sub clusters. The sub clusters were Balami - Uda at node 1 with no distance and Yankassa with a distance of 0.0238 at node 2 (including Balami Uda and Yankassa). The WAD was totally separated from the two sub clusters with a distance of 0.5802 at node 3 (including Balami, Uda and Yankassa). The WAD separated completely at node 2 with no distance and Yankassa with a distance of 0.0238 at node 2 (including Balami Uda and Yankassa). The WAD separated completely at node 3 with a distance of 0.4347 (including Balami, Uda and Yankassa) while the WAD separated completely at node 2 with a distance of 0.3045 (including Balami, Uda and Yankassa). The WAD separated completely at node 3 with a distance of 0.4347 (including all four populations) (Figure 1). The tree topology of the dendrogram of genetic distance between Balami, Uda, Yankassa and WAD sheep populations at the Transferrin locus revealed two sub clusters; Balami-Uda at node 1 corresponding to a distance of 0.1258, and Yankassa at node 2 having a distance of 0.3045 (including Balami, Uda and Yankassa) while the WAD separated completely at node 3 with a distance of 0.4347 (including all four populations) (Figure 2).

The Phylogenetic tree of the genetic distances pooled for the two loci supports the genetic distance estimates where the Balami population is the most genetically distant from the WAD population. The Balami and Uda populations formed a different cluster at node 1, indicating a closer relationship between the two populations, whereas the WAD separated completely at node 3, the Uda formed a sub cluster with the Balami-Yankassa group at node 2 (Figure 3).

DISCUSSION

Haemoglobin

All of the breeds in this study were polymorphic for Hb
Figure 1. Dendrogram of genetic distance between four Nigerian indigenous sheep populations at the haemoglobin locus.

Figure 2. Dendrogram of genetic distance between four Nigerian indigenous sheep populations at transferrin locus.

Figure 3. Dendrogram of genetic distance between four Nigerian indigenous sheep based on haemoglobin and transferrin.
with frequencies of Hb\textsuperscript{B} considerably higher than those of Hb\textsuperscript{A} in Balami, Uda and Yankassa. Similar results have been obtained by Bunch and Foote (1976), Zanotti et al.(1988), Clarke et al. (1989), Bunge et al. (1990), Mwacharo et al. (2002), Boujenane et al. (2008), and Shahrbabak et al. (2010) who reported that Hb\textsuperscript{B} is generally the most occurring allele in most sheep breeds. However, in contrast, the Hb\textsuperscript{A} was higher for the WAD population in this study; this variation may be attributed to the selective advantages of Hb in different geographical regions. The WAD being predominant in the wet humid regions may have Hb\textsuperscript{A} conferred on it for survival, while the Balami and Uda breeds may need of Hb\textsuperscript{B} for survival in the drier savannah regions where they are found. The Yankassa however is most widely spread and had about 60.0% of its members having Hb\textsuperscript{B} while the other 40.0% were found to have Hb\textsuperscript{A}. This may have given it the advantage of survival in the regions between the extremes of the wet humid regions and the drier savannah regions. Similar results of predominance of Hb\textsuperscript{A} had been reported for other sheep populations. Buis and Tucker (1983) found that in some Dutch breeds (Friesian, Schoonebeker, Drente and Kempen), Hb\textsuperscript{A} was the more common allele compared to Hb\textsuperscript{B}. In France, Nguyen et al. (1992) also made the same observations in Rambouillet breed. Tella et al. (2000) in a study of West African Dwarf and Yankassa sheep in South West Nigeria reported that Hb\textsuperscript{A} occur at higher frequency in the two breeds with Hb\textsuperscript{A} occurring in 98.8% of the WAD population and 78.78% of the Yankassa population sampled. However, Schillhorn and Folaranmi (1978) reported that haemoglobin allele types have selective advantages in different geographical regions, while Hb\textsuperscript{A} has been shown to have advantage in sheep at higher altitudes; Hb\textsuperscript{B} occurs more commonly in lowland breeds. In Nigeria, Hb\textsuperscript{B} type has a very high frequency in sheep of the northern savannah zone, the region in which the Balami and Uda breeds are predominantly found. This predominance appears to be of adaptive significance in the arid regions to which these breeds demonstrated fitness. This is due to the decreased haematocrit values, lower blood viscosity and more availability of water associated with Hb\textsuperscript{B} blood types compared to Hb\textsuperscript{A} types. This is buttressed by the reports of Tsunoda et al. (2006) that Hb\textsuperscript{A} allele has a high affinity for oxygen and is important for survival in mountain areas at altitudes over 3000 m and Pieragostini et al. (2006) who reported that the Hb\textsuperscript{A} is more frequent in sheep living in areas above 40°C latitude.

Furthermore, Ordas (2004) reported that Hb\textsuperscript{A} has a higher affinity for molecular oxygen than Hb\textsuperscript{B} because of differences in oxygen dissociation rates. The higher availability of molecular oxygen in erythrocytes with Hb\textsuperscript{A} may be responsible for the higher incidence of parasitism. This may be due to the fact that the Hb\textsuperscript{A} erythrocytes may support parasite establishment and propagation more than those with Hb\textsuperscript{B} which have lower diffusible intra erythrocytic oxygen. Thus, Altaif and Dargie (1978) and Buwanendran et al. (1980) reported a possible correlation between haemoglobin polymorphism and genetic resistance to helminth infection in sheep and goats. The results obtained in this study demonstrates that extreme temperatures (acute cold or sultry heat), extreme relief forms (desert or mountain), precarious nutrition and breeding conditions favour the fixing of the Hb\textsuperscript{A} allele and that the temperature situated in the biological comfort zone, moderate relief forms (forest, steppe hill) or the breeding techniques or methods are correlated with a more emphasized fixing of the Hb\textsuperscript{B} allele. Thus, in the biological respect, the allele Hb\textsuperscript{A} is characterised by a great selection advantage in comparison with the allele Hb\textsuperscript{B}. In a great measure, the selective advantage of Hb\textsuperscript{A} is due to the biophysical, biochemical and physiological peculiarities of the haemoglobin molecule type A (saturation capacity with oxygen, dissociation curve of oxyhaemoglobin, erythrocyte load with haemoglobin and metabolic profile of the erythrocyte) (Raushenbach and Kamene", 1978).

**Transferrin**

The seven alleles observed at the Tf locus were dispersed, in terms of their frequencies and number within each breed, and in respect of their distributions among breeds. The differences observed at the Tf alleles indicate clear genetic differentiation between the Nigerian breeds studied. The Tf\textsuperscript{E} allele is a rare allele exclusively found in the Balami breed at very low frequency and the Tf\textsuperscript{G} was found only in three of the four breeds studied (Uda, Yankassa and WAD). Manwell and Baker (1977) suggested that electrophoretic variants with low frequencies may represent, in many cases, relative recent mutations occurring after divergence of the breeds; this could be the case with the Balami breed. Akinyemi and Salako (2012) also reported Tf\textsuperscript{D} in Balami breed and the same was also reported for SardiandBeni Ashen sheep breeds in Morocco (Boujenane et al., 2008), and it is reported to be more widely distributed in European sheep breeds (Buis and Tucker, 1983; Zanotti et al., 1990).

The gene frequencies at Tf locus were compared with those reported by other researchers to obtain information on the degree of divergence between breeds. Ashton and Ferguson (1962) reported the frequencies of alleles Tf\textsuperscript{A}, Tf\textsuperscript{B}, Tf\textsuperscript{C}, Tf\textsuperscript{D}, Tf\textsuperscript{E} and Tf\textsuperscript{G} in three different populations of Australian Merino. Stormont et al. (1968) reported the frequencies of these alleles in Mailliard Merino and Nguyen et al. (1992) published results on these alleles in Spanish Merino.

Higher frequency of Tf\textsuperscript{A} and Tf\textsuperscript{C} in Yankassa is supported by the report of Ibeagha-Awemu and Erhardt (2004) on the same breed, where Tf\textsuperscript{C} was reported to have the highest frequency in Yankassa and by the
report of Akinyemi and Salako (2012), who reported the Tf^A allele as the highest in the Yankassa. The presence of Tf^D and Tf^E alleles in this study was also reported by Ibeagha-Awemu and Erhardt (2004). The occurrence of Tf^E in Yankassa in this study was also reported by Akinyemi and Salako (2012) and was observed in some Moroccan sheep breeds (Boujenane et al., 2008) but was not reported by Ibeagha-Awemu and Erhardt (2004). The presence of the alleles Tf^E, Tf^D and Tf^D in the studied breeds were also reported by Akinyemi and Salako (2012) in similar breeds but were totally absent in a report on Kenyan breeds (Mwacharo et al., 2002). Ibeagha-Awemu and Erhardt (2004) posited that the absence of these alleles may not totally exclude their occurrence in the breeds but may have exposed the limitation of the method of starch gel electrophoresis in separating Tf variants.

Observation at Transferrin locus are generally difficult to compare with the result obtained in other studies because of the different electrophoresis media used by other researchers and subsequently different resolution power, that is, starch gel and poly acrylamide gel electrophoresis (Akinyemi and Salako, 2012). However, significant deviations of allele frequencies may occur owing to crossing and linking, inbreeding, sample error, population bottlenecks and random genetic drift. The genetic differences between the breeds are to be expected for breeds studied since they are found in separate locations throughout Nigeria, where little or no gene flow occurs.

Hardy-Weinberg equilibrium

The significant deviations from HWE (P < 0.05) observed for both locus within the WAD breed could be attributed to unobserved null alleles, excess of heterozygote individuals than homozygote individuals, migration, high mutation rate and artificial selection in the breeds (Aminafshar et al., 2008). Significant deviations of allele frequencies may occur owing to crossing and linking, inbreeding, sample error, population bottlenecks and random genetic drift. Ideal Hardy-Weinberg’s populations do not actually occur in nature owing to various factors, which can shift the equilibrium and disrupt the stability of a population, giving rise to change in the genetic structure (Sargent et al., 1999). Deviation from HWE at protein loci have also been reported in studies such as in Southern Africa sheep (Sargent et al., 1999). Since on the overall data set, there were no significant deviations from HWE, it may be suggested that there are no biological phenomena or sampling error biases with a net effect for sufficient differences between observed and expected proportions.

Gene flow and F- statistics

F-Statistic values of $F_{ST}$ and $F_{IT}$ are measures of deviation from Hardy-Weinberg’s proportions and total populations respectively. Positive values indicate a deficiency in heterozygotes and negative values indicate an excess of heterozygotes. $F_{IS}$ can be interpreted as a measure of inbreeding (the measure of allelic fixation of individuals relative to the subpopulations). Thus, the negative values of $F_{IT}$ observed at the two loci in the four breeds studied and the overall negative value of -0.047 and the negative value of $F_{IS}$ showed the deficiency of homozygotes in the populations and that mate were less related in comparison with the average relationship of the population. This observed excess of heterozygotes could be due to non-random mating and genetic exchange between populations.

Estimated $F_{ST}$, which corresponds to the proportion of genetic variability accounted for by the differences among breeds, was 0.123. Thus, a large part of the total genetic diversity can be explained by the variation within breeds (0.877) and to a smaller extent by the variation among breeds. This result indicates that genetic diversity quantified by allozyme markers shows little genetic differentiation among Nigerian sheep breeds studied. The degree of differentiation observed between the Nigerian sheep breeds could be due to geographic proximity, similarities in environment and breeding practices, but most likely due to past genetic exchange among them since mean gene flow over all loci was 1.791.

Genetic distance and genetic identity

Nei (1972) standard genetic distance (D) obtained in this study (0.034-0.640) indicates the level of genetic differentiation between the breeds. Buis and Tucker (1983) reported D values of 0.181 to 0.308 between different sheep breeds and an average D value of 0.248. Different authors have reported different values of D in different sheep breeds. Ordas and Primitivo (1986) estimated the genetic distance between Spanish dairy sheep breeds and reported 0.0094-0.055 using data from 8 loci. Zanotti et al. (1990), using data from four blood groups and six protein loci, reported genetic distance ranging between 0.012 and 0.060 in five Italian sheep breeds. Mwacharo et al. (2002) obtained a closer estimate of genetic distance between Kenyan sheep breeds (0.044 - 0.169) than between Kenyan and the exotic Merino sheep (0.044 - 0.283) in a study using data on five protein-coding loci. Among six Moroccan local sheep, namely, D’man, BeniAhsen, Sardi, Timahdite, BeniGuil and Boujaad, Boujenane et al. (2008) reported a genetic distance range of 0.006 to 0.026. Distances obtained in this study between breeds were higher than those by Akinyemi and Salako (2012) who reported a range of 0.003 to 0.015. The distances obtained from this current study indicate that that the Balami and Uda which are predominantly northern breed are more closely related to each other than they are to the WAD which is a southern breed. The Yankassa however has adaptive
futures which make it the breed in-between.

**Dendrogram**

The phylogenetic tree constructed separated the WAD from other indigenous sheep, suggesting either early prehistoric separation of the WAD sheep or separate historical origin. The close genetic relationship between the Balami and Uda breed may be attributed to possible interbreeding between these two populations which are predominantly Northern breeds to form a homogenous population separated by administrative boundaries. Furthermore, the close genetic relationship between the breeds may also be attributed to similarity in ecological zones and production systems as well as the incidents of cross border livestock rustling contributing to the migration and movement of livestock and subsequent interbreeding between such livestock (Mwacharo et al., 2002). Based on the highest value of Nei's genetic distance (0.640), breeding programs involving the crossing of the Balami and WAD is recommended, since the crosses between breeds which are homogenous but distinctly different in their relationship would produce more hybrid vigour in the crossed progeny.

**Conclusion**

The populations were characterized by the presence of 7 transferrin alleles Tf\(\alpha\), Tf\(\beta\), Tf\(\gamma\), Tf\(\delta\), Tf\(\phi\), and Tf\(\phi\)\(\gamma\), controlling 23 genotypes, 6 of which were homozygous and 17 heterozygous. Two haemoglobin alleles, Hb\(\alpha\) and Hb\(\beta\) controlling 3 genotypes were found. Two of the haemoglobin genotypes were homozygous. According to the transferrin system all the breeds were in genetic equilibrium except for the WAD which had large variation in number between the observed and expected genotypes and the high \(\chi^2\) value of 29.61. According to the haemoglobin system, the WAD population was not in genetic equilibrium as revealed by the \(\chi^2\) test of Hardy Weinberg Equilibrium. All other breeds were in genetic equilibrium at the haemoglobin system. The presence of differences between the frequencies of the alleles by categories could be a source of genetic diversity.

**Conflict of Interests**

The authors have not declared any conflict of interests.

**REFERENCES**


Schillhorn van Veen TW, Folaramni DOB (1978). The haemoglobin