BIODIVERSITY AND POPULATION STRUCTURE OF INDIGENOUS BREEDS OF SHEEP IN NIGERIA

BY

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DEDICATION

TO THE FOND MEMORIES OF MY FATHER

PA. EHIGIATỌ OSAIYUWU

ABSTRACT

Genetic characterisation is the basis for selective breeding, crossbreeding, conservation, utilisation, improvement and rational management of animal genetic resources. Indiscriminate crossbreeding has led to erosion of genetic resources of indigenous domestic animals in Nigeria. Information on the genetic biodiversity of the indigenous sheep in Nigeria has not been adequately documented. Therefore, the population structure and biodiversity of indigenous breeds of sheep in Nigeria were assessed in this study.

Twenty-five sheep each of Balami, Uda, Yankassa and West African Dwarf (WAD) breeds were purposively sampled from Lokoja, Iwo, Okene and Ibadan for biochemical studies. Blood (5 mL) samples were collected to determine variations at four structural protein loci: Albumin (Alb), Transferrin (Tf), Carbonic Anhydrase (CA) and Haemoglobin (Hb), using cellulose acetate electrophoresis. Blood (5 mL) from 24 Balami, 25 Uda, 23 Yankassa and 19 WAD sheep were sampled from Shika, Guga and Ibadan for microsatellite loci analysis. Using real time PCR, 13 microsatellite marker loci: CSRD247, HSC, INRA63, MAF214, OARAE129, OARCP49, OARFCB304, BMS4008, D5S2, OARFCB20, MAF65, MCM527, and SPS113 were genotyped. The populations were characterised for genetic variability using Mean Number of Alleles (MNA), allele frequencies, Number of Unique Alleles (NUA), Polymorphic Information Content (PIC), observed Heterozygosity (H_o), genetic distance (D). Data were analysed using F-statistic (F_{it}, F_{is}, F_{st}), Analysis of molecular variance, cluster analysis and test of Hardy-Weinberg Equilibrium (HWE) at $\alpha_{0.05}$.

Thirteen allelic variants (Hb^A, Hb^B, CA^F, CA^S, Alb^A, Alb^B, Tf^A, Tf^B, Tf^C, Tf^D, Tf^E, Tf^G and Tf^P) were observed at the four protein loci. Modal number (seven) occurrence of alleles was at the Tf locus while two were observed in other loci. The H_o were 0.52, 0.59, 0.61 and 0.52 for Balami, Yankassa, WAD and Uda respectively. The closest D (0.05) was between Balami and Yankassa, while between Balami and WAD was farthest (0.44). Homozygote deficiency (F_{is} = -0.25; F_{it} = -0.05) was observed within breeds. Significant HWE were observed in Yankassa (Hb and Tf) and WAD (CA). A

total of 149 alleles were observed at the microsatellite loci. The MNA per locus was 11.4±4.0 and ranged between 5.9±2.3 and 8.5±3.4 among breeds with NUA of 45. The PIC observed across loci was 0.65, while the H_o ranged from 0.63 (Balami) to 0.69 (Uda). The D was least (0.09) between Balami and Uda, and highest (0.31) between WAD and Uda. Observed inbreeding within populations (F_{is} = 0.05) resulted in heterozygote deficiency and low genetic differentiation among breeds (F_{st} = 0.06). Only 4.5% of the total genetic variation was explained by population differences, 2.6% by variation within population and 92.9% by differences among individuals. Yankassa clustered with Balami at protein loci, while Balami and Uda clustered at microsatellite loci. The HWE was significant for BMS4008, CSRD247, HSC, INRA63, MAF65, MAF214, MCM527, OARAE129, OARCP49 and OARFCB304 microsatellite loci in at least one population.

Genetic exchange was present at biochemical loci, whereas breed homogeneity was supported at microsatellite loci. Selection and crossbreeding between West African dwarf sheep and any of Uda, Balami or Yankassa will improve breed crosses.

Keywords: Nigerian sheep diversity, Heterozygosity, Nigerian indigenous sheep, Sheep protein loci

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CERTIFICATION

I certify that this study was carried out under my supervision by Osamede Henry OSAIYUWU in the Department of Animal Science, University of Ibadan, Ibadan.

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INTRODUCTION

Livestock domestication by man introduced a major cultural revolution. Hominids and early man were hunters and gatherers for millions of years. The climatic fluctuations, which followed the end of the glacial period some 14,000 years ago, may have been instrumental in forcing man to domesticate animals. Records of the domestication of sheep dates back to as early as 7000 BC in the Near East (Plug and Badenhorst, 2001).

In the past years, selection programs have mainly put high emphasis on production traits, which led to an increased specialization for traits such as milk yield and quality, meat, and wool. This happened sometimes even by crossbreeding the local breeds with exotic ones, to generate populations with the desired phenotypes. This hybridization process has resulted in an increased reliance on a small number of breeds to meet the local's food requirements, which could lead to the disappearance of local breeds. The conservation of local breeds has received greater interest in the last years, based on the awareness that indigenous and locally developed sheep breeds are important assets, because of the unique combinations of adaptive traits developed to respond effectively to the pressures of the local environment (Buduram, 2004). From these considerations and given the importance of the local genetic resources, it is easily understandable why considerable interest is given nowadays to genetic diversity studies in domestic animals in general and, recently, in small ruminants (Baumung *et al*., 2004).

Genetic diversity studies in domestic animals aim at evaluating genetic variation within and between breeds, since the breed is the management unit for which factors such as inbreeding are controlled (Tapio *et al*., 2005). However, the definition of a breed, as applied by Food and Agricultural Organisation (FAO), frequently does not reflect the underlying genetic population structure. Therefore, a molecular genetic study of the

population diversity and structure improves the understanding of the actual genetic resources.

The domestic sheep (*Ovis aries*) has, during the last 10,000 years attained a relatively significant increase in body size, a decrease in horn size and a change from a hairy, moulty fleece to a white woolly fleece (Ryder, 1983). Hundreds of local breeds and strains have been developed for different production systems throughout the world. Further genetic improvement has occurred in the last 50 years as a result of the application of quantitative genetics and selective breeding methods (Adebambo *et. al*., 2003). In addition, studies of the physiology of the sheep has contributed to improved agricultural production and highlighted the sheep as a useful model in the study of mammalian reproduction and neurology (Crawford, *et al*., 1995). Many economically important traits in sheep such as growth rate, body composition, and disease resistance are multigenic in nature. If we are to understand the genetics of these complex traits, we need to know and exploit the wide genetic diversity that is present in the domestic sheep (Adebambo *et. al*., 2003). With a relatively unselected sheep population of more than 14 million in Nigeria, widely dispersed throughout the country, yet highly localized in their existence and adaptation, the need to characterize and exploit these diverse genotypes cannot be over-emphasized (Adebambo *et. al*., 2003).

Nigeria has basically four definitive sheep breeds – the West African Dwarf (WAD), the Uda, the Balami and the Yankassa, all of which are well adapted to different ecological niches within the country's geographical sphere, except for the Yankassa which has a very wide spread across the country (Adu and Ngere, 1979).

The characterization and conservation of domestic animal diversity is essential to meet future needs in Africa and in Nigeria in particular. In order to cope with an unpredictable future, genetic reserves capable of readily responding to directional forces imposed by a broad spectrum of environment must be maintained. Maintaining genetic diversity is an insurance package against future adverse conditions. Due to diversity among environments, nutritional standards and challenges from infectious agents, a variety of breeds and populations are required. These act as store houses of genetic

variation which form the basis for selection and may be drawn upon in times of biological stress such as famine, drought or disease epidemics.

The need for characterization and conservation comes from the potential rate of decrease of genetic variation. The loss of genetic variation within and between breeds is detrimental not only from the perspective of culture and conservation but also utility, since lost genes may be of future economic interest. Within breeds, high rates of loss of genetic variation leads to reduced chances of breed survival due to decreased fitness through inbreeding depression. These breeds become subject to faster changes in gene frequencies, greater rate of loss of gene and genetic constitutions (haplotypes). These are all due to small effective population size or equivalently high rates of inbreeding (Meuwissen, 1991).

Once animal genetic diversity has been lost, it cannot be replaced. Advances in biotechnology offer possibilities of improving, utilising, characterising and conserving present domestic animal diversity. The economic implications of maintaining existing farm animal genetic resources in their natural environment are negligible as compared to the costs involved in biotechnology development (FAO, 2000).

Animals, as compared to plants, are more complicated and more expensive to manipulate. Animals have hundreds of thousands of genes which interact in a complex way (Weller, 2001). It is this unique combination of genes, their interaction with each other and the environment that determines an animal's ability to reproduce or adapt itself to a particular environment.

To be able to distinguish between breeds for conservation and utilization purposes, the determination of the genetic variability, population structure and phylogenetic relationships using Biochemical and Deoxyribonucleic acid (DNA) microsatellite markers becomes imperative to support existing phenotypic data. In the past few years, microsatellite-based studies on genetic characterization, establishment of genetic relationships and differentiation have concentrated mainly on different European sheep populations (Arranz *et al.*, 1998; Diez-Tascon *et al.*, 2000). Unfortunately, only a few such studies have considered sheep breeds across Africa and Nigeria in particular.

Microsatellites are highly polymorphic two to six nucleotide repeat sequences that are widely dispersed in the mammalian genome. They have been shown to be powerful tools in the genome mapping of man and his animals (Cornall, *et al*., 1991; Estoup *et al*., 1993).

1.1 Objective of the Study

1.1.1 Main Objective:

The main objective of this research is to explore genetic diversity within and between the Nigerian indigenous sheep populations.

1.1.2 Specific Objectives

The specific objectives of this research are to:

1. estimate the allelic and genotypic frequencies of hemoglobin, transferrin, albumin and carbonic anhydrase in Balami, Uda, Yankassa and West African Dwarf (WAD) sheep breeds.
2. evaluate breed differences from genetic, distance and the degree of heterozygosity of alleles at the hemoglobin, transferrin, albumin and carbonic anhydrase locus in Balami, Uda, Yankassa and WAD sheep breeds
3. determine and document genetic variability and differentiation of the sheep through microsatellite analysis of PCR product
4. estimate allelic and genotypic frequencies of various microsatellites in Balami, Uda, Yankassa and WAD sheep breeds
5. evaluate breed differences from genetic, distance and the degree of heterozygosity of alleles at various microsatellite locus in the different breeds

1.2 Justification

Characterization of breeds is the first step towards conservation and utilization of Animal Genetic Resource (AnGR) which makes the determination of the genetic variability, population structure and phylogenetic relationships using Biochemical and DNA microsatellite markers an important data source to support existing phenotypic data.

A large number of studies have documented the characterization of blood group and allozyme systems of livestock (Baker and Manwell, 1980; Shamsuddin *et al.*, 1986; Nguyen *et al.*, 1992; Canatan and Boztepe, 2000; Akinyemi *et.al*, 2010, Bindu and Raghavan, 2010). However, the level of polymorphism observed in proteins is often low which has reduced the general applicability of protein typing in diversity studies. With the development of Polymerase Chain Reaction (PCR) and sequencing technologies, DNA-based polymorphisms are now the markers of choice for molecular-based surveys of genetic variation. Importantly, polymorphic DNA markers showing different patterns of Mendelian inheritances can now be studied in nearly all of our major livestock species.

Microsatellites have now been isolated in large numbers from most livestock species and a recommended list (ISAG/FAO, 2004) of markers for genetic characterization studies is publicly available. Important assumptions on the use of genetic markers include: (i) that the polymorphisms observed at the molecular markers are neutral; (ii) that the use of a relatively small number of independently segregating marker loci will be a good predictor of the overall genomic diversity of a population; in other words that variation in allele frequencies between populations will reflect the distribution of genetic diversity within and among populations.

Microsatellite markers were chosen for this study as they are available and are recommended by the Food and Agricultural Organization (FAO) for animal genetic resources studies (FAO, 2011).

CHAPTER TWO

LITERATURE REVIEW

2.1 Origin of Sheep

Sheep (*Ovis aries*) are quadrupedal, ruminant mammals typically kept as livestock. Like all ruminants, sheep are members of the order *Artiodactyla*, the even-toed ungulates. Although the name "sheep" applies to many species in the genus *Ovis*, in everyday usage it almost always refers to *Ovis aries*. Numbering a little over one billion, domestic sheep are also the most numerous species of sheep.

Sheep are most likely descended from the wild mouflon of Europe and Asia. Sheep are raised for fleece, meat (lamb, hogget or mutton) and milk. Sheep's wool is the most widely used animal fibre, and is usually harvested by shearing. Ovine meat is called lamb when from younger animals and mutton when from older ones. Sheep continue to be important for wool and meat today, and are also occasionally raised for pelt, as dairy animals, or as model organisms for science.

Sheep were among the first animals to be domesticated by humankind; sources provide a domestication date between nine and eleven thousand years ago in Mesopotamia (Ensminger and Parker, 1986; Weaver, 2005).Their wild relatives have several characteristics—such as a relative lack of aggression, a manageable size, early sexual maturity, a social nature, and high reproduction rates—which made them particularly suitable for domestication (Budiansky, 1999). Today, *Ovis aries* is an entirely domesticated animal that is largely dependent on man for its health and survival (Budiansky, 1999). Feral sheep do exist, but exclusively in areas devoid of large predators (usually islands) and not on the scale of feral horses, goats, pigs, or dogs, although some

feral populations have remained isolated long enough to be recognized as distinct breeds (Budiansky, 1999).

The exact line of descent between domestic sheep to their wild ancestors is presently unclear (Hiendleder, *et al*., 2002). The most common hypothesis states that *Ovis aries* is descended from the Asiatic (*O. orientalis*) species of mouflon. It has been proposed that the European mouflon (*O. musimon*) is an ancient breed of domestic sheep turned feral rather than an ancestor, despite it commonly being cited as ancestor in past literature (Ensminger and Parker, 1986). A few breeds of sheep, such as the Castle milk Moorit from Scotland, were formed through crossbreeding with wild European mouflon.

The urial (*O. vignei*) was once thought to have been a forebear of domestic sheep, as they occasionally interbreed with mouflon in the Iranian part of their range (Ensminger and Parker, 1986). However, the urial argali (*O. ammon*), and snow sheep (*O. nivicola*) have a different number of chromosomes than other Ovis species, making a direct relationship implausible, and phylogenetic studies show no evidence of urial ancestry (Hiendleder, *et al*., 2002). Further studies comparing European and Asian breeds of sheep showed significant genetic differences between the two. Two explanations for this phenomenon have been posited. The first is that there is a currently unknown species or subspecies of wild sheep that contributed to the formation of domestic sheep (Hiendleder, *et al*., 2007). A second hypothesis suggests that this variation is the result of multiple waves of capture from wild mouflon, similar to the known development of other livestock (Meadows, *et al*., 2007).

Initially, sheep were kept solely for meat, milk and skins. Archaeological evidence from statuary found at sites in Iran suggests that selection for woolly sheep may have begun around 6000 BC (Ensminger and Parker, 1986; Weaver, 2005), but the earliest woven wool garments have only been dated to two to three thousand years later (Smith, *et al*., 1997). By that span of the Bronze Age, sheep with all the major features of modern breeds were widespread throughout Western Asia (Ensminger and Parker, 1986). However, one chief difference between ancient sheep and modern breeds is the

technique by which wool could be collected. Primitive sheep cannot be shorn, and must have their wool plucked out by hand in a process called "rooing". This is because fibres called kemps are still longer than the soft fleece. The fleece may also be collected from the field after it falls out. This trait survives today in unrefined breeds such as the Soay and many Shetlands. Indeed, the Soay, along with other Northern European breeds with short tails, unshearable fleece, diminutive size, and horns in both sexes, are closely related to ancient sheep. Originally, weaving and spinning wool was a handicraft practiced at home, rather than an industry. Babylonians, Sumerians, and Persians all depended on sheep; and although linen was the first fabric to be fashioned into clothing, wool was a prized product. The raising of flocks for their fleece was one of the earliest industries, and flocks were a medium of exchange in barter economies. Numerous Biblical figures kept large flocks, and subjects of the kings of Israel were taxed according to the number of rams they owned (Ensminger and Parker, 1986).

Sheep entered the African continent not long after their domestication in western Asia (Blench and Kevin, 1999). A minority of historians once posited a contentious African theory of origin for *Ovis aries* (Blench and Kevin, 1999). This theory is based primarily on rock art interpretations, and osteological evidence from Barbary sheep (Blench and Kevin, 1999). The first sheep entered North Africa via Sinai, and were present in ancient Egyptian society between eight and seven thousand years ago (Blench and Kevin, 1999). Sheep have always been part of subsistence farming in Africa, but today, the only country that keeps an influential number of commercial sheep is South Africa (Ensminger and Parker, 1986).

2.2 General Description

Domestic sheep are relatively small ruminants, usually with a crimped hair called wool and often with horns forming a lateral spiral. Domestic sheep differ from their wild relatives and ancestors in several respects, having become uniquely neotenic as a result of selective breeding by humans (Budiansky, 1999). A few primitive breeds of sheep retain some of the characteristics of their wild cousins, such as short tails. Depending on breed, domestic sheep may have no horns at all (i.e. polled), or horns in both sexes, or

in males only. Most horned breeds have a single pair, but a few breeds may have several (Ensminger and Parker, 1986).

Another trait unique to domestic sheep as compared to wild ovines is their wide variation in colour. Wild sheep are largely variations of brown hues, and variation within species is extremely limited. Colours of domestic sheep range from pure white to dark chocolate brown and even spotted or piebald (Rocky Mountain Natural Colored Sheep Breeders Association (RMNCSBA), 2007; British Coloured Sheep Breeders Association (BCSBA), 2012). Selection for easily dyeable white fleeces began early in sheep domestication, and as white wool is a dominant trait it spread quickly. However, coloured sheep do appear in many modern breeds, and colour may even appear as a recessive trait in white flocks (RMNCSBA, 2007; BCSBA, 2012). While white wool is desirable for large commercial markets, there is a niche market for coloured fleeces, mostly for hand-spinning (Weaver, 2005). The nature of the fleece varies widely among the breeds, from dense and highly crimped, to long and hair-like. There is variation of wool type and quality even among members of the same flock, so wool classing is a step in the commercial processing of the fibre.

Suffolks are medium wool, black-faced breed of meat sheep that make up 60% of the sheep population in the U.S (Simmons and Ekarius, 2001). Depending on breed, sheep show a range of heights and weights. Their rate of growth and mature weight is a heritable trait that is often selected for in breeding (Simmons and Ekarius, 2001). Ewes typically weigh between 45 and 100 kilograms (99 and 220 lb), and rams between 45 and 160 kilograms (99 and 350 lb) (Melinda, 2004). When all deciduous teeth have erupted, the sheep has 20 teeth (Frandson and Spurgeon, 1992). As with other ruminants, the front teeth in the lower jaw bite against a hard, toothless pad in the upper jaw. These are used to pick off vegetation, and then the rear teeth grind it before it is swallowed. There are eight lower front teeth in ruminants, but there is some disagreement as to whether these are eight incisors, or six incisors and two incisor-shaped canines. This means that the dental formula for sheep is either $\frac{0.0.3.3}{4.0.3.3}$ or $\frac{0.0.3.3}{3.1.3.3}$

(Melissa, 2001). There is a large toothless gap between the front "biting" teeth and the rear "grinding" teeth.

For the first few years of life it is possible to calculate the age of sheep from their front teeth, as a pair of milk teeth is replaced by larger adult teeth each year, the full set of eight adult front teeth being complete at about four years of age. The front teeth are then gradually lost as sheep age, making it harder for them to feed and hindering the health and productivity of the animal. For this reason, domestic sheep on normal pasture begin to slowly decline from four years on, and the average life expectancy of a sheep is 10 to 12 years, though some sheep may live as long as 20 years (Smith *et al.*, 1997).

Sheep have good hearing, and are sensitive to noise when being handled (Smith *et al.*, 1997). Sheep have horizontal slit-shaped pupils, possessing excellent peripheral vision. With visual fields of approximately 270° to 320°, sheep can see behind them without turning their heads (Weaver, 2005; William, 2005). Many breeds have only short hair on the face and some have facial wool (if any) confined to the poll and or the area of the mandibular angle; the wide angles of peripheral vision apply to these breeds. A few breeds tend to have considerable wool on the face. For some individuals of these breeds, peripheral vision may be greatly reduced by "wool blindness", unless recently shorn about the face (Terrill and Hazel, 1946). Sheep have poor depth perception; shadows and dips in the ground may cause sheep to baulk. In general, sheep have a tendency to move out of the dark and into well-lit areas, and prefer to move uphill when disturbed. Sheep also have an excellent sense of smell, and, like all species of their genus, have scent glands just in front of the eyes and interdigitally on the feet. The purpose of these glands is uncertain (Smith *et al.*, 1997), but those on the face may be used in breeding behaviours (Simmons and Ekarius, 2001). The foot glands might also be related to reproduction (Simmons and Ekarius, 2001), but alternative reasons, such as secretion of a waste product or a scent marker to help lost sheep find their flock, have also been proposed (Smith *et al.*, 1997).

2.3 Domestication

The region and time of domestication were largely determined by the development of various preconditions for it. To these belong, among others, the following steps and factors (Reed, 1984):

1. Contacts of human with sheep. Humans started to hunt for sheep for meat and skins. The two species learned features of each other's social behaviour. Each social group learned to tolerate members of the other species as a part of a larger social group. This led to close contacts and symbiotic relationships between humans and animals, a mutual understanding of each other's behaviour and the taming of newborn lambs and using them as pets (Reed, 1984).
2. The worldwide change in environment that accompanied and followed the end of the last glacial period in the twelfth to ninth millennia BC. South western Asia had not been covered by glaciers, so that people can live there through the ice age. Wild sheep have adapted to high altitudes and cold climates by delaying the breeding season or by extending gestation length. Growth of wild grasses (ancestors of wheat and barley) in the hills of south-western Asia, the gathering and preserving of their grains by people and the development of cultivation of these grasses, of little villages and of sedentary lifestyle were important factors. The crops and leftovers attracted sheep, bringing them close to humans (Reed, 1984).
3. Increased birth rates and lowered mortality of humans, increased sizes of settlements and diminishing supply of large game resulting from overhunting.
4. Physiological ability of ruminants developed during millions of years, to eat, digest and convert cellulose –rich grasses and straws to products useful for humans as a ruminant, the sheep was able to recycle its urea and thus to survive on protein-poor diets (Reed, 1984).

These preconditions prevailed especially in south-western Asia, thus explaining why that region became centre of domestication of many ruminant species including sheep. The behaviour and intelligence of these animals could have made their domestication possible at any time during the preceding several millions of years (Reed, 1984)

2.4 Places and Dates of Domestication

The cultivation of plants and the domestication of herd animals began on the grassy, open-forested hills on the crescent shaped fertile area extending from Palestine northwards to Lebanon and southern Turkey, and then southwards through the foothills of the Zagros Mountains on the Iraq-Iran border. The three neighbouring areas are especially associated with sheep domestication: the Kermanshah plateau Andluristan, which descend from there to the plain in Iran, and the western foothills of the Zagros Mountains in Iraq (Ryder, 1984).

The time of domesticating sheep was the prehistoric period at the end of the middle Stone Age, by the primitive peoples settling there and cultivating plants (Ryder, 1984) .The first evidence for sheep domestication comes from bone remains dated 900BC at Zawi Chemi Shanidar in Iraq, where 50% of the bones were from sheep (>40% of them immature). The high proportion of young animals is considered a sign of a systematic influence of humans on the deaths of sheep and of economic development. Other sites and dates of remains showing early domestication are Tepe Sarab in Iran (8000BC), Tebe Sabz and Jarmo in Iran and Afghanistan (7000BC), Turkey, Baluchistan and the Indus valley in India (6500BC) and Syria, Crete, Greece and the Quetta valley in Pakistan (6000BC). By 5500BC, domestic sheep had evidently reached Corsica and by 400BC by the Atlantic Coast, Britain and Scandinavia.

Relatively, few bone remains are adequate to show age and sex ratios or the relative importance of sheep as compared with other livestock. In the Zawi Chemi Shanidar excavations, 50% and 42% were from sheep and goats, respectively. At Tepe Sabz, the goat predominated until 5500BC, when both species became more common and only 40% reached the age of 3 years. Thereafter, skin working tools decreased and spindle whorls used in wool spinning increased. After 4000BC, sheep dominated. The excess of young males on many early sites can be interpreted as differential mortality rather than killing, but it might have created a surplus of ewe's milk, leading to the origin of milking. The knowledge obtained from the sheep remains can be supplemented, for

example, by rock paintings in Tassili, Sahara, which show horned sheep about 4000BC, with smooth coat very similar to that of wild type (Ryder, 1984).

2.5 Motives for Domesticating Sheep

Only assumptions are available about the reason for domesticating sheep, since it happened long before any records were kept (Ryder, 1984). The first domesticators may not have known what they were doing but they had known animals as living creatures to be hunted and killed or to be avoided in the case of large predators. The change from aggressive hunting to protective keeping was primarily a change in attitude of humans. This change left no direct traces for the archaeologist to discover, but only indirect results of changes in human behaviour following modifications in human thinking. The process was probably gradual, beginning long before there is evidence for it.

It may have been preceded by a game-management phase, and was an unconscious, unplanned and gradual beginning of an association between humans and sheep, both of which were social species, pre adapted by their evolutions of benefit to each other (Ryder, 1984). The process can be understood on the basis of ethnographic and animal behaviour studies. Men may have caught newborn lambs of killed ewes as hunting decoys or as pets and brought them with the children. The young lamb might then have become attached to the foster mother by imprinting. The lamb had to be handled and fed at a personal and individual level, to avoid its running wild. Those lambs that inherited genetic combinations for continuing wildness either escaped or were killed, so that their genes did not persist in the population undergoing domestication. Thus women may have had a decisive role in sheep domestication (Ryder, 1984).

It is often easier to obtain food by hunting than by farming; for example, North American Indians did not domesticate the bighorn. Thus, the motives may have been largely ecological; for example, the desiccation caused by the retreating ice cap forced humans to share food sources with the decreasing numbers of wild animals and at the same time search for alternative sources. Another motive was supplied by the extinction of the Pleistocene mega fauna, causing a shift to the hunting of smaller animals for food and thus a closer association of humans with sheep (Ryder, 1984).

The hunter gatherers might have seen the uses for meat, bones and skins. After long experience and the development of a more sedentary lifestyle by humans and the accumulation of random mutations in sheep, their secondary uses (milk and wool) could have been realized. After blood and milk were developed as sources of food, the animals became more valuable alive, so that meat consumption probably declined after domestication. The same is true of wool, which in the wild animals is obscured by the hairy outer coat, demanding to be made finer before one can obtain fleece suitable for textile use.

Zeuner (1963) considered confinement and breeding in captivity, with separation from the wild type, an important stage of domestication, since it allowed the emergence of a distinct domestic type. After this came the selective breeding for certain features, with occasional mating to wild forms. This was already practised in the Bronze Age since in the Middle East distinct breeds' can be recognized by 3000BC. This was followed by gradual intensification of the development of different breeds with desired economic characters and elimination of wild and primitive domestic types.

2.6 Effect of Domestication on Genetic and Physiological Mechanisms

The mechanism of evolution that led to the development of, for example, wild sheep were mutations and chromosomal rearrangement, selection, migration, geographical isolation and fixation of diversity by inbreeding and random genetic drift in small population (Dobzhansky, 1951). Domestication increased the speed or the size of the effects of many factors (e.g. inbreeding, outbreeding, genetic drift) and added to the forces changing gene frequencies (Lush, 1945).

The change to domesticated conditions did not create new inheritance, but the environmental changes following domestication allowed many genetic differences to show themselves more clearly than before and thus to be more readily and accurately selected. Examples that can be mentioned include growth rate, fattening ability and milk yield in which good feeding increases differences. The change permitted a greater range of variation to survive through reduced natural selection (Lush, 1945).

2.7 Classification of Sheep Breeds

2.7.1 Definition of Breed

The concept of breed was first used in animal breeding in the sixteenth century, but its meaning has undergone many changes with time and is still different in the minds of different people. Among others, the following definitions have been used.

1. a breed is a population or a group of populations which can be distinguished from other populations of the same species on the basis of different allele frequencies, chromosome changes or genetically determined phenotypic characteristics (Ryder, 1984).
2. Turton (1974) used two alternative definitions:

a. a homogenous sub specific group of domestic livestock with definable and identifiable external characteristics that enable it to be separated by visual appraisal from other similarly defined groups within the same species; and

b. a homogenous group for which geographical separation from phenotypically similar groups has led to general acceptance of its separate identity.

3. Carter and Cox (1982) defined a breed as a subgroup of a species possessing certain recognizable characteristics and maintained as a closed breeding population, historically in a single geographical area, after which it is frequently named.

The Food and Agriculture Organization of the United Nations (FAO) has adopted Turton's definition in its programme on animal genetic resources, especially in developing countries, which do not have breeding organisations (Loftus and Scherf, 1993). It has also realized that breeds have been developed according to geographical and cultural differences and to meet human food and agricultural requirements. It considers that breed is more a cultural than a strict term. Much of the development of animal breeds took place in Britain in the eighteenth and nineteenth centuries.

Ryder (1964) considered that breed differences have resulted partly from man's attempts to select, transport and crossbreed for obtaining desired animals and partly from natural process of selection and genetic drift. New breeds originated as strains of existing breeds. A too heavy emphasis on breed purity is not well-founded, since

today's crossbreds often can become pure breeds of tomorrow. The term has obviously received too much attention in animal breeding where the concept of breed purity was central for a couple of centuries from the middle of the eighteenth century and was largely based on morphological traits that are now known to be determined by only a few of the 100,000 genes. With the development of animal genetics and breeding, the term has lost some of its value. For the purposes of conservation and use of breeds, it would be important to measure their mutual relationships.

2.7.2 Classification and Characterization of West African Sheep

Hair sheep of West Africa have been classified into two types: a larger longer legged, loop eared type found in the drier Sahelian zone and a smaller horizontal eared type found in the humid forest and savannah zone (Bradford and Fitzhugh, 1983). Epstein (1971) referred to the large long-legged type as the savannah and the smaller type as the West African Dwarf (WAD). These names are confusing on two accounts; the larger long-legged sheep are primarily associated with the Sahelian zone and the smaller types are not true dwarfs (Bradford and Fitzhugh, 1983). ILCA (1979) divided wooled thin tailed sheep in West Africa into two types: Sahel and forest or savannah. These types of designation referred to the ecozones in which each type is commonly found. Sahel and savannah and forest are usually used names but forest and savannah types are usually discussed together.

1. Larger longer legged, loop eared type found in the drier Sahelian zone, also called Sahel, are taller and heavier than the savannah types, generally associated with nomadic or transhumant production system. Their long legs facilitate travel over long distance and browsing on trees and shrubs (Bradford and Fitzhugh, 1983). They are generally taller and heavier than the forest types perhaps because of less stress from climate and disease. Also, sheep raised at high altitude tend to be larger than those raised at lower latitude (ILCA, 1979). Wattles, also called throat tags, lappets, toggles, tassels or appendices colli, are common among Sahel sheep, but less so among the forest savannah types. They are generally white or white and brown. Bradford and Fitzhugh (1983) observed that Sahel type rams are maneless and horned; some ewes are

horned with long thin tails that often extend below the hocks. The long spiral shape horn is common among the Sahel types. Wither height of matured female is over 60cm (ILCA, 1979). Ears are long and pendulous in Sahel types. Mane and throat ruff are absent in Sahel type but present in forest and savannah type.

2. Also called the forest/ savannah type, the WAD are more compact than the Sahel type. Epstein (1971) called this type dwarf on the basis of its small matured height which is typically less than 60cm at withers. However, Bradford and Fitzhugh (1983) noted that although they were very small they were not achondroplastic. They are commonly white with black spotting although there are some solid black and white sheep. Red, tan, brown (solid or spotted) and black belly pattern are found in the Senegal-Nigeria region. Bradford and Fitzhugh (1983) observed that the forest type rams were horned but some polled rams were also seen. They observed no horned ewes. The horn shape was a single curve or crescent. Typically, forest savannah rams have mane and throat ruff of long coarse hair. Tail rarely extends below the hock. Weight of matured females is 20-30kg in forest type, height at withers is 40-55cm. Forest and savannah types are trypanotolerant but Sahel types are not (ILCA, 1979).

Mason (1996) classified Uda, Yankassa and Balami as Sahel types and West African Dwarf as forest or savannah types.

2.7.3 Nigerian Sheep Breeds: Description and Distribution

Sheep are raised all over Nigeria, with a broad distinction between their rifeness and importance in the north, and the more dispersed populations of the humid zone. Sheep and goats are seen as having secondary importance in relation to crops. There are generally considered to be four breeds or races of sheep native to Nigeria: the Balami, Uda, Yankassa and West African Dwarf (WAD) (Adu and Ngere, 1979).

2.7.4 Classification of Nigerian Sheep

The different breeds of sheep found in Nigeria are of the hairy type and are broadly grouped into large, long legged types found in the northern part of the country and the dwarf type of the hot humid coastal areas (Adu and Ngere, 1979).The sheep and goat found in the south are small bodied and trypanotolerant while in the north, sheep and

goats are large bodied and particularly in the Sahel savannah, the desert adaptation of long limbs and tail and long pendulous ears are to be seen (Hall, 1991).

2.7.4.1 Balami

The Balami is the largest bodied native sheep in Nigeria and it is named after the tribe – Bornu that owns it in large numbers. As a pastoral animal, it is confined to the semi-arid north, but it is favoured as a stall-fed breed by Muslims throughout the Nigerian Middle Belt. It is white and hairy with pendulous ears and a long thin tail; rams have a throat ruff and are horned but ewes are normally polled. Another feature that makes the Balami distinctly recognisable is its Roman nose, a large bulbous nose that distinguishes it from the Yankassa.

The Balami grows fast and attains a weaning weight of 18kg in 12 weeks. The yearling weights are 35-40kg for ewes and 45-60kg for rams (Adu and Ngere, 1979; Oni, 2002). Birth weight of 3.5kg for singles and 3.1kg for twins have been reported for the breed at Katsina, Nigeria. The Balami has a good potential as a meat producer and its ability to survive under arid conditions is a characteristic that can be exploited in the utilization of non-arable land.

Plate 1: Balami Ram

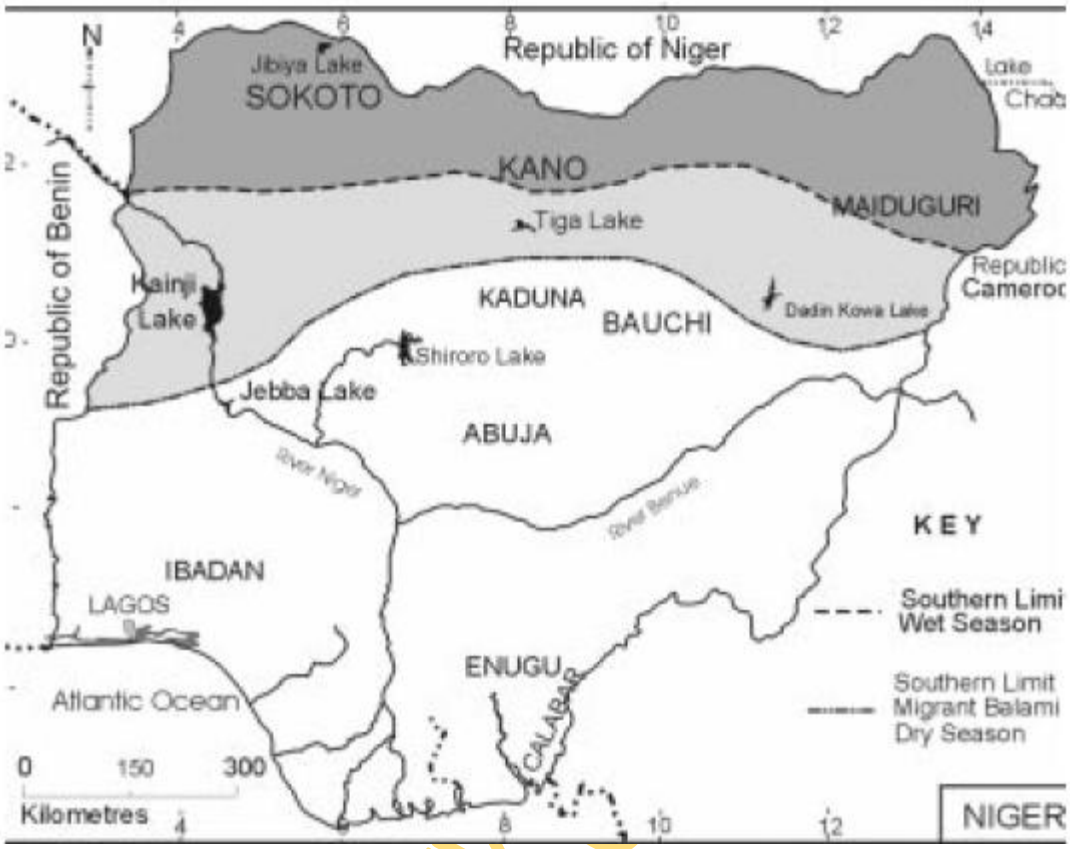

Figure 1: Approximate Range of Distribution of Balami Sheep in Nigeria

Source: Roger (1999)

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2.7.4.2 Uda

The Uda is slightly smaller-bodied than the Balami, although their size ranges overlap. It is easily recognised by a distinctive coat colour pattern; entirely brown or black forequarters and white behind. The Bali-Bali variety is all white; the coat is short and coarse; the head is long and heavy with flat forehead and a slightly convex profile; horns present in males but may be absent in females; male horns are spirally twisted, growing horizontally out from the head; female horns are short and fine; ears are pendulous and long; back line is long and dipped (Wilson, 1991).Tails are long and thin. The ram carry horns which become, wide and spiral as they mature. Horns are usually absent in the ewes.

Mature live weights are 30 to 45kg in ewes and 30 to 60kg in rams. Uda sheep give their name to a Fulani clan, the Uda'en, who herd large flocks of this breed between Niger and the northern reaches of the Nigerian Middle Belt. The breed is more predominantly found in the north western part of the country. It is also found in southern Niger, central Chad to western Sudan and Cameroun called Udah, Peul, Balibali, Bororo (western Sudan) of Fellata (Salako and Ngere, 2002). Haumesser and Gerbaldi (1980) studied traditionally-managed Uda flocks in Niger Republic; Wilson and Durkin (1983a, b) and Wilson and Light (1986) report on related sheep production systems in central Mali.

The Uda is adapted to extensive grazing and survives best under hot and dry environment (Oni, 2002) and suffers from poor survival outside this ecologic zone. It is renowned for its trekking abilities (Adu and Ngere, 1979). Wattles are present occasionally in both sexes. In general, the Uda sheep inhabit the semi-arid mono-modal rainfall lowlands and adjoining arid areas of Southern Niger, Northern Nigeria, Central Chad, Western Sudan and parts of Cameroon. The production systems are agro-pastoral and pastoral transhumance. The Ara Ara variety inhabits the semi-arid areas of South-Central and Central Niger under pastoral and agro-pastoral management (Wilson, 1991).

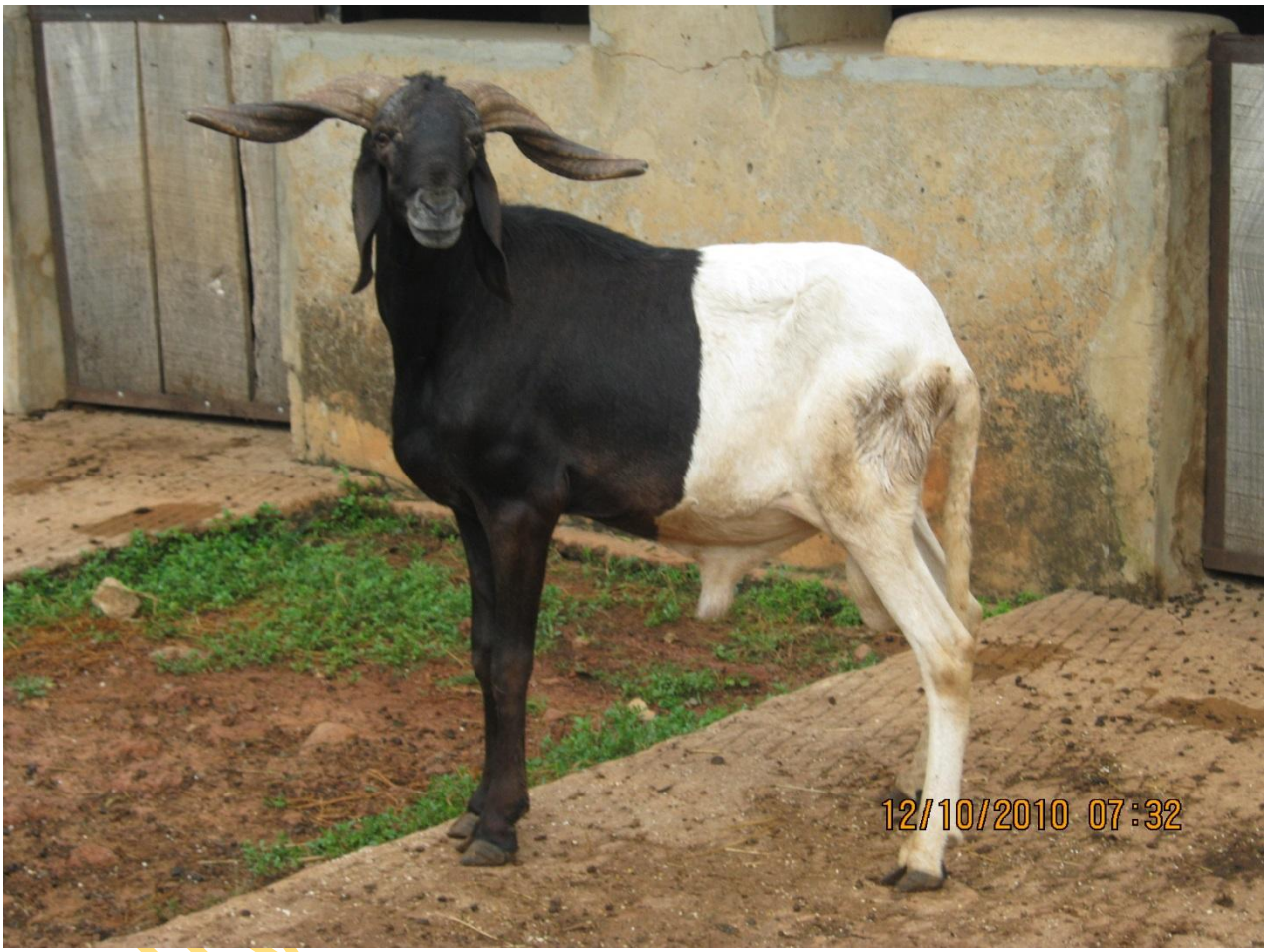


Plate 2: Uda Ram

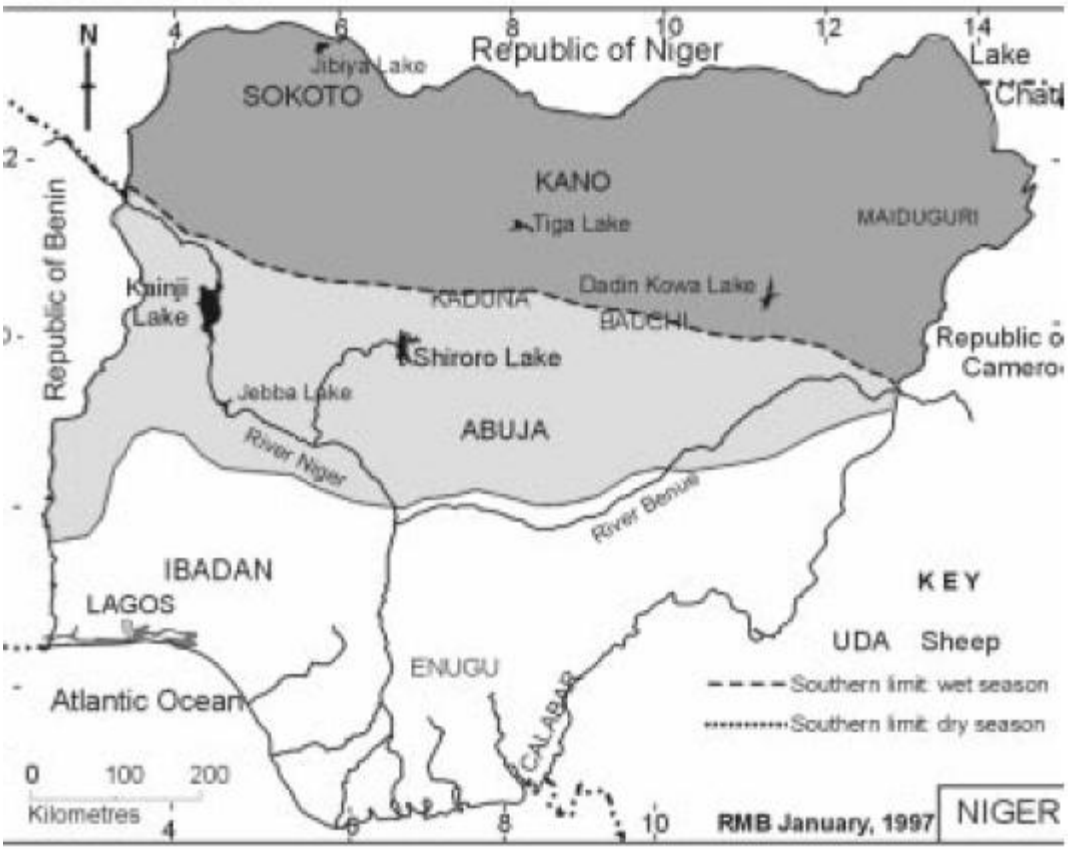


Figure 2: Approximate Range of Distribution of Uda Sheep in Nigeria

Source: Roger (1999)

2.7.4.3 Yankassa

The Yankassa breed has been the most extensively studied in Nigeria. It is the most widely distributed and most numerous sheep breed. It is found throughout the Sahel, Sudan and Guinea savannah zones. Yankassa inhabits semi-arid and sub humid areas of Northern and North-Central Nigeria; Yankassa is the Hausa word for local (Wilson, 1991).

The Yankassa is intermediate in size between the West African Dwarf sheep and the long-legged Uda (Adu and Ngere, 1979). The coat colour is typically white with black patches around the eyes, ear, muzzle and sometimes feet. Such black spots are rarely found elsewhere on the body. Some of the ewes have wattles. The rams have curved horns and heavy hairy white mane and ewes are polled. Mature live weights are 30 to 45 kg in rams and 25 to 40kg in ewes. At 3months, males are 14.9kg and females 13.9kg (range12-29.81kg). Average daily gains are 148g and 116g for singles and twins, respectively, between 0-3 months of age, 99g and 82g for animals between 0-6months and at 12months, 80 and 72g, respectively (Ngere *et al.*, 1979; Kwatu *et al.*, 1983; Otchere *et al.*, 1987). A strain of Yankassa called Biu local occurs mainly in Borno state. They are becoming semi nomadic to completely sedentary as grazing land continues to shrink (Devendra and Mcleroy, 1982).

Yankassa sheep have been recorded in all parts of Nigeria, though the populations attenuate towards the northern border and the sea-coast. Some tentative studies have been made of its ecological adaptations. Yankassa sheep do not need daily watering in the wet season and watering once a day suffices in the dry season (Aganga *et al*., 1988).

Plate 3: Yankassa Ewes

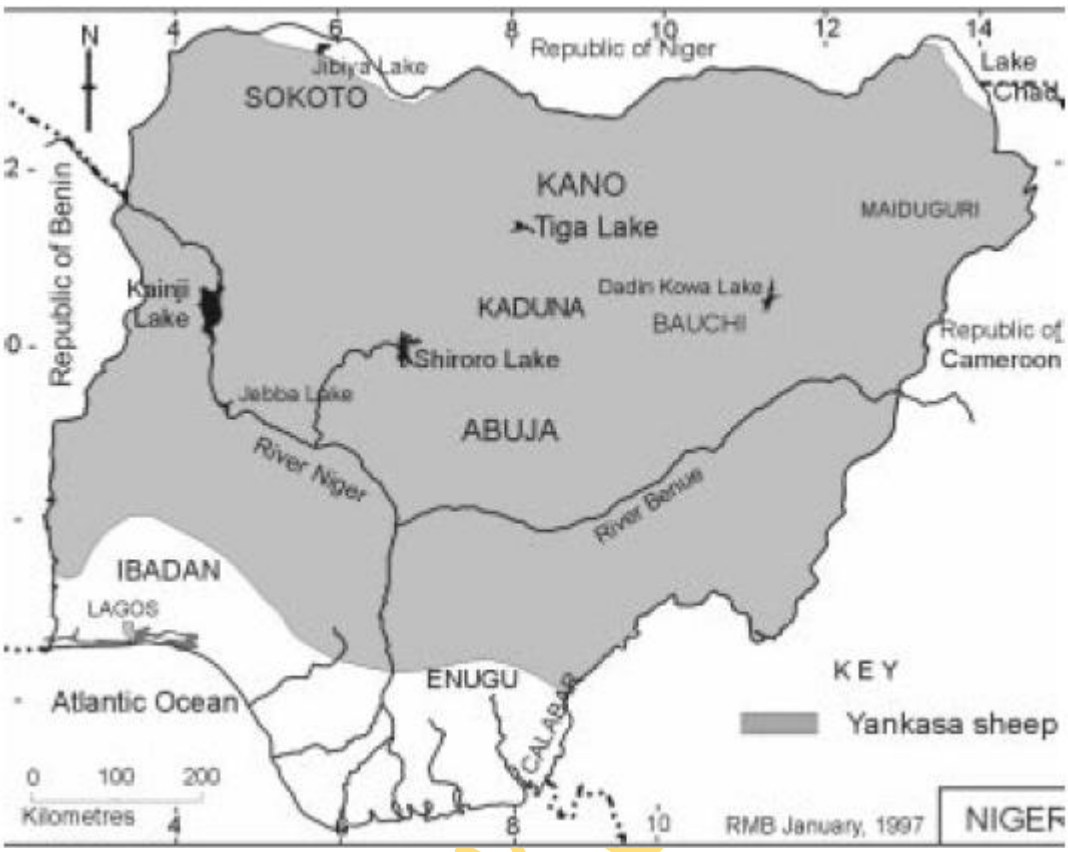


Figure 3: Approximate Distribution of Yankassa Sheep Breed in Nigeria

Source: Roger (1999)

2.7.4.4 West African Dwarf

The West African Dwarf is a small-bodied, compact breed which may be all white, black, brown, or spotted black or brown on a white coat. Its variation in colour and patchy distribution make it difficult to distinguish clearly from the Yankassa. The breed is widely distributed and thrives in the area of south of latitude 14^0N that is known to be heavily tsetse fly infested. This probably lends support to the conviction that the breed is trypanotolerant. Adu and Ngere (1979) say that different types exist, mentioning the 'Pagan' variety on the Jos Plateau, and the 'Umuahia' variety near the Confluence, but there are no other accounts of such varieties. Devendra and McLeroy (1982) argue that the WAD breed cannot be subcategorised on the basis of appearance, and no performance data is available. Mature rams have close spiral horns and a heavy mane of hair. Mature weights are 20-25 kg for ewes and 25-30 kg for rams.

The West African Dwarf sheep has not been kept on large scale except on some government farms: Agege, Onitsha, Umuahia, Fashola, upper Ogun and some teaching and research stations. FAO (1991) reported a modal flock size in South Eastern Nigeria where 28% of families own 11.4 sheep each. The WAD sheep is reported to have high reproductive performance even under village conditions.

Plate 4: West African Dwarf Lambs

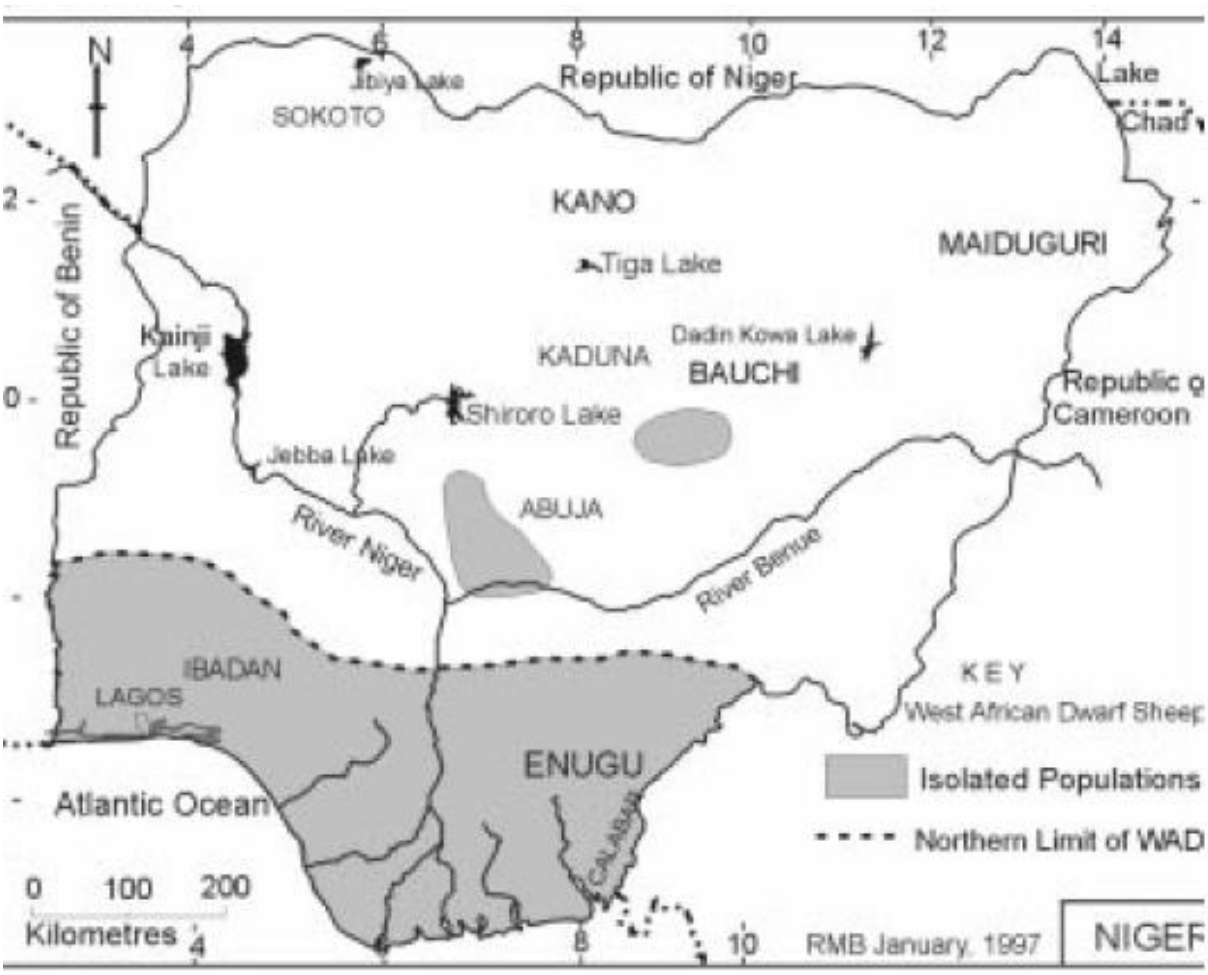


Figure 4: Approximate Range of Distribution of West African Dwarf Sheep in Nigeria

Source: Roger (1999)

2.8 Population Density

Africa is estimated to produce about 16.3% of the world sheep population. The centres of concentration of sheep in Africa are Ethiopia, South Africa, Somalia, Kenya and Sudan. Reynolds (1986) estimated that about 104 million sheep found in tropical Africa are kept permanently within small farming sector. It is estimated that 94% of the world's total live in the developing countries and there are approximately 0.5 goats and sheep per head of rural dwellers in the humid West Africa (RIM, 1992). Nigeria sheep population has been estimated to be about 22million (RIM, 1992) a third of which are found in the humid southern states (ILCA, 1979). Areas of highest sheep density are Benue, Plateau, and Kano, North Central, North Eastern and North Western states which fall within Sudan and Guinea savannah zones and are most favourable for livestock production (Adu and Ngere, 1979).

2.9 Production Systems in Nigeria

In Nigeria, livestock species are kept mainly under the traditional extensive system of rearing except in the case of pigs and poultry where commercial rearing in the intensive system has some significance. This traditional system is composed of the pastoral, village and urban smallholder farmers. Adu and Ngere (1979) reported that raising of sheep in Nigeria has traditionally been part of the farming system of the people. There are sheep rearing systems that closely relate to the farming and cultural life of the people. They are: the Fulani system, the compound system, government and research farms, and commercial production system.

2.9.1 Fulani System

The Fulani being semi nomads graze their sheep with their cattle together in herds throughout the year moving the herd from north to south of the country in response to rainfall pattern and availability of pasture at different times and seasons (Akinyemi, 2010).

2.9.2 Compound System

This is practiced by the Hausa, another major tribe in Nigeria. These people are more settled and therefore keep the animals in their compound during the wet season and feed them on freshly cut grass. During the dry season, animals are allowed to roam and fend for themselves. Some of the farmers feed their animals on crop residues such as groundnut haulms, husks, grain offal and cottonseed. In the south, sheep are left to roam, especially during the day, browsing roadside herbage and consuming kitchen wastes like yam and plantain peels. During the cropping season, the animals are tethered either on fallow fields or in the compound to prevent damage to crops by the animals. In the evenings or early morning, they may be given supplementary feeds in the form of chopped cassava roots, cassava peels, cassava leaves, plantain leaves, grains and some other forms of browse herbage.

In all the local systems, specific housing is not provided for these animals which spend the night either in porches of houses, in streets or on motor roads where they are exposed to night colds and accidents. Health care is poor and records are virtually non-existent. Breeding is not controlled, the male run freely with the females except in few cases in the compound system where the best ram is only allowed to go with the females. The criteria for the selection of such rams are often based on fanciful colours, horns and on vigour and size.

2.9.3 Government and Research Farms

Under this system sheep are better managed and provided with better housing, feeding and health care. The system is mostly semi extensive where the animals are grazed on improved pastures or herded on semi-improved shrub savannah and given conserved pasture in the form of hay or silage especially during the dry season. Supplements are fed in the form of compounded concentrate mixture. There are routine programmes for deworming, spraying and/or dipping. Adequate records are kept and breeding is controlled. Sheep in government and research farms are under improved environment and therefore perform better than animals under local systems (Akinyemi, 2010).

2.9.4 Commercial Production System

On government farms, research farms and commercial ranches, sheep are managed and provided with better housing, feeding and health care. The system is mostly semi-intensive where animals are grazed on improved pastures or herded on semi-improved shrubs, savannah and given conserved pasture in the form of hay or silage especially during the dry season when supplements are fed in the form of concentrate mixtures. There are definite programmes for deworming, spraying, dipping etc. Adequate records are kept and breeding is controlled (Adu and Ngere, 1979).

These sheep perform better than the ones under the local system (Hall, 1991; Adu and Ngere, 1979). Available information on the production characteristics of the indigenous sheep are those from government and research farms (Adu and Ngere, 1979).

2.10 Breed Characterization

Characterization of Animal Genetic Resource (AnGR) encompasses all activities associated with the identification, quantitative and qualitative description, and documentation of breed populations and the natural habitats and production systems to which they are or are not adapted. The aim is to obtain better knowledge of AnGR, of their present and potential future uses for food and agriculture in a defined environment, and their current state as distinct breed populations (FAO, 1984; Rege, 1992).

National-level characterization comprises the identification of the country's AnGR and the surveying of these resources. The process also includes the systematic documentation of the information gathered so as to allow easy access. Characterization activities contributes to objective and reliable prediction of animal performance in defined environments, so as to allow a comparison of potential performance within the various major production systems found in a country or region. It is, therefore, more than the mere accumulation of existing reports (FAO, 2007).

The information provided through the characterization process enables a range of interest groups, including farmers, national governments and regional as well as global bodies to make informed decisions on priorities for the management of AnGR (FAO,

1992; FAO/UNEP, 1998). Such policy decisions aim to promote further development of AnGR while ensuring that these resources are conserved for the needs of present and future generations.

2.11 Characterization – as the Basis for Decision-Making

According to FAO (2007), a key consideration for the management of AnGR at the national level is whether, at a given point in time, a particular breed population is self-sustainable or whether it is at risk. This primary assessment (baseline survey) of breed/population status is based on information on:

1. population size and structure;
2. geographical distribution;
3. within-breed genetic diversity; and
4. the genetic connectedness of breeds when populations are found in more than one country (e.g. the Djallonke sheep of West Africa).

If a breed/population is not at risk, no immediate steps to implement conservation measures are necessary. Nevertheless, as part of national livestock development plans, decisions have to be taken as to whether a genetic improvement programme is needed – in response, for example, to changing market conditions. Decisions regarding such improvement programmes are mainly guided by information on long-term benefits to livestock keepers and society (FAO, 2007).When a breed/population is found to be at risk, active conservation strategies have to be implemented or the potential loss of the breed must be accepted.

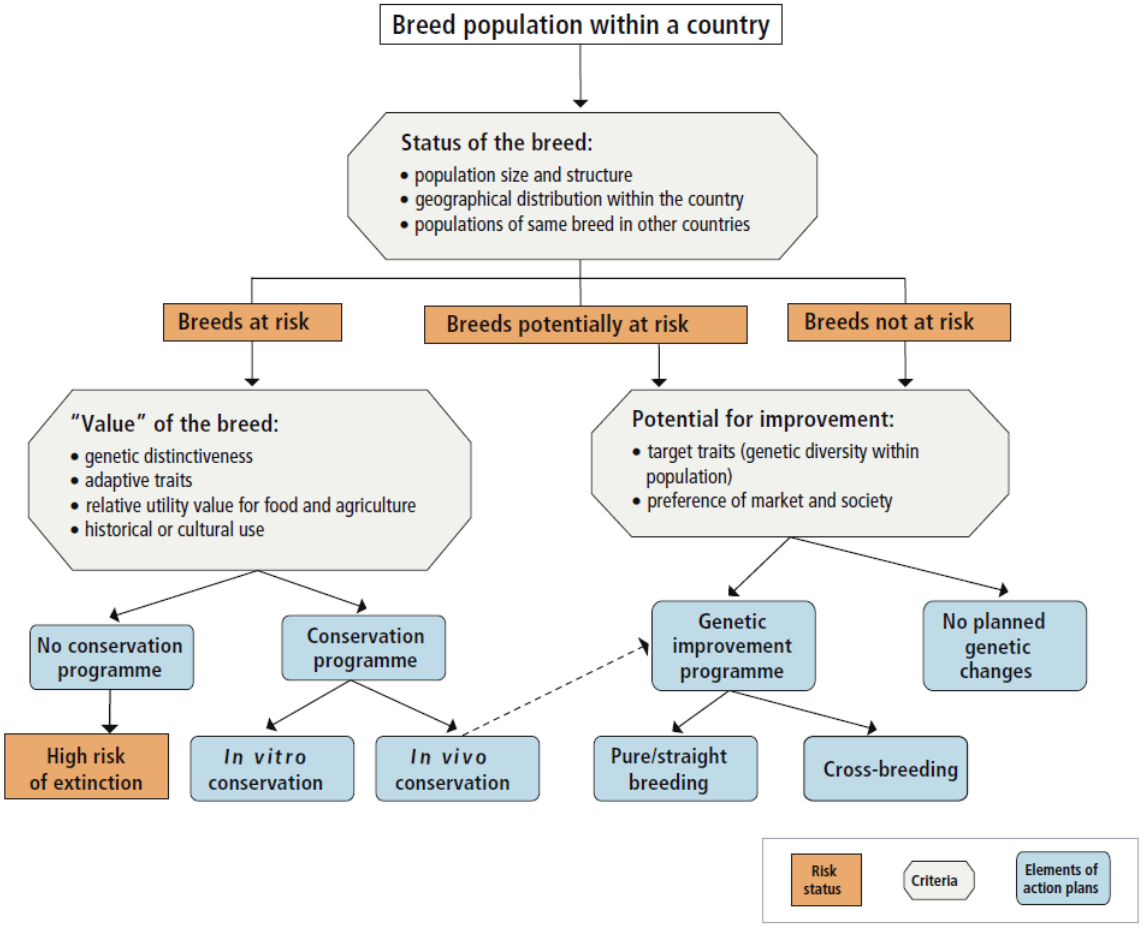


Figure 5: Information Required for Designing Management Strategies

Source: FAO, 2007

FAO, (2007) opined that decisions on conservation strategies and on development programmes for self-sustainable breeds, comprehensive information is needed and should include:

1. description of the typical phenotypic characteristics of the breed population, including physical features and appearance, economic traits (e.g. growth, reproduction and product yield/quality) and some measures (e.g. range) of variation in these traits – the focus is generally on the productive and adaptive attributes of the breed;
2. description of the production environments, both the original habitat and the current production system in which the population is kept – some breeds are kept in more than one production environment, in a number of countries, and sometimes outside their original geographical area;
3. documentation of any special characteristics (unique features) of the population in terms of adaptation and production – including responses to environmental stressors (disease and parasite challenge, extremes of climate, poor feed quality, etc.);
4. images of typical adult males and females in their typical production environment;
5. relevant indigenous knowledge (including but not limited to gender-specific knowledge) of traditional management strategies used by communities to utilize the genetic diversity of their livestock;
6. description of on-going management (utilization and conservation) actions and the stakeholders involved; and
7. description of any known genetic relationships between breeds within or outside the country.

In addition to the information listed for both pathways (conservation and development), the following supplementary information is useful to guide the choice of priority breeds and geographic areas for conservation programmes:

1. genetic distinctiveness of the breeds and their significance with respect to the total genetic diversity among the breeds under consideration (in order to maximize the diversity conserved for the benefit of future human generations);
2. origin and development of the breeds; and
3. unique genetic (or phenotypic if genetic attributes are not known) characteristics and their significance in current or anticipated production settings.

National decision-makers need to identify the breeds in which genetic improvement programmes would be most beneficial. Such programmes could include breeds classified as at risk, and form part of a conservation programme (FAO, 2007).

The set of information needed for the development of appropriate breeding programmes also allows the choice of breed to be reconsidered as the production environment evolves, whether through changes to husbandry practices, market conditions, cultural preferences, or biophysical (e.g. climatic stress or disease challenge) factors. Similarly, this information is needed in the design of AnGR restocking schemes undertaken following natural disasters (drought, floods, etc.), disease outbreaks or civil unrest (FAO, 2007).

Management decisions may differ in type and scope at sub-national, national, regional and international levels. It is, therefore, important that relevant information on breed characteristics is made accessible to decision-makers at all levels. For example, it may happen that a country decides not to invest in the conservation of a specific local breed, but a regional or international organization decides that the breed is a unique genetic resource, and that it is in the global interest to conserve it (FAO, 2007).

2.12 Tools for Characterization

2.12.1 Survey

Surveys are undertaken to systematically collect data needed to identify breed populations and describe their observable characteristics, geographical distribution, uses and general husbandry, as well as their production environments. Full baseline surveys need to be undertaken once; some elements of the survey may be repeated when significant changes are observed in the livestock sector.

As part of the effort to develop global databanks for the management of AnGR, FAO developed a comprehensive list of animal and environment descriptors to serve as a guide for standardized characterization activities at various levels (FAO, 1986a, b, c). However, these descriptors were far too complex for universal application. In recognition of this fact, FAO developed simplified formats for data collection for mammalian and avian species. This was based on the experience of the European Association for Animal Produuction (EAAP), which started collecting data in the 1980s and later built the first computer-based information system (Animal Genetic Data Bank) known as EAAP–AGDB. International Livestock Research Institute (ILRI), in collaboration with FAO (Rowlands *et al.*, 2003) has developed and tested an approach for collecting and analysing on-farm breed-level information in Zimbabwe. The same approach has been applied in Ethiopia. A key lesson from this work is that logistic and time requirements for extensive livestock surveys, data management and analysis, can be grossly underestimated. It was also found that the outcomes of multivariate survey techniques need to be verified by complementary molecular genetic studies (Ayalew *et al.*, 2004).

Based on the Global Strategy for the management of AnGR, ten categories of variables are covered in AnGR surveys, including basic and advanced breed population information, main uses of the breed, origin and development/evolution of the breed, typical morphological features, average performance levels, special characteristics, and on-going conservation activities.

2.12.2 Monitoring

Changes in population size and structure need to be documented regularly for all breeds. This should be carried out on a yearly or biennial basis, as the application of modern reproductive technologies, global trade, market demands, and policies favouring particular breeds, can lead to rapid changes in the size and structure of breed populations (FAO, 2007). Monitoring should be conducted at least once per generation of the species, particularly for breeds classified as at risk or potentially at risk. This requires surveys at intervals of about eight years for horses and donkeys, five years for

cattle, buffalo, sheep and goats, three years for pigs and two years for poultry species. At present, most national livestock censuses do not contain breed-level data, and so regular reporting of breed population numbers does not take place. Species and breeds that have been classified as at risk should be monitored on a regular basis. This monitoring should serve as the basis for national early warning (FAO, 2007).

Information collected during monitoring activities enables adjustments to be made to management plans for AnGR. Monitoring programmes need to be carefully designed so that they provide feedback to farmers, managers and other stakeholders. Monitoring approaches need to be flexible, and activities by different players need to be well coordinated, as different groups will monitor different parameters. For example, farmers may wish to monitor production parameters; resource managers may wish to monitor completion of breed inventories; and administrators may wish to monitor the cost effectiveness of various programmes. Monitoring is also necessary to evaluate progress in the implementation of action plans, and to identify new priorities, issues and opportunities (FAO, 2007).

Monitoring can be an extremely expensive aspect of AnGR management. However, if countries are strategic in their approaches to monitoring, and take advantage of existing resources, it can be cost effective. For managing genetic resources at high risk, data on current population size and geographic location are required. For such populations, regular and simple quantification and reporting of actual population sizes by those directly involved may be adequate and achievable. Large and widely dispersed populations may require the establishment of stratified samples, where a portion of the population in each major geographical region of the country is monitored. Lack of easy-to-apply tools for collecting such data, general lack of trained persons to undertake assessments, and lack of awareness on the part of policy-makers and implementers regarding the importance of such information, represents important challenges (FAO, 2007).

In every country there may be opportunities to monitor AnGR by taking advantage of existing activities, and thereby avoiding significant additional costs. National livestock

censuses offer good opportunities. It may also be possible to set up effective monitoring stations in locations where livestock are sold or traded, such as auctions and local markets. This approach can greatly reduce costs by bringing the livestock to the monitors. However, a focus on traded animals may not accurately reflect the structure of the target populations on the farms. In countries where farmer groups, breed societies, or herd or stud books exist, tracking registrations can be a very effective means to monitor particular breeds. There may also be opportunities to combine monitoring activities with the tasks of existing government offices. For example, wildlife biologists could assist in monitoring livestock populations as part of wildlife surveys (FAO, 2007).

Health officials could record livestock population numbers by breed when conducting food processing inspections or delivering veterinary services. All these options, however, have to be treated with caution and potential biases need to be considered. The value of the information obtainable on the basis of existing activities has to be weighed against the additional information, but also greater costs, associated with surveys specifically designed and conducted to monitor AnGR (FAO, 2007).

2.12.3 Information Systems

Information systems or databases can serve a variety of different purposes, but collectively they contain important information for decision-making, research, training, planning and evaluation of programmes, progress reporting and public awareness. An information system normally includes hardware, software (applications), organized data (information) and facilities for communication. It can be operated either manually, electronically using computers, or through a combination of both. The information may be on a single desktop machine, or a network of computers. Alternatively, it may be on the Internet, allowing external access to view or, in case of interactive dynamic systems, update the information (FAO, 2007).

The overall purpose of information systems is to enable and support decision-making regarding the present value and potential future uses of AnGR, by a range of stakeholders, including policy-makers, development practitioners, farmers and

researchers. Thus, they need to incorporate essential decision-support tools to meet the needs of stakeholders at sub-national, national, sub-regional, regional and global levels. However, users operating at these different hierarchies or levels will each have different objectives, and be interested in different aspects of the data contained within the information system. For instance, users operating at regional or global levels will be more interested in the cross-border distribution of breeds, cross-border livestock markets, trans-boundary disease risks, and germplasm exchange across borders. Conversely, more relevant issues for users at national and sub-national (local) levels are breed population size, herd/flock structures, production levels, and stressors associated with local environments (FAO, 2007).

Linkages and information exchange between the hierarchies, as well as with external information sources can add value to information systems. Complementary databases may exchange information through a system of data transfer, or can serve as "gateways" to each other through electronic links via the Internet. For instance, national and sub-national AnGR databases could be linked to geophysical databases (climate, soils, water or landscape). Functional linkages between these sets of data could lead to the generation of animal disease risk maps, and information on specific adaptations of particular breeds to stressful environments. National databases of domestic animal diversity are essential planning tools. They present the current state of knowledge on the size, distribution, status, and utility value of AnGR. They allow access to information on planned and on-going management activities. Moreover, they facilitate the identification of gaps in existing information (FAO, 2007).

At present, a number of public-domain electronic information systems for animal genetic diversity are globally accessible and contain data from more than one country. Two of these – the Domestic Animal Diversity Information System (DAD-IS) and the European Farm Animal Biodiversity Information System (EFABIS) (previously EAAP–AGDB) – are related to the FAO global information system for AnGR. The Domestic Animal Genetic Resources Information System (DAGRIS, 2004), managed by ILRI is a database of synthesized research information from published and grey literature.

Oklahoma State University's (2005) Breeds of Livestock information system provides brief summaries of breed origins, characteristics and uses.

Currently, the information resources have facilities for simple searches by country or breed only. Ideally, they should have as much research information as is available, and enable users to make informed judgments about the value of each item of information. If researchers and decision-makers are to have the information they require, the functionality of the existing information systems will need to be greatly increased, to allow extraction and customized analysis of various categories of information within and between data sources. The scope of data acquisition also needs to be expanded so that breed information can be linked to geographical information system (GIS)-based environment and production system mapping. This will allow poorly documented adaptation traits such as disease resistance to be predicted from past and current breed distribution and use (Gibson *et al.*, 2007).

Information systems for AnGR have been developed and administered as global public goods, and have limited ability to attract investment from the private sector or major funding agencies. This explains the very limited information that the systems contain compared to that which is potentially possible and which would be necessary for them to effectively achieve their stated purposes. One possibility to circumvent such limitations is to establish functionalities for interconnectivity and interoperability between information systems. This has been achieved with FABIS net (a distributed information system for AnGR) which enables countries to set up national Web-based information systems that can exchange core data with the higher levels of the network – regional systems (such as EFABIS) and the global system (DAD-IS).

2.13 Molecular Genetic Characterization

Molecular genetic characterization explores polymorphism in selected protein molecules and DNA markers in order to measure genetic variation at the population level. Because of the low level of polymorphism observed in proteins, and hence limited applicability in diversity studies, DNA-level polymorphisms are the markers of choice for molecular genetic characterization (FAO, 2007).

The process of molecular genetic characterization comprises field sampling of biological material (often blood or hair root samples), laboratory extraction of DNA from the samples, DNA storage, laboratory assaying (e.g. genotyping or sequencing), data analysis, report writing, and maintenance of a molecular genetic information database. Sampling for molecular analysis may be combined with surveying and/or monitoring, as molecular information on its own cannot be used for utilization and conservation decisions.

In the document on the state of the art in the management of animal genetic resources (FAO, 2007), characterization at the molecular genetic level is said to be undertaken mainly to explore genetic diversity within and between animal populations, and to determine genetic relationships among such populations. More specifically, the results from the laboratory work are used to:

1. determine within and between-breed diversity parameters;
2. identify the geographical locations of particular populations, and/or of admixture among populations of different genetic origins;
3. provide information on evolutionary relationships (Phylogenetic trees) and clarify centres of origin and migration routes;
4. implement gene mapping activities, including identification of carriers of known genes;
5. identify parentage and genetic relationships (e.g. DNA fingerprinting) within populations;
6. support marker assisted genetic improvement of animal populations; and
7. develop DNA repositories for research and development (FAO, 2005).

In populations with limited or no information on pedigrees and population structure, molecular markers can also be used to estimate the effective population size.

In the absence of comprehensive breed characterization data and documentation of the origin of breeding populations, molecular marker information may provide the most easily obtainable estimates of genetic diversity within and between a given set of populations.

The Food and Agricultural Organization (FAO) of the United Nations has proposed a global programme for the management of genetic resources using molecular methodology for breed characterization (Bjornstad and Roed, 2001). This strategy places a strong emphasis on the use of molecular markers to assist in the conservation and assessment of breeds and to determine the genetic status of these breeds**.**

The study of the structure and function of genes at the molecular level in a breeding population can help determine the similarity of the genetic material carried by populations and the genetic variation they possess. Several techniques have been developed to estimate the genetic variation or polymorphisms in populations and hence the genetic relationship among populations. These methods include biochemical polymorphisms, immunological methods and molecular methods (DNA hybridization, RFLP, RAPDS, mtDNA, microsatellites and SNP).

2.13.1 Blood Typing and Protein (Biochemical) Polymorphisms

Blood and protein polymorphisms were used during the 1960's but revealed a limited number of loci and alleles at a locus (Nei, 1987; Tanabe *et al*., 1991). This method is rapid, affordable and reliable, but requires fresh blood samples. Detection of protein polymorphisms involves the electrophoretic separation of proteins based on the differences of their molecular weight followed by histochemical recognition of differences in banding patterns for particular proteins between individuals (Baker *et al*., 1966).

In studies of the haemoglobin locus, Boujenane *et al.* (2008) reported three alleles: A, B and H in Moroccan local sheep. However, the presence of A, B and H alleles at the haemoglobin beta locus in Moroccan local breeds is not in agreement with reports of fixation of the B allele in Yankasa, Uda, Mbororo and West African Dwarf breeds (Ibeagha-Awemu and Erhardt, 2004), and Kwale, Makueni and Siaya fat-tailed breeds of Kenya (Mwacharo *et al*., 2002). Nevertheless, the H allele was also found in the Welsh Mountain breed (Kilgour *et al*., 1990).

Missohou (1999) reported that in the Senegalese Djallonke sheep breeds the haemoglobin locus is monomorphic B. This result differed from those reported in the

same breed in Nigeria by Olusanya (1975) but is in agreement with those reported by Ndamukong (1995) in the dwarf sheep of Cameroon. For them HBB frequency ranged from 0.66 to 0.99. It is well known that the control of anaemia induced by trypanosome infection is a key trait of trypanotolerance. The fact that only the B allele is present in this trypanotolerant breed confirmed the low blood viscosity and better tolerance to anaemia associated with this allele.

After electrophoresis on cellulose acetate, Morhi *et al.* (2005) observed two haemoglobin phenotypes in Baloochi and Kordi breeds: AA and AB phenotypes. AA was commonest in the two breeds. The incidence of type AB haemoglobin in Baloochi and Kordi breeds was 26.5% and 9.5%, respectively. BB phenotype was not seen in Baloochi or Kordi breeds. In sheep with AB phenotype, haemoglobin B was dominant.

Mwacharo *et al.* (2002) reported a fixation of HbB in five sheep populations in Kenya. This agrees with results obtained on the fat-tailed Namaqua sheep (Clarke *et al.*, 1989) and in other breeds of sheep (Zanotti *et al*., 1990).

Imumorin *et al.* (1999) reported the distribution of Hb genotypes AA, AB and BB were 35.5%,22.4% and 42.1% in Red Sokoto goats while the corresponding values in West African Dwarf goat were 30%,46% and 12%. The frequency of HbA was 0.467 in Red Sokoto which was slightly lower than 0.530 in West African Dwarf goats.

Salako *et al.* (2007) in a preliminary study of Haemoglobin polymorphism in Nigerian indigenous small ruminant populations reported two codominant alleles (A and B) with allele frequencies 0.48 and 0.52, respectively, that gave rise to three phenotypes (AA, AB and BB) with genotype frequencies of 0.4, 0.17 and 0.4, respectively, in the West African Dwarf sheep population.

In a study on the genetic variability in nineteen South African sheep breeds, Sargeant *et al.* (1999) reported that six of the breeds were polymorphic at the carbonic anhydrase locus, C allele was found in Russian Red Wooled Persian and Border Leicester breeds at low frequencies, A allele was found in the remaining four i.e. Landrace, Afrino, Namaqua and Vendor also at low frequency. In a study by Ordas and Primitivo (1983) on Churra sheep breeds, a new allele M was found at the carbonic anhydrase locus, Ca^{S}

was also found in all the breeds studied (Churra, Lacha and Machenga), the allele was reported fixed in most of the breeds.

Ca^{S} was observed to be fixed in Yankassa, Uda, Mbororo and West African dwarf sheep breeds and in Merino, East Friesian Milk and German Grey Heath sheep breeds (Ibeagha-Awemu and Erhadt, 2004).

Ibeagha-Awemu and Erhardt (2004) reported six alleles at transferrin ($Tf^{A, B, C, D, M}$ and ^{E}) locus in Yankassa, Uda, Mbororo and West African dwarf sheep. In Yankassa, Uda, Mbororo and West African Dwarf breeds Tf ^{A} was the most frequent allele (0.250, 0.3, 0.491 and 0.643, respectively), while Tf ^{E} was the least in Yankassa, Mbororo and West African Dwarf (0.013, 0.009 and 0.012, respectively). Tf^{E} was however absent in Uda. In the same study, eight alleles were observed in five German sheep breeds, namely: Merino land, Black Faced Mutton, East Friesian Milk, Rhon sheep and German Grey Heath. Tf^{A} (0.278), Tf^{C} (0.293), Tf^{D} (0.679), Tf^{E} (0.292) and Tf^{E} (0.312) were the most common alleles in Merino land, Black Faced Mutton, East Friesian Milk, Rhon sheep and German Grey Heath, respectively. Tf P was observed in Black Faced Mutton and East Friesian Milk Sheep but was absent in Merino land, Rhon sheep and German Grey Heath. This rare allele is also widely distributed in some European sheep breeds (Buis and Tucker, 1983; Zanotti *et al.*, 1988, 1990).

Mwacharo *et al.* (2002) in a study on Kenyan sheep commented that of all the five proteins typed transferrin was the most polymorphic locus, exhibiting five alleles in the Merino (Tf ^{A,,B,C,D} and ^{E}) and four in the fat-tailed sheep. Tf^{D} was the most common in all the breeds: Kwale (0.443), Makueni (0.5), Siaya (0.6), Kakamega (0.506), Kajiado (0.432) and Merino breeds (0.475) while Tf ^{B} was lowest in Kwale (0.033), Makueni (0.031) and Kajiado (0.097). They further reported that the occurrence of Tf^{C} in Kwale than Tf^{D} can be due to genetic drift and/or the small sample size used. The observation of Tf^{E} in the Merino has also been observed in other flocks of Merino and breeds of sheep in Europe (Ordas and Primitivo, 1986; Nguyen *et al.*, 1992). The fact that all alleles observed in the fat tailed sheep were also found in the Merino, whereas those which were absent in the former occurred in the latter indicates that the indigenous

breeds are markedly divergent from the Merino. The fixation of certain alleles and the loss of others within the fat tailed sheep are indicative of evolutionary change either as a result of natural selection or random genetic drift.

On the genetic structure of Ile de France sheep breed, Slavov *et al.* (2004) reported five alleles that produced a total of eleven genotypes. Four of these were homozygous while the remaining seven were heterozygous. The Tf^{DD} genotype constituted the highest proportion, 12.5% of the total 264, among the homozygous genotypes. This translated to a proportion of 26.99% homozygous animals. Thus, of all the five transferrin alleles Tf^{D} allele was the most frequent with 0.365 while Tf^{E} was the least frequent with 0.002.

The theoretical basis of the study of polymorphic proteins is that breeds can be defined as populations that differ from each other in the relative distribution and frequencies of genes (Hasselholt, 1969).

2.13.2 DNA Hybridization

DNA hybridization was developed in the 1960's and was the first technique used to study the organization of eukaryotic genomes and was applied in molecular evolution and systematics studies (Sibley and Ahlquist, 1990). After denaturation, DNA from two genomes is combined and allowed to re-anneal on cooling. The extent of nucleotide differences between the two different strands can then be approximated upon reheating and measuring the temperature at which the double strands dissociate, provides an index of relatedness and conversely of genetic distances. This technique has been used to estimate the genetic distance between species of higher primates and carnivores (O' Brien *et al*., 1985) as well as birds (Sibley and Ahlquist, 1990).

2.13.3 Restriction Fragment Length Polymorphisms (RFLP'S)

In RFLP, genomic DNA is isolated, cut using restriction enzymes, size fractionated on gels and transferred to a filter by blotting (Southern, 1975) and probed with clones from the genomic region of interest (Aquadro *et al*., 1992). The advantage of RFLP's is that it can be used to screen a large number of individuals without requiring complicated molecular techniques (Aquadro *et al*., 1992).

2.13.4 Random Amplified Polymorphic DNA (RAPD)

RAPD (Williams *et al*., 1990) is a polymerase chain reaction (PCR) based technique that has been used for the study of populations. It uses one short oligonucleotide (±10 – 12 bp long) to amplify random segments of DNA. The polymorphisms generated by this technique indicate dominant-recessive characters (presence or absence of a band). This technique has been used successfully in the study of plants (Kantanen *et al*., 1995) but was found to be not highly reproducible in animals.

2.13.5 Mitochondrial DNA (MTDNA)

In animal cells, DNA is also found outside the nucleus in small oblate bodies known as the mitochondria. These maternal markers have been instrumental in identification of wild ancestors, localization of domestication centres and reconstruction of colonization and trading routes (Bruford *et al.,* 2003; Groeneveld *et al*., 2010). Most studies with mtDNA target the hyper variable control region (D-loop), but complete mtDNA sequences add substantial information by establishing the relation between haplo groups (Achilli *et al*., 2008). A caveat is the artefactual amplification of nuclear copies of mtDNA, which can be minimized by using long range PCR amplifications and homologous primers that are complementary to their target regions without mismatches.

The reason for the increased use of mtDNA in population studies is that it is transmitted only through the maternal line in most species (Avise *et al*., 1987; Gyllensten *et al*., 1985; 1991), evolves more rapidly than nuclear DNA (Brown, 1985; Stoneking*et al*., 1991), is considerably smaller than nuclear DNA with a size of approximately 15-20 kilo bases (kb) in length, comprises approximately 37 genes (Wallace, 1986), is present in multiple copies in each eukaryotic cell, is easily isolated and purified and, there is general conservation of gene order and composition (Wilson *et al*., 1985). The fact that mtDNA shows haplotype diversity within species makes it a useful tool in establishing phylogenetic relationships at or below the species level (Avise *et al*., 1987). mtDNA has been used in studies of the origins of sheep (Hiendleder *et al*., 1998; 2002). There are however a few drawbacks of using mtDNA for population studies. The lack of recombination makes the mitochondrial genome a single heritable unit; potentially

producing gene diversity estimates that have larger standard errors than those determined using nuclear loci (Dowling *et al*., 1990). An occasional bi-parental inheritance has been reported (Hoeh *et al*., 1991) that could also complicate mtDNA analysis.

2.13.6 Y- Chromosomal Markers

Y-chromosomal variation is a powerful tool to trace gene flow by male introgression (Petit *et al.*, 2002). It is the most powerful marker in human population genetics and is used more and more in domestic animal species.

2.13.7 Genome Sequencing

"Next-generation" genomic technologies, several of which have already passed the proof-of-principle stage, will expand further the scope of molecular studies and likely allow in the near future the affordable whole-genome sequencing of individual animals.

Predictably, this will open new avenues of research that lead to new insights into diversity and the estimation of conservation values. Most notably, dense genetic maps allow the demarcation of "footprints" or "signatures" of selection, while the growing amount of knowledge on genotype phenotype relationships will also reveal novel aspects of functional diversity. Clearly, this will ask for new software and hardware for extracting and storing meaningful information for the huge amount of DNA sequence. The building of a bioinformatics infrastructure will thus be a potentially limiting factor in the exploitation of this variation. .

2.13.8 Copy Number Variations (CNVs)

Genetic studies of the human genome indicate the presence of variation in copy number of certain chromosomal segments, as well as a relationship between copy number and phenotypic variation. It is anticipated that this category of genetic variation will also prove to be relevant for studying the diversity of livestock (FAO, 2011).

2.13.9 Single Nucleotide Polymorphisms (SNP's)

DNA microarrays or "chips" have been used in studies ranging from gene expression to identification of single nucleotide polymorphisms (SNP's) or differences in DNA sequences amongst genotypes (Wang *et al*., 1998). The microarray technology allows the simultaneous analysis of thousands of parameters within a single experiment, thus generating large amounts of genomic data within a single experiment (Templin *et al*., 2002). The potential use of DNA chips and SNP's in the characterization of livestock has already been identified (Altshuler *et al*., 2000).

At present, the unavailability of SNP markers in most species remains an obstacle to their systematic employment in population genetic studies (Zhang and Hewitt, 2003). This technique is unaffordable at this stage, but should be kept in mind for future sheep genetic resources studies.

SNPs have the following advantages relative to microsatellites (Landegren, *et. al*., 1998; Vignal, *et. al.*, 2002; Morin, *et. al.*, 2004)

1. Automatic allele scoring is unambiguous and facilitates integration with datasets from other laboratories.
2. The cost of genotyping on a per marker basis is much less than with microsatellites.
3. The high number of SNPs can allow a description of individual and breed relationships with unprecedented accuracy and have the potential to supplement or substitute pedigree data.
4. Markers can reveal functional, as well as neutral, genetic variation, which may lead to the identification of gene variants corresponding to specific phenotypes (Kohn *et al*., 2006).
5. High density SNP screens can identify multiple SNPs in linkage disequilibrium with any form of DNA variation that is involved in phenotypic variation. This allows for the use of genomic information for the prediction of breeding values within and possibly even across breeds.

A number of caveats must be considered with SNPs (FAO, 2011), however. First, no commercial low-density panels are currently available for AnGR characterization. Although the cost per SNP is low relative to microsatellites and this cost decreases with the number of SNPs that are analysed, the costs for the high-density assays (currently $200-300) are nonetheless prohibitive for many applications. The equipment for high-throughput SNP panels is still quite expensive, so outsourcing is often the most feasible option. Also, because costs per sample are decreased if many samples are analysed, collaboration with other scientists to combine the assays in one run is warranted.

A second caveat is that high-density SNP analyses yield a large amount of data, requiring specialized skills and computing infrastructure for bioinformatics, genetic analysis and data management. These latter requirements often represent the limiting factor in full exploitation of genomic analysis. The costs and need for specialized technical capacity have particularly been barriers for the use of SNP in developing countries for genetic characterization of AnGR (FAO, 2011).

A final obstacle in the use of SNP for the study of AnGR diversity is the ascertainment bias, which is a source of inaccuracy in evaluation of diversity as a consequence of the population in which the SNP were discovered. The commercially available SNP panels were largely constructed with the objective to support genomic selection and thus a majority of SNP in these panels originate from the international trans-boundary breeds. Some of these SNP may be monomorphic in local breeds, whereas loci that are polymorphic only in local breeds are likely to be excluded (FAO, 2011).

In addition, the SNP in commercial panels have been selected to have high minor-allele frequency (i.e. greater variability) in the international trans-boundary breeds, without considering variability in other breeds. As a consequence, diversity in the other breeds, including those located close to the domestication centres, can be underestimated and estimates of relationships among breeds can be distorted. Development of standard SNP panels for diversity studies that are not biased by ascertainment protocols would require additional SNP discovery in a more representative group of breeds covering most of the existing diversity within a species (FAO, 2011).

2.13.10 Microsatellite Markers

Molecular genetics is revealing new facets of genetic variation, both on the standing variation and on the new variation generated by mutations. Microsatellites are a new class of marker that has become the preferred technique for population studies. Microsatellites are short tandem repeats (STR's) of genomic sequences. The repeated unit can be a mono-, di-, tri- or tetra nucleotide with di- repeats being most common. They generally occur in non-coding regions of the genome. DNA microsatellite sequences are valuable genetic markers due to their dense distribution in the genome, they are highly variable, co-dominantly inherited and relatively easy to detect. As hyper variability is highly significant for detecting differences in a population and between individuals, microsatellite typing can reveal degrees of polymorphism that is easy to interpret (Buduram, 2004).

Microsatellites offer several advantages. They are relatively easy to isolate in different species, different loci can be used according to the level of variation, which ranges from very low to extremely high (Beaumont and Bruford, 1999), they can be easily amplified by PCR and thus used on a wide range of sample material such as blood, hair, meat, saliva and skin, and their genetic systems are easily automated enabling the analysis of a large number of samples (Luikart *et al*., 1999). However, microsatellites do have several disadvantages: they are difficult to isolate from certain groups of organisms (Beaumont and Bruford, 1999); there are some technical challenges of microsatellite analysis for some types of samples such as saliva, hair or faecal material (Gagneux *et al*., 1997); and data generated in different laboratories using different methods have proved difficult to amalgamate (Beaumont and Bruford, 1999).

After standardization through international societies within different laboratories, the use of microsatellites for genetic characterization of livestock including cattle (MacHugh *et al*., 1997; Hanotte *et al*., 2000), goat (Chenyambuga, 2002), camels (Nolte, 2003), horses (Botha, 2001) and sheep (Crawford and Littlejohn, 1998) is now accepted worldwide. The popularity of microsatellites remains undiminished, as most

researchers are of the opinion that the advantages such as the resolving power, outweighs their disadvantages.

2.14 Genetic Variation Parameters

2.14.1 Number of Alleles/Frequency

Chen *et al.* (2011) reported 167 alleles detected at 10 loci in four sheep populations of China. The number of alleles per locus varied 12 to 22, with at least 8.6 alleles observed in each of the population studied. Gustavo *et al.* (2000) in a study of desert bighorn sheep using nine dinucleotide microsatellite loci reported average number of alleles per population of 2.0 to 4.38 with MAF65 having the most allele overall with 10 alleles. In a study of two predominant Uruguayan sheep populations using 10 microsatellite loci, Ivanna, *et al.* (2002) reported that the markers were highly variable, showing between 7 and 15 alleles each.

D' Angelo *et al.* (2008), in a study of the genetic variability of the Gentile di Puglia sheep breed reported that the mean number of alleles per locus in the population was 9.68, ranging from 4 (BM1824) to 15 (OarJMP29 and MAF70) while a remarkable proportion of all alleles (23%) were found in only 1 of the 6 farms investigated, although at a low frequency (<18%). The mean number of alleles per locus was less on the single farms with respect to the whole population, and ranged from 5.63 (farm A) to 6.11 (farm C).

Adebambo *et al.* (2003) study of the genetic relationships between Native Sheep breeds reported that all loci sampled were found to be polymorphic with allele ranging from 2.85 to 11.5 in the sheep breeds except Hel 9 which is monomorphic in most breeds. The only allele found in all the breeds was 102/103 which may be one and the same allele in the Yankassa, Balami and the Uda x Yankassa cross. Allele 102 alone was found in the Merino and the West African Dwarf breeds whilst the Uda is the only breed in which both alleles 94 and 102 were found. A total of 285 alleles were

generated ranging from 3 in Hel 9 to 24 in Hel 1, a large percentage of which showed very high degree of variation.

Al-Atiyat, *et al.* (2012), in the study of Fat Tailed-Sheep in the Southern region of Jordan, observed that average number of alleles per loci for all population studied was 6.17, whereas the number of alleles per each locus ranged from 5 to 8. The authors reported that the average number of alleles for each flock was 5.5, 4.7 and 5.5 for Flocks1, 2, and 3, respectively. However, the microsatellite (MS) loci varied in number of described alleles from 7 (OARHH55) in Flock 1 to 3 (OARAE101) in flock 3. Kevorkian *et al.* (2010), reported that all 11 loci studied in four Romanian autochthonous sheep breeds were successfully amplified with a total of 197 alleles detected. The authors reported that MAF70 showed the highest number of alleles per locus with 30 alleles while OarCP20 had the lowest number of alleles per locus with 11 alleles with a global mean number of alleles of 17.9±5.87.

In a study to investigate the Bhutanese sheep biodiversity using eight microsatellite markers, Dorji *et al.* (2010) observed total numbers of alleles at the eight microsatellite locus to be 85, while 107 alleles were reported for all samples. The authors reported mean number of alleles per locus to be 6.65, with a range from 9 to 25.

2.14.2 Heterozygosity

The average expected (Hardy-Weinberg) heterozygosity at n loci within a population is the best general measure of genetic variation within-populations (Allendorf and Luikart, 2007).

$$H_e = 1 - \sum_{i=1}^{n} p_i^2$$

Square of p gives the expected frequency of homozygotes for p^{th} allele of i^{th} locus and by introducing all of the loci (1 to n), total amount of expected homozygosity is subtracted from 1, which gives expected heterozygosity.

Estimation of H_e generally is not affected by sample size and even a few individuals are sufficient for estimating H_e if a large number of loci are examined (Gorman and Renzi, 1979). Furthermore, it is robust to the presence of null alleles (Drury *et al.,* 2009).

The average observed and expected heterozygosity for 4 sheep populations was reported as 0.2879 and 0.8050, respectively with 0.3237 as the highest observed heterozygosity value and 0.2585 as the lowest value of observed heterozygosity (Chen, *et al.,* 2011). Alvarez *et al.,* (2004) reported expected heterozygosity values of 0.619 to 0.708 and observed heterozygosity values of 0.572 to 0.712 in a study of the genetic diversity of six sheep breeds from Northern Spain using 14 microsatellite. Ivanna *et al.* (2002) reported observed heterozygosity values in the range of 0.62 to 0.84 for whole population studied.

D' Angelo *et al.* (2008), in a study of the genetic variability of the Gentile di Puglia sheep breed reported that observed heterozygosity averaged over loci was 0.683, whereas expected heterozygosity was 0.767. Observed heterozygosity averaged over loci was less than expected heterozygosity on all farms, ranging from 0.664 (farm B) to 0.707 (farm D). Moioli *et al*. (2006) investigated genetic diversity among Gentile di Puglia (25 animals), Sopravissana (20 animals), and Sarda (15 animals) sheep breeds by using a set of 13 microsatellite loci. They observed heterozygosity for the Gentile di Puglia sheep breeds (0.59% and 0.127) compared with the Sopravissana (0.51% and 0.122) and Sarda (0.53% and 0.121) sheep breeds.

Adebambo *et al*. (2003) in the study of the genetic relationships between native Sheep breeds in Nigeria reported average heterozygosities of 0.57 to 0.72. In the study of Fat Tailed-Sheep in the Southern region of Jordan, Al-Atiyat *et. al.* (2012) reported overall observed heterozygosity as 0.67 for all flocks. They also reported that in most cases observed heterozygosity was lower than expected heterozygosity which resulted overall in slightly higher average expected heterozygosity of 0.70 for all flocks. The average expected heterozygosity for each flock was 0.667, 0.652 and 0.716 for flocks 1, 2 and 3, respectively.

Al-Atiyat *et al.* (2012), further reported that the results showed higher expected heterozygosity in all flocks and for most of the studied loci except for OARHH30 where expected heterozygosity was 0.55 and observed heterozygosity was 0.47. In a similar study, based on three microsatellite loci, the heterozygosities of Awassi, Kivircik, and Akkaraman breeds of turkey as well as two of their crossbreeds were high and ranged from 0.667 to 0.782 (Soysal *et al.*, 2005). The average expected heterozygosity was 0.72 in Afshari sheep breed of Iran (Qanbari *et al.*, 2007). Arora *et al.* (2011) reported that both observed heterozygosity and expected heterozygosity averaged 0.665 and 0.786, respectively.

Kevorkian *et. al.* (2010), reported mean observed heterozygosity of 0.611±0.17 overall with a range of 0.52 (OarCP20) to 0.87 (HSC) and expected heterozygosity overall mean of 0.733±0.09 and ranged from 0.34 (MAF33) to 0.81 (OarCP34) in 11 loci studied in four Romanian autochthonous sheep breeds. They further reported mean estimates of observed and expected heterozygosity over all loci and breeds to be 0.64±0.06 and 0.74±0.05, respectively. Dorji *et al.* (2010) reported that observed heterozygosity ranged from 0.506 for ILSTS005 to 0.732 for TGLA0053.

2.14.3 Polymorphic Information Content (PIC)

Polymorphic information content is calculated using total number of alleles and allele frequencies in a population. If it is above 0.75 the locus is regarded as much more informative. The polymorphism information content (PIC) for each marker is determined separately using the following equation:

$$PIC = 1 - \sum_{i=1}^{n} p_i^2 - 2\left[\sum_{i=1}^{n-1}\sum_{j=i+1}^{n} p_i^2\, p_j^2\right]$$

Where p_i is the frequency of the i^{th} allele, and n is the number of alleles (Botstein *et al.*, 1980).

Chen, *et al.* (2011) reported mean PIC values of 0.7223 to 0.8385 for 10 microsatellite markers in 4 sheep populations indicating that the genetic diversity of the sheep

populations was high. Ivanna *et al.* (2002) in their study of Uruguayan sheep populations using 10 microsatellite loci observed that all loci were highly polymorphic, showing PICs between 0.63 and 0.87 for the whole sample. The average PIC was 0.67 in Afshari sheep breed of Iran (Qanbari *et. al.,* 2007).

Al-Atiyat *et al.* (2012) reported PIC average of 0.65 for overall of three flocks. There were 0.639, 0.643 and 0.714 for Flocks 1, 2 and 3, respectively. The lowest value (0.483) was for ILSTS30 in Flock 2 and the highest value (0.737) was for BM143 in Flock 3. In the report of Arora *et al.* (2011), PIC values ranged from 0.543 to 0.929 and averaged 0.775.

Kevorkian *et al.* (2010) reported that the majority of the markers were found to have high polymorphism, with PIC ranging from 0.621 (OarCP20) to 0.86 (HSC) with the mean PIC across all 11 loci being 0.732, indicating high level of information of the chosen microsatellite set. PIC values for individual Bhutanese sheep breeds ranged from 0.529 for Tsirang to 0.658 for Jakar while the values for the reference populations ranged from 0.644 for Karakul to 0.708 for Lanzhou sheep breeds (Dorji *et al.*, 2010).

2.14.4 Hardy-Weinberg Equilibrium (HWE)

In a large random breeding population with no selection, mutation or migration, the gene and genotype frequencies are constant from generation to generation. A population with constant gene and genotype frequencies is said to be in Hardy-Weinberg Equilibrium (Falconer, 1989).

In a study of the genetic diversity of six sheep breeds from Northern Spain using 14 microsatellite, Alvarez *et al.* (2004) reported that the number of markers that show significant deviation from Hardy-Weinberg equilibrium within breeds ranged from one to four. However up to nine markers were found to be in Hardy-Weinberg disequilibrium across populations. Gustavo *et al.* (2000) observed in their study of desert bighorn that none of the loci or studied sites differed significantly from the Hardy-Weinberg proportions. In a study of two Uruguayan sheep populations, Ivanna *et al.* (2002) reported that 3 out of the 10 microsatellite loci studied showed significant departure from Hardy-Weinberg equilibrium in the whole population.

D' Angelo *et al. (2008)* reported that of the 19 microsatellites loci studied, 4 microsatellites (OarAE129, ILSTS28, ILSTS5, MAF33) showed significant deviations from Hardy-Weinberg proportions ($P < 0.01$) and were associated with heterozygote deficiency in the Gentile di Puglia sheep breed. Nevertheless, on farm C none of the 19 microsatellites showed significant deviations from Hardy-Weinberg proportions ($P < 0.01$), whereas on farms A, B, and F only 1 microsatellite locus and on farms D and E 2 microsatellite loci showed significant heterozygote deficiency ($P < 0.01$). Al-Atiyat *et al.* (2012) reported that analysis of deviation from HWE for each locus revealed that only locus, INRA40, in Flock 3 of fat tailed-sheep in southern region of Jordan significantly deviated from HWE (Chi-square = 8.23, $p< 0.004$) however, the studied sheep flocks were in HW equilibrium even though they were small in size and non-random mating took place with a few sires.

In order to test possible deviations from Hardy-Weinberg equilibrium, Kevorkian *et al.* (2010) reported that exact p-values for single breeds were pooled and all investigated breeds were in Hardy-Weinberg equilibrium. However, when the Hardy-Weinberg testing was performed for individual locus, deviations from Hardy-Weinberg equilibrium were found to be significant ($p<0.05$) in OarCP20, MAF70, MAF214, MAF33 and highly significant ($p<0.001$) in OarCPB11.

The deviations from HWE were statistically significant ($p<0.05$) for all 11 locus-population combinations or 27.5% of all Bhutanese sheep pair wise comparison. These deviations involved four loci in Jakar (OARFCB0020, MCM0042, TGLA0053 and OARFCB011) and three loci (OARFCB0129, MCM0527 and OARFCB011) in Sarpang populations (Dorji *et al.*, 2010).

2.14.5 Genetic Distance

Genetic distance is the degree of gene difference (genomic difference) between species or populations that is measured by some numerical method. Thus, the average number of codon or nucleotide differences per gene is a measure of genetic distance (Nei, 1987).

Theoretically, the genetic distance between two populations is defined in terms of the poplation gene frequencies for all loci in the genome. In practice, however, it is virtually impossible to examine all genes in the populations for all loci. Therefore, we must estimate the genetic distance by sampling a certain number of individuals from the populations and examining a certain number of loci (Nei, 1987).

To obtain correct tree topology from microsatellite data, the Nei's (D_A) genetic distance is accepted as the most appropriate method (Takezaki and Nei, 1996), hence it includes assumptions about some of the evolutionary forces: genetic drift and mutations.

This method is based on infinite allele model and calculated as:

$$D_A = 1 - \frac{1}{r}\sum_{j}^{r}\sum_{i}^{mj}\sqrt{x_{ij}y_{ij}}$$

where,

x_{ij} = Frequencies of the i^{th} allele at the j^{th} locus in sample X;

y_{ij} = Frequencies of the i^{th} allele at the j^{th} locus in sample Y; and

m*j* =Number of alleles at the j^{th} locus. r =Number of loci examined.

Nei's Genetic Distance (D_A) varies between 0 and 1. "0" stands for identical populations and "1" is for populations that share no alleles.

The genetic distances reported for 4 sheep population in China were (0.6888, 0.6485 and 0.6807) and highest DA was observed between Tan sheep and Small- tailed Han sheep. Similarly, the smallest DA (0.1341) was observed among two White Tan sheep populations (Chen, *et al.*, 2011). The genetic distance between each pair of sampling locations ranged from a minimum of 0.020 between Mt. Davis and Lost Cabin to a maximum of 0.870 between San Ysidro and Albertia (Gustavo *et al.*, 2000). They also reported that all the genetic distances were highly significant ($p < 0.001$) except for two smallest genetic distances ($D_{Davis,\ Cabin} = 0.02$, $D_{Kofa,\ Castle} = 0.04$).

Adebambo *et al*. (2003) in the study of the genetic relationships between Native Sheep breeds in Nigeria reported that the breeds were clearly separated from each other forming three distinct groups on the evolutionary scale. The authors reported that the Uda and Balami which are basically Northern breeds are clustered together with a 65% occurrence bootstrap value, whilst the Yankassa stood between the extremes of the West African Dwarf (a Southern humid zone breed) and the Uda and Balami (Northern arid Zone breeds). The Merino, an exotic breed, clustered with the Uda x Yankassa cross (73% occurrence) probably indicating a closer association between the two breeds despite the fact that the Uda cross is a Nigerian breed whilst the Merino is exotic. Whilst genetic distances of 0.36 and 0.43 were found between the Uda and Yankassa and the Uda and Balami, respectively, distances of 0.66 were found between the West African Dwarf and the Balami as well as 0.53 between the West African Dwarf and the Uda, and as high as 0.71 to 0.80 between the Merino and these three breeds, showing that they might have diverged relatively early during evolution.

Al-Atiyat *et al.* (2012) in a study of fat-tailed sheep in Southern Jordan reported estimated distance from allele difference between two flocks as 0.026 between Flock 1 and Flock 2, 0.035 between Flock 1 and Flock 3 and 0.033 between Flock 2 and Flock 3. The genetic distance between breeds studied ranged from 0.263 for Milk Line Palas and Meat Line Palas to 0.606 for Karabash and Meat Line Palas (Kevorkian *et al.*, 2010). The lowest genetic distance was found between Sipsu and Tsirang (0.041) and the highest between Tsirang and Karakul (0.705) (Dorji *et al.*, 2010).

2.14.5 Neighbour Joining (NJ) Tree

Population relationships are often visualized by constructing a dendrogram based on the genetic similarity of breeds. After obtaining a genetic distance matrix, a clustering algorithm is used to group the populations.

The most widely used clustering algorithms are UPGMA (Unweighted Pair-Group Method with Arithmetic Mean) and NJ (neighbour-joining). The trees constructed by UPGMA algorithm are ultra-metric, i.e. distances from root to all leaves (populations at the end of the lines) are equal. This algorithm starts by finding the two populations with

minimum distance and combines them into an internal node. Distance of new node to the leaves is half of the original distance between two populations and to other populations are weighted mean of original pair wise distances. The process continues in this manner till the resulting tree is completed (Mattiussi, *et al.,* 2004)

NJ algorithm (Saitou and Nei, 1987) is different from UPGMA in that branch lengths of the tree can be different (non-ultra metric), therefore providing additional information about the relationship between populations. It combines populations that are closest to each other and also furthest from the rest. It is a fast method even for very large data sets. NJ tree construction, by sequentially finding the neighbours helps to minimize the total length of the tree. Since NJ tree does not assume equal rate of evolution of the breeds after the divergence, NJ method performs better under non-uniform rates either among lineages or among sites.

2.14.6 F- Statistics

Wright's (1978) fixation index is a measure to describe the level of differentiation between populations (i.e. a test whether or not they are from the same gene pool). F–Statistic values of FST and FIT are measures of deviation from Hardy Weinberg proportions and total populations, respectively. Positive values indicate a deficiency in heterozygotes and negative values indicate an excess of heterozygotes. FIS can be interpreted as a measure of inbreeding (the measure of allelic fixation of individuals relative to the subpopulations).

F-statistics (inbreeding coefficients) developed by Wright (1965) and extended by Nei (1977) is the oldest and most widely used method to measure the genetic differentiation within and between populations (Allendorf and Luikart, 2007). Usually, the genotype frequencies in populations do not follow Hardy-Weinberg equilibrium frequencies in nature and F statistics uses these deviations to measure the inbreeding (which is the tendency for mates to be closely related) within populations. One of these inbreeding coefficients, F_{is} is a measure of departure from Hardy- Weinberg proportions within local subpopulations and estimated by the formula:

$$F_{is} = 1 - \frac{H_o}{H_s}$$

where H_o is the mean observed heterozygosity over all sub-populations and H_S is the mean expected heterozygosity over all sub-populations. When the value of F_{is} is positive, it is an indication that there is inbreeding in the examined population which causes heterozygotes deficiency. Whereas, F_{is} will be negative when there is migration from outside of the population causing an excess of heterozygotes.

F_{st} is a measure of genetic divergence among sub-populations and can be used as a distance measure. It can be calculated by the formula:

$$F_{st} = 1 - \frac{H_s}{H_t}$$

where H_t is the expected heterozygosity if the entire base population were panmictic (random mating is observed) and H_s is the mean expected heterozygosity over all sub-populations. If two populations are considered each time, it can be used as a distance matrix to compare pairwise differences among sub-populations.

F_{st} will be between 0, when populations have equal allele frequencies, and 1, when populations are fixed for different alleles. That is why F_{st} is sometimes referred to as fixation index. F indices as proposed by Wright (1965) does not consider the unequal finite sample sizes and hence some disagreement on the interpretation of the quantities and on the method of evaluating them. Weir and Cockerham (1984) revised the F coefficients in order to unify various estimation formulas so that they are suited to small data sets.

The values of F_{it}, F_{is} and F_{st} reported for 10 markers in 4 sheep populations were 0.674, 0.642 and 0.0893, respectively. The mean genetic differentiation among breeds, measured as the F_{st} value was 8.93%, thus 91.07% of the population diversity resulted from differences among individuals, indicating a close relationship among population or migration among sheep populations (Chen, *et al.*, 2011).

Mean estimates of F- statistics obtained over 11 loci (Weir, 1990) were: F (F_{it}) = 0.247±0.13, f (F_{is}) = 0.182±0.13, θ (F_{ST}) = 0.082±0.039. The genetic differentiation F_{st} among the breeds studied was 8.2% while the values ranged from 0.031 (OarCP34) to

0.386 (MAF33) for $F_{is,}$ 0.015 (OarCP34) to 0.143 (OarCP20) for F_{st} and 0.045 (OarCP34) to 0.471 (MAF33) (Kevorkian *et al.*, 2010). F_{st} values ranged from 0.031 between Sipsu and Tsirang to 0.226 between Jakar and Tsirang and were significantly different ($p<0.05$) for all pair wise combinations (Dorji *et al.*, 2010).

2.14.7 Unique Alleles (Private Alleles)

A private allele is one found in only one population. The presence or absence of private alleles gives an idea about the migration rates between populations. Slatkin (1985) proved that there is a linear relationship between Nm (the actual number of immigrants entering to a subpopulation at each generation) and the average frequency of private alleles at equilibrium. For instance, if gene flow is small, several private alleles will be found in populations that developed by mutations. The length of the time a new allele stays private is primarily determined by the migration rates, such that the proportion of alleles that are private decreases as migration rate increases (Lowel and Allendorf, 2010).

Gustavo *et al.*, (2000) reported alleles unique to single populations (MAF65-121 in castle Dome, OarFCB128-112 in San Ysidro, MAF65-133 and MAF209-111 in Old Dad and OarFCB128-118, MAF48-134 and MAF65-119 in Alberta) in a study of desert bighorn sheep populations of Canada. The number of unique alleles found for Bhutanese and foreign sheep were 11 and 16, respectively (Dorji *et al.*, 2010).

2.14.8 Gene Diversity

Average gene diversity of 0.51 for 11 desert study sites, 0.57 for 2 rocky mountain study sites and 0.52 overall with a range of 0.36 in Red rock to 0.63 in Eagle population were reported in the study of desert bighorn sheep from 13 study sites in Canada (Gustavo *et al.*, 2000).

In a study of genetic diversity of four Romanian autochthonous sheep breeds, Kevorkian *et al.* (2010) reported gene diversity ranging from 0.531 (OarCP20) to 0.892 (HSC) and the mean gene diversity was 0.754 for 11 microsatellite loci studied.

2.14.9 Structure Analysis

The STRUCTURE software provides an effective way to illustrate the presence of population structure and to distinguish distinct genetic populations (Pritchard *et al.*, 2000). The underlying assumptions of the model in which there are K populations (where K may be unknown), each of which is characterized by a set of allele frequencies at each locus. If their genotypes indicate that they are admixed, the individuals in the sample are assigned jointly to two or more populations. With the version 2.2 of Structure software, microsatellites, Amplified Fragment Length Polymorphisms (AFLP), Restriction Fragment Length Polymorphisms (RFLP) and Single Nucleotide Polymorphisms (SNPs), data sets could be used and analysed (Falush *et al.*, 2007).

The most significant factors to determine for STRUCTURE analysis are the burn in length, the ancestry model and estimation of *K* (number of populations). Burn in length explains how long to run the simulation before collecting data to make sure that the simulated population reached to drift-mutation equilibrium which minimizes the starting configuration. Typically a burn in length of $> 5N_0$ is used, where N_0 indicate the initial population size and 10,000 - 100,000 burn-in length is more than adequate (Falush *et al.*, 2003). For the ancestry of individuals, admixture model is performed. This model is reasonably flexible for many of the complexities of real populations (Falush *et al.*, 2003). It assumes that individuals may have mixed ancestry.

There are several methods suggested to estimate K (number of populations). One of them is the method suggested by Evanno *et al.* (2005). For the true K, the distribution of Ln P(D) (or L(K), according to Evanno *et al*. (2005), do not indicate a clear mode, but at the true value of K the second order rate of change of the likelihood function (ΔK = m|L‟(K)|/ s[L(K)]) with respect to K ('K) does show a clear peak. Another method recently developed and widely used (Tapio *et. al.*, 2010) is based on testing the similarity between the results of individual runs for each different value of K. At the maximum similarity, it is argued that the correct K is obtained.

2.14.10 Factorial Correspondence Analysis (FCA)

In multidimensional space to see the individuals and to investigate the relationships between the individuals, the Factorial Correspondence Analysis (FCA) (Lebart *et al.*, 1984) is used. Each individual is represented with respect to the alleles. For each of the allele the diploid individual can assume 0, 1 or 2. Hence string of "0, 1, 2" will represent the individuals. The program finds independent axes which are the linear combinations of the alleles such that the maximum genetic diversity observed within the total data could be explained by the first axis. The most informative axes are first three ones (Machugh *et al.,* 1994). Visualizing on the independent axes for how individuals are related to each other is an informative way to see the amount of inertia, distinctness of the breeds and yet relative similarity between the breeds.

2.14.11 Analysis of Molecular Variance (AMOVA)

In *F* statistics, gene frequencies are compared among breeds. However, from molecular data, not only the frequency of molecular markers but also the amount of mutational differences between different genes can be obtained. Instead of Mendelian gene frequencies, a method that analyses differences between molecular sequences is very useful to estimate the population differentiation. One can achieve this by using Analysis of Molecular Variance (AMOVA) which estimates population differentiation directly from molecular data and testing hypotheses about such differentiation. Several kinds of molecular data, such as microsatellite based data or direct sequence data can be analysed with this method (Excoffier *et al.,* 1992).

With AMOVA, any kind of raw molecular data is analysed as a Boolean vector p***i***, that is, a "1 x n" matrix of 1s and 0s, where 1 indicates the presence of a marker and 0 its absence. By subtracting the Boolean vector of one haplotype from another, Euclidean distances between pairs of vectors are then calculated. For all pair wise arrangements of Boolean vectors, squared Euclidean distances are calculated and then set into a matrix, and divided into sub-matrices corresponding to subdivisions within the population. The data can then be analyzed in a nested analysis of variance framework. A nested

ANOVA differs from a simple ANOVA in that data is arranged hierarchically and mean squares are computed for groupings at all levels of the hierarchy. This allows for hypothesis tests of between-group and within-group differences at several hierarchical levels (Excoffier *et al.*, 1992)

The design and formulas of the calculation for AMOVA for genotypic data with several groups of populations, within-individual level is as can be seen in the Excoffier *et al.*'s (2006) Arlequin package program and is presented in the table below:

Table 1: General AMOVA Table for Genotypic Data

Source of Variation	**Degree of freedom**	**Sum of squares (SSD)**	**Expected mean Squares**
Among Groups	G-1	SSD(*AG*)	$n''\sigma_a^2 + n'\sigma_b^2 + 2\sigma_c^2 + \sigma_d^2$
Among Populations/Within Groups	P-G	SSD(*AP/WG*)	$n\sigma_b^2 + 2\sigma_c^2 + \sigma_d^2$
Among Individuals/ Within Populations	N-P	SSD(*AI/WP*)	$2\sigma_c^2 + \sigma_d^2$
Within Individuals	N	SSD(*WI*)	σ_d^2
Total	2N-1	SSD(*T*)	σ_T^2

where;

SSD (AG): Sum of squared deviations among groups of populations

SSD (AP/WG): Sum of squared deviations among populations, within groups

SSD (AI/WP): Sum of squared deviations among individuals, within populations

SSD (WI): Sum of squared deviations within individuals

SSD (T): Total sum of squared deviations

G: Number of groups in the structure

P: Total number of breeds

N: Total number of gene copies

Source: Excoffier *et al*., 2006

The variance components can be used to calculate a series of statistics called phi-statistics (Φ), which summarize the degree of differentiation between population divisions and are analogous to *F*-statistics, such as Φ_{CT}, Φ_{SC}, Φ_{IS} and Φ_{IT} corresponds to the differentiation among groups, among populations-within groups, among individuals-within populations and within individuals, respectively. Hypothesis about differentiation at corresponding level of a population can be constructed by *F* -statistic. Furthermore, these hypotheses can be tested using the null distribution of the corresponding variance components; if the variance of the subpopulations does not significantly differ from the null distribution of the variance of the population, the hypothesis that those subpopulations are differentiated from the larger population would be rejected. The data do not perfectly follow a normal distribution; hence, the molecular data consist of Euclidean distances obtained from vectors of 1s and 0s. Therefore, resampling of the data is used to compute the null distribution (Excoffier *et al.*, 1992). In each iteration, individuals are assigned to a randomly chosen population while holding the sample sizes constant. Many permutations are made to build the null distribution to which hypothesis will be tested.

Since the null distributions are obtained by resampling, the individuals from which haplotypes are sampled should be chosen independently and at random. Because of genetic drift, any one haplotype should not be assumed to be completely representative of variation among the whole genome. It is therefore important that the data are derived from an adequate number of markers. Using neutral, non-selected genetic markers can be a useful means of avoiding the confounding effects of selection, if neutral markers can be identified. Populations are assumed to be panmictic and there is no inbreeding. Violation of these assumptions will result in heterozygote deficiency and if the rates of non-random mating or inbreeding differ between populations, fixation estimates will be confounded.

CHAPTER THREE

MATERIALS AND METHODS

3.1 STUDY 1: BLOOD PROTEIN POLYMORPHISM AND GENETIC VARIATION IN BALAMI, UDA, YANKASSA AND WEST AFRICAN DWARF SHEEP POPULATIONS

Assessment of genetic diversity within and between indigenous domesticated animal populations has recently become a matter of intense research. Information on the 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. Once lost, genetic diversity is irreplaceable, hence the need for appropriate characterization and conservation.

Indigenous breeds in developing countries are continuously being replaced at a faster rate, by exotic, high producing breeds in spite of their excellent adaptation to prevailing environmental conditions. This poses the danger of losing valuable genes for adaptation to extreme environmental and disease conditions which may be of value in the very near future.

Polymorphism of blood protein first offered the possibility to study genetic differentiation before the advent of molecular markers. Consequently, several livestock breeds including the domestic sheep have been characterised for variations in major blood proteins (Di Stasio, 1995). Information on blood proteins has also been used to study genetic relationship among sheep breeds (Mwacharo *et al*., 2002; Ibeagha-Awemu and Erhardt, 2004; Esharatkhah *et al*., 2007). This study was therefore undertaken to analyse the genetic diversity at four loci controlling blood polymorphism in Balami, Uda Yankassa and West African Dwarf (WAD) sheep breeds and to estimate the genetic distances between them.

3.1.1 Animals and Management.

The animals used for the biochemical evaluation, were raised semi intensively at the Small Ruminant Unit of the University of Ibadan Teaching and Research Farm and the Osun State Government cattle hob in Iwo. Other animals were raised extensively by Nomads. Animals were allowed to graze in the morning and fed Agro by-products in the evening while in the shed. The sheds were open-sided with wire nettings and metal poles used as fence to keep the animals in under the shed. The shed were made of steel poles and aluminium/corrugated roofing sheets. The animals had free access to water and occasional access to salt licks.

3.1.2 Sampling: Breed Recognition, Location and Sample Size

Documented morphological features described by Adu and Ngere (1979) were used as base line markers to ascribe sampled animals to a breed. Individuals that do not strictly conform to primary breed characters were excluded from the sample.

Animals were sampled from four different locations and flocks (Figure 6). Each location was selected because of the availability of the breed and willingness of the owners to allow for sampling. Balami population was sampled from the sheep market, Felele area in Lokoja, Kogi state. Yankassa was sampled from two different nomadic flocks around Okene in Kogi state. Samples of Uda were obtained from the Osun State government cattle hob in Iwo, Osun State, while the West African Dwarf sheep were obtained from the available flock at the University of Ibadan.

A total of one hundred (100) mixed sex, unrelated, adult indigenous sheep comprising twenty five of each of Yankassa, Balami, Uda and West African Dwarf sheep were sampled. Five (5ml) of blood was collected from each animal by jugular vein puncture into 10ml vacutainer tubes containing Heparin as anti-coagulant.

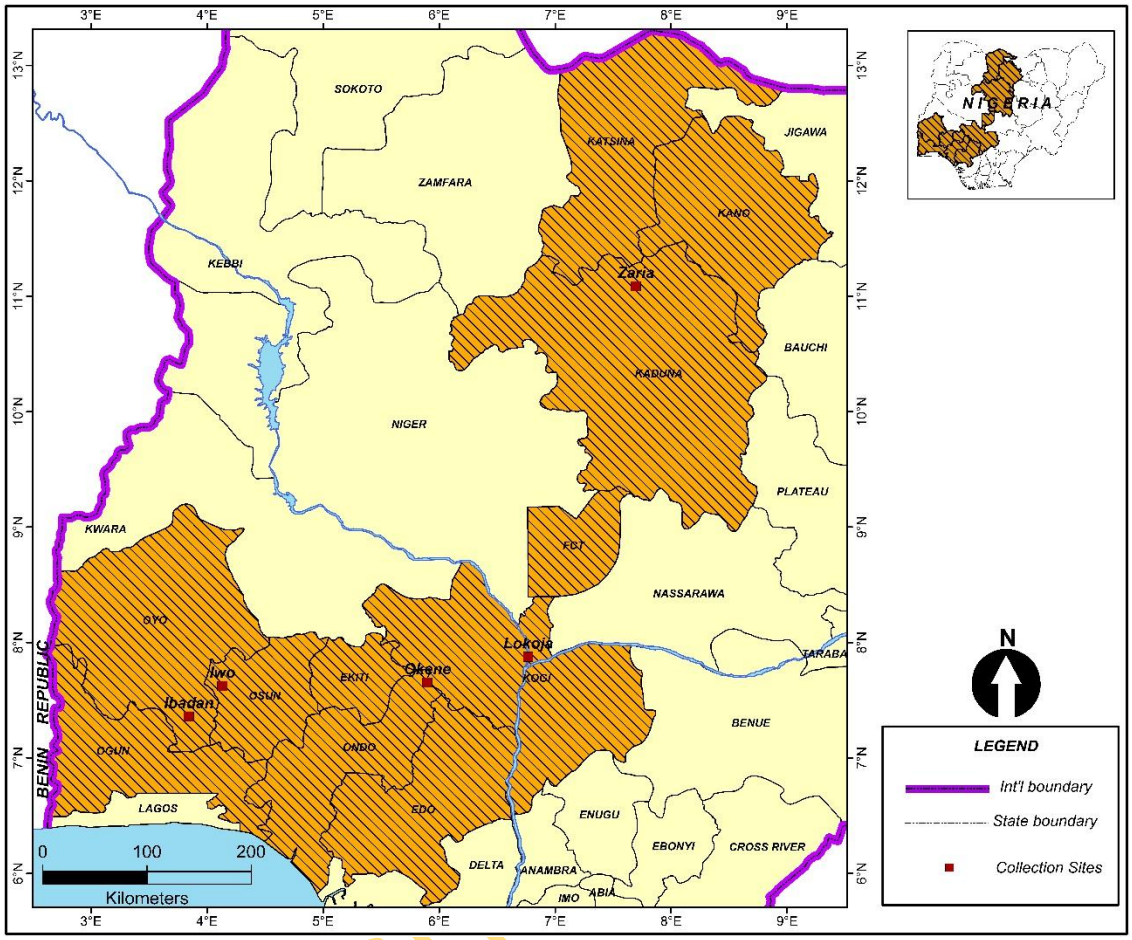


Figure 6: Sampling Locations for Sheep Used Characterisation

3.1.3 Sample Preparation

3.1.3.1 Blood Haemolysates

Red blood cell was prepared from the erythrocyte fraction of heparinized blood by centrifugation at 2500-3000rpm for 10mins at 4^{o}C. The RBC was washed in normal saline (0.155M NaCl) three times and centrifuged at 2500-3000rpm for 5mins at 4^{o}C.The RBCs were lysed with a fourfold volume of distilled H_2O.

3.1.3.2 Plasma

The plasma fraction was separated from the erythrocyte fraction of heparinized blood by centrifuging at 2,500-3,000 rpm for 10 min at 4℃. The supernatant is used.

3.1.3.3 Gel Soaking

Cellulose acetate plates were soaked in the same buffer as the electrode buffer. This is often referred to as a continuous buffer system. Multiple gels were simultaneously soaked in an 800 ml beaker with individual gel plates separated by glass rods to ensure complete soaking of each plate. Care was taken, however, to prevent the formation of bubbles on the gel plate as it was immersed. This was accomplished by submerging the plates at a slow, constant rate into the gel buffer. Plates were soaked for at least 20 minutes.

3.1.3.4 Sample Loading

Prepared blood samples were added to the wells of the sample plate, a cellulose acetate plate was removed from the soaking buffer and blotted dry between sheets of filter paper to remove excess moisture from the gel surface, which may impair resolution. The plate was placed on the aligning base. Care was taken to ensure that the cellulose acetate plate lay flat and did not shift when the extracts were loaded. To prevent movement, the aligning plate was moistened with a drop of gel buffer before the cellulose acetate plate was set on it. The plate was centred on the aligning base to ensure that all samples were applied.

Using the applicator, samples were applied one or more times to the same position on the plate. The optimal number of applications to each load zone varied, depending on the amount of enzyme activity.

Once loaded, plates were rested on the wicks in the tank (without electric current being applied) while subsequent plates were loaded. The applicator was cleaned by blotting its teeth on filter paper before applications were made with other extracts.

3.1.3.5 Gel Running

The plates were placed acetate side down on the wicks in the electrophoresis tank. The load zone located near the end of the gel was not allowed to come into contact with the wicks. Since the current runs from the cathode to the anode (negative to positive), the load zones 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, extracts were loaded near the centre of the gel. Care was taken to ensure that there was no air between the wicks and the cellulose acetate plates (i.e. the plates were made to lie flat). A finger was run along the length of the gel at both ends to ensure complete contact and displacement of air and microscope slides were used as weights to ensure complete contact.

3.1.3.6 Gel Staining

When the gel run was complete, the final stain ingredients were added and the plates removed from the tank and placed label side down on a petri dish. Again, care was taken to ensure that the cellulose acetate plate lay flat. Once plates were removed from the tank, they were stained immediately with the appropriate stain (Table 2) before they dried out. After the stain had been poured, the plates were incubated in the dark at room temperatures to accelerate the staining process. Since several plates were run together, the current was turned off and the plates were removed and stained one at a time to prevent drying of the plates.

3.1.3.7 Gel Scoring

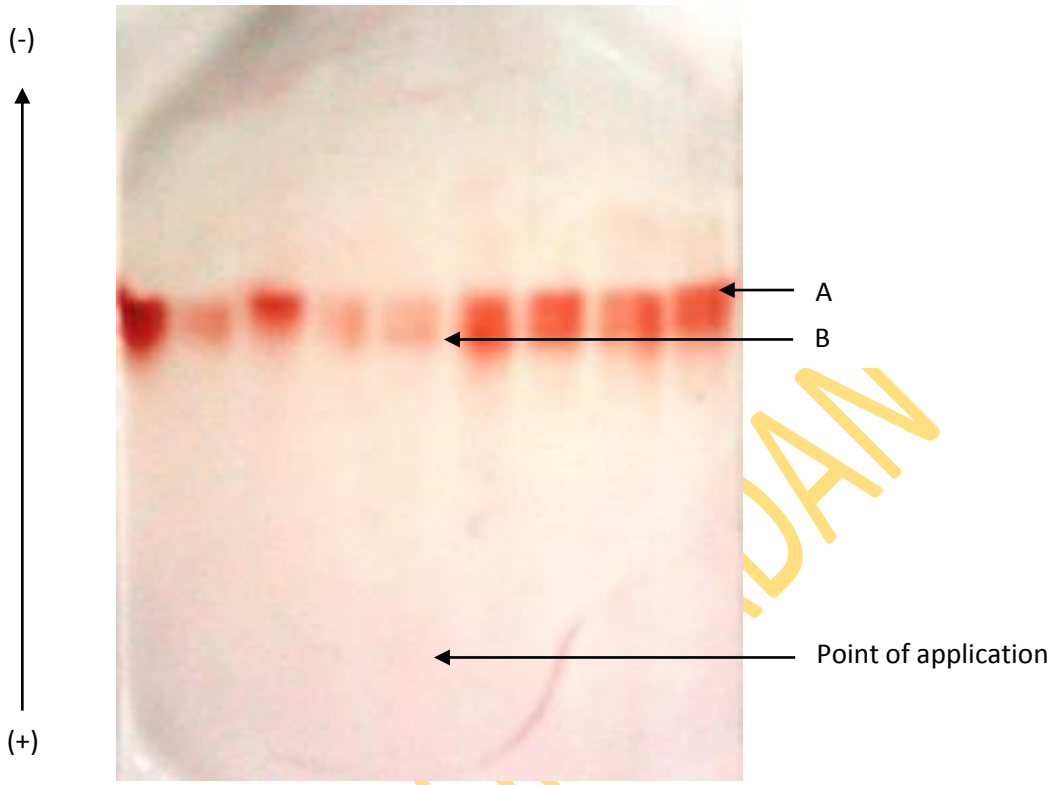
Once the plate had stained sufficiently to resolve the band, the stain was removed by washing and rinsing the gel plate several times with the specified destaining solution (Table 2) until sharp bands were visible. Plates were soaked in petri dishes containing the destaining solution until scored. The bands were scored visually based on their migratory pattern as described by RIKEN (2006).

3.1.3.8 Electrophoretic Conditions

The method used was as described by RIKEN (2006) with minor modifications to suit the samples used in this study.

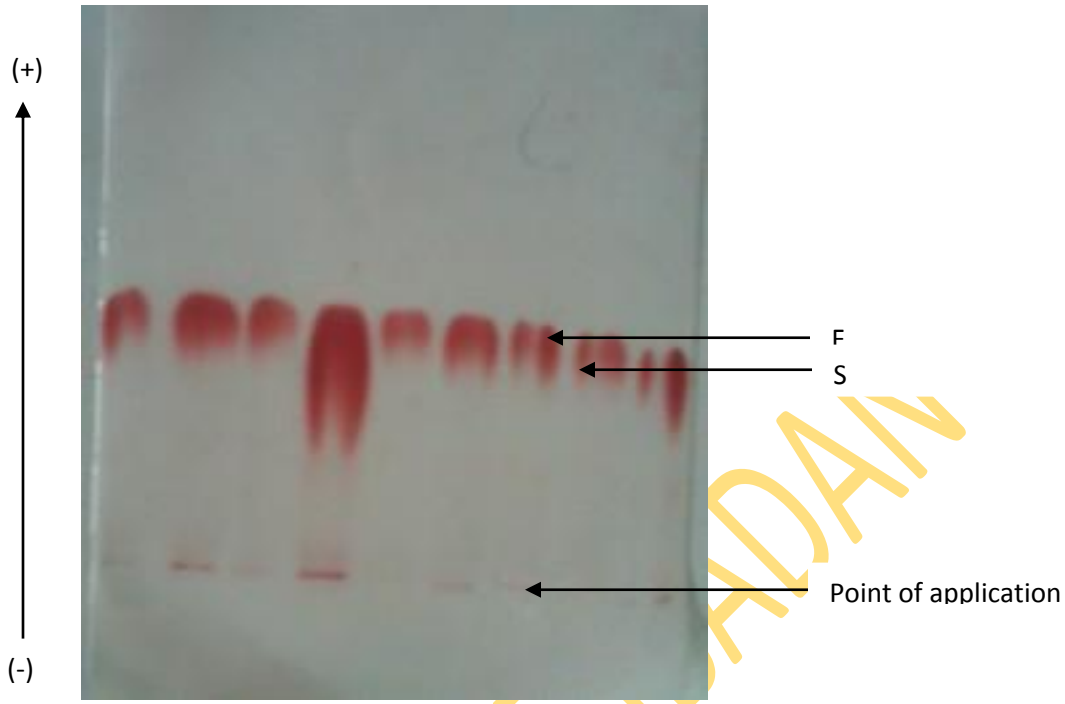
3.1.3.9 Band Scoring

Bands were scored visually as described by RIKEN (2006) according to the migration of the bands. Direct counting was used for calculating gene frequencies. Frequencies generated were used to compute genotypic frequencies.



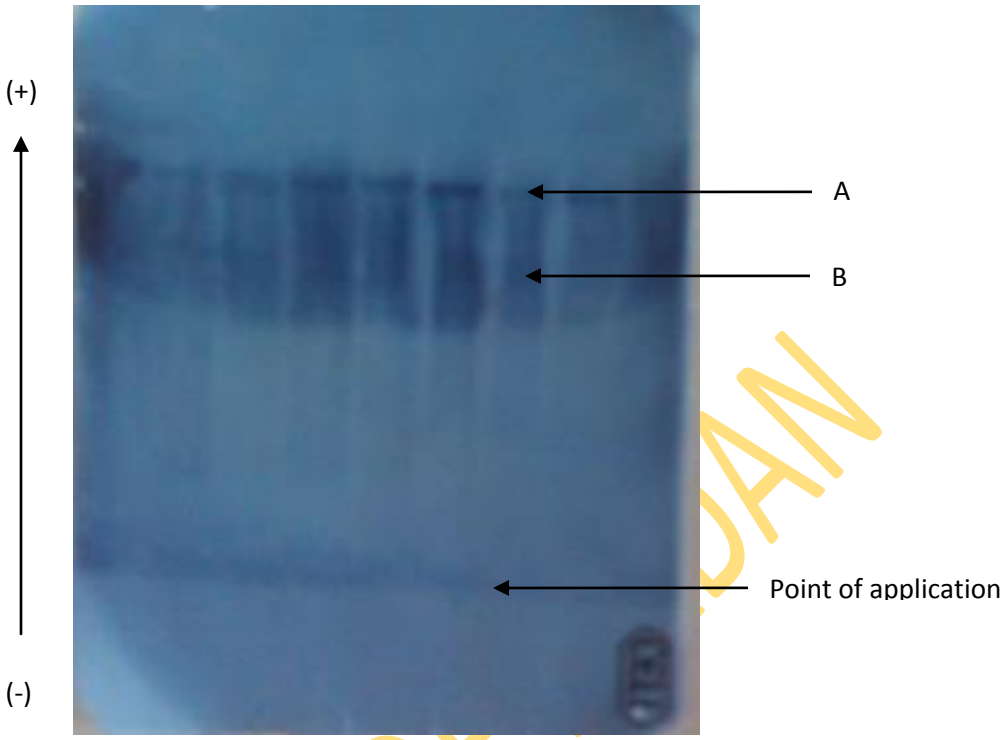
A and B are alleles scored based on the rate of migration

Plate 5: Electrophoregram Showing Migration of Haemoglobin on Cellulose Acetate Gel



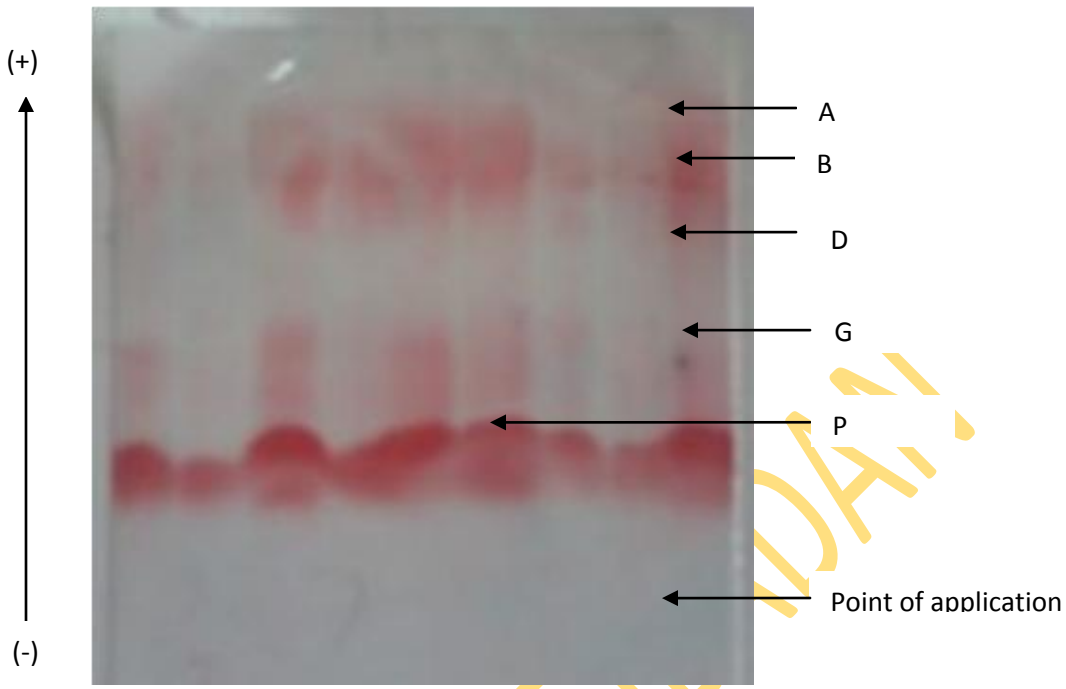
F (Fast) and S (Slow) are alleles scored based on the rate of migration

Plate 6: Electrophoregram Showing Migration of Carbonic Anhydrase on Cellulose Acetate Gel



A and B are alleles scored based on the rate of migration

Plate 7: Electrophoregram Showing Migration of Albumin on Cellulose Acetate Gel



A, B, D, G and P are alleles scored based on the rate of migration

Plate 8: Electrophoregram Showing Migration of Transferrin on Cellulose Acetate Gel

Table 2: Electrophoretic Conditions

Protein/Enzyme	Sample	Buffer	Time(mins)	ph	Voltage	Stain	Destain
Haemoglobin	RBCs in 8 volumes dH_2O 0.3μl	Tris EDTA borate	30	8.4	250	Ponceau S	5% acetic acid
Carbonic Anhydrase	RBCs in 4 volumes dH_2O 1.2μl	EDTA sodium acetate 1 : 4 dilution	35	5.6	220	Ponceau S	1% acetic acid
Transferrin	Plasma undiluted 0.6μl	Tris glycine	20	8.5	150	Ponceau S	5% acetic acid
Albumin	Plasma	Tris citrate	25	5.6	150	Aniline Blue	water

3.1.4 Statistical Analysis

Allelic variants or allozyme bands for each locus were marked in the order of increasing mobility, A being the allele with slowest mobility for Haemoglobin (Hb), Transferrin (Tf) and Albumin (Al) while for Carbonic anhydrase (Ca) S was the slow allele. Allele frequencies and genotypic frequencies for each locus in each sample were computed by direct counting and tested for fit to Hardy-Weinberg ratios using χ^2 goodness– of – fit test. The observed and expected heterozygosity (H_o and H_e, respectively) were calculated according to Nei (1973) with the correction for small samples (Levene, 1949). The genetic identity (I) and genetic distance (D) were calculated using Nei's (1978) formular. Subsequent UPGMA (Sneath and Sokal, 1973) was used in a cluster analysis to produce a dendogram showing the relationship between populations. The genetic distance estimates were used to construct dendogram based on individual locus and the pooled distances for the four loci studied. The genetic differentiation among populations and fixation indices F_{st}, F_{is}, and F_{it} were analysed according to Nei (1987) by Wright's (1978) F-statistics. All computations were performed using Popgene (Yeh *et al*., 1997) and Tools for Population Genetic Analyses (TFPGA; Miller, 1997).

3.2 STUDY 2: MICROSATELLITE VARIATIONS AMONG BALAMI, UDA, YANKASSA AND WEST AFRICAN DWARF SHEEP POPULATIONS OF NIGERIA

Selection programs in the past have mainly put emphasis on production traits, which led to an increased specialization for traits such as milk yield and quality, meat, wool, etc. This sometimes is achieved even by crossbreeding the local breeds with exotic ones, to generate populations with the desired phenotypes. This process has resulted in an increased reliance on a small number of exotic breeds to meet the local's food requirements, which could lead to the disappearance of local breeds. However, this aspect has received greater interest in the last few years, based on the awareness that indigenous and locally developed sheep breeds are an important asset, because of the unique combinations of adaptive traits developed to respond effectively to the pressures of the local environment (Buduram, 2004). From these considerations and given the importance of the local genetic resources, it is easily understandable why considerable interest is given nowadays to genetic diversity studies in domestic animals in general and, recently, in small ruminants (Baumung *et al.*, 2004). Genetic diversity studies in domestic animals aim at evaluating genetic variation within and across breeds, since the breed is the management unit for which factors such as inbreeding are controlled (Tapio *et al.*, 2005). However, the definition of a breed, as applied by FAO, frequently does reflect the underlying genetic population structure. Identification of private alleles whic can be used to estimate mutation rates in a population, contribute to indicators of gene flow, and can be related to the mean number of migrants exchanged per generation between populations is made possible during genetic diversity studies at microsatellite locus.

Wright's *F*–statistics and other similar indices that describe the partitioning of genetic variance at different hierarchical levels can be estimated for natural populations using a variety of molecular marker data (Nei, 1973). *F*–statistic values F_{st} and F_{it} are measures of deviation from Hardy Weinberg proportions and total populations, respectively; where 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. Therefore, a molecular genetics study of the population diversity and structure may improve the understanding of the actual genetic resources. Thirteen microsatellite markers were used to investigate the Nigerian sheep biodiversity within and among populations.

3.2.1 Study Area

Nigeria is divided into five grassland zones: Sahel, Sudan Savannah, Northern Guinea, Southern Guinea and the Derived Savannah. Nigeria occupies 923,768 km^2 (FOS, 1989) and is a country of marked ecological diversity and climatic contrasts. The overall physiography is described by Buchanan and Pugh (1955) and Udo (1970). The predominant soils are ferruginous tropical, with alluvial deposits along the Niger and Benue. Together with the Lake Chad Basin, these rivers constitute the major drainage basins of the country, with several important catchment areas, such as the Sokoto-Rima system in the north-west, and the Donga and Taraba in the extreme east. The other major topographical features are the high altitude grasslands of the Jos and Mambila Plateaux, which despite occupying a relatively small area, are of considerable significance to the livestock populations.

The climate is determined largely by the seasonal movement of the Inter-Tropical Convergence Zone (ITCZ) which leads to contrasting dry and wet seasons and a marked north-south rainfall gradient. Northern areas receive substantially less rainfall and have a much shorter wet season: mean annual rainfall is less than 500 mm in north-eastern Nigeria with only 2 months a year receiving above 100 mm. In the south-east, rainfall exceeds 4000 mm annually, with more than 100 mm falling during at least 9 months of the year. The rainfall patterns are modified, in the centre of the country, by the Jos Plateau to the south-west, precipitation rises to 1500–2000 mm annually; to the north-east, the highlands cast a rain-shadow which significantly reduces rainfall (Barbour *et al.*, 1982).

3.2.2 Animals and Management

The animals were semi intensively managed at the Small Ruminant Research section of the National Animal Production Research Institute, Shika Zaria and the University of Ibadan Teaching and Research Farm. Animals were allowed to graze in the morning and fed *Dussa*, a by-product of millet fermentation, in the evening. They were housed in cross ventilated pens and had free access to water and salt licks. The routine health management and system involves regular deworming and de-ticking (dip bath).

3.2.4 Sampling: Breed Recognition, Location and Sample Size

Documented morphological features described by Adu and Ngere (1979) were used as base line markers to ascribe sampled animals to a breed. Individuals that do not strictly conform to primary breed characters were excluded from the sample.

Animals were sampled from three different flocks at the National Animal Production Research Institute, (NAPRI) Shika, and Guga village, Zaria, Kaduna state, and the University of Ibadan Teaching and Research Farm. These locations were chosen purposely because of the availability of pure breeds of sheep in them. NAPRI was selected for the Northern breeds since the Institute and the surrounding villages has a large number of the Balami and Uda population that were identified and acquired from the local farmers. It also has a large flock of Yankassa which has been well established over time with records of ancestry. The University of Ibadan and its environ were chosen as representative of the southern breed (WAD) since it believed that genetic admixtures due to indiscriminate cross breeding with the aim to improve the size and body confirmation characteristics of the Dwarf sheep was not likely.

A total of one hundred indigenous (100), mixed sex sheep comprising twenty five of each of Yankassa, Balami, Uda and West African Dwarf sheep were sampled. The animals sampled were adult and unrelated.

Five (5) millilitres (ml) whole blood was collected from each animal by jugular vein puncture into 5ml vacutainer tubes containing Ethylenediamine Tetra Acetic acid (EDTA) as anti-coagulant. The blood samples were kept cold by placing them into ice packs and care was taken to prevent exposure to high temperature.

3.2.5 DNA Extraction and Purification

DNA was extracted from whole blood using MagneSil® KF, Genomic System kit (Promega, 2003) for automated system using the KingFisher®96 instrument (Thermo Scientific, 2006). The laboratory procedures were carried out at the Veterinary Genetics Laboratory of the University of Pretoria in South Africa.

The following procedure was followed:

1. Sample sheet was scanned into the database and printed out
2. Using a deep well plate the following were added
    a. 150µl whole blood (well mixed)
    b. 1000µl RBC Lysis solution
3. The mixture was sealed with a PCR foil seal and mixed on a shaker for 5 minutes at speed dial 1000
4. The mixture was then centrifuged at 3500rpm for 10 minutes
5. 1000µl of supernatant was removed from each well by pipetting
6. 1000µl RBC solution was then added to each well and sealed with a PCR foil seal and mixed on a shaker for 5 minutes at speed dial 1000
7. The mixture was then centrifuged at 3500rpm for 10 minutes
8. 1000µl of supernatant was removed from each well by pipetting
9. To the remaining liquid in each well, the following was added
    a. 150µl Lysis solution of promega MagneSil® KF, Genomic System
    b. 40µl Binding beads of promega MagneSil® KF, Genomic System (to ensure that the beads were well suspended, the mixture was vortexed)
10. 4 KingFisher plates were labeled as follows
    a. 1X Salt wash
    b. 2X Alcohol wash (1 and 2)
    c. 1X Elution plate,

d. Tray number was added onto the plate for storage purposes.

11. 200µl Salt wash solution was added to salt wash plate
12. 200µl Alcohol wash solution was added to each well of Alcohol wash plate 1 and 2
13. 50µl Elution mix (Nuclease free water) was added to the Elution plate
14. The OPVGL_Blood_V01 protocol was selected using the arrow keys and the START button was pressed to start the protocol
15. The plates were loaded according to protocol request as follows
    a. 1st is Tip comb + holder (deep well plate)
    b. 2nd is Elution mix
    c. 3rd is Alcohol wash 2
    d. 4th is Alcohol wash 1
    e. 5th is Salt wash
    f. 6th is Lysis plate
16. After loading each plate the START button was pressed to confirm that the required plate was loaded
17. The DNA purification protocol was initiated after loading the required plates and confirming the positions by pressing the START key
18. After purification process was completed, the plates were removed according to instructions shown on the instrument screen
19. The START button was pressed after each plate removal to confirm the action
20. The STOP button was pressed to complete the run after the last plate has been removed and the text End_of_run appeared on the screen.
21. The quantity of DNA yielded was determined using a Nano Drop® ND- 1000 Spectrophotometer.
22. Extracted DNA was stored at -20ºC until analysis in the Polymerase Chain reaction (PCR)

Plate 9: KingFisher 96 Well Genomic DNA Purification Instrument

Plate 10: ND- 1000 Spectrophotometer used for DNA Quantification

3.2.6 Characteristics of Selected Microsatellite Markers

Table 3: Characteristics of Selected Microsatellite Markers

Locus	Chromosome Number	Sequence (F-Forward and R- Reverse)	Flouorescent Label	Product size range (bp)
PLEX 1				
CSRD247	14	F:GGA CTT GCC AGA ACT CTG CAA T R:CAC TGT GGT TTG TAT TAG TCA GG	HEX	219 – 253
HSC		F:CTG CCA ATG CAG AGA CAC AAG A R:GTC TGT CTC CTG TCT TGT CAT C	6'FAM	268 – 302
INRA63	18	F:GAC CAC AAA GGG ATT TGC ACA AGC R:AAA CCA CAG AAA TGC TTG GAA G	6'FAM	174 – 208
MAF214	16	F:AAT GCA GGA GAT CTG AGG CAG GGA CG R:GGG TGA TCT TAG GGA GGT TTT GGA GG	TET	179 – 209
OARAE129	5	F:AAT CCA GTG TGT GAA AGA CTA ATC CAG R:GTA GAT CAA GAT ATA GAA TAT TTT TCA ACA CC	TET	144 – 180
OARCP49	12	F:CAG ACA CGG CTT AGC AAC TAA ACG C R:GTG GGG ATG AAT ATT CCT TCA TAA GG	HEX	89 – 133
OARFCB304	19	F:CCC TAG GAG CTT TCA ATA AAG AAT CGG R:CGC TGC TGT CAA CTG GGT CAG GG	HEX	157 – 197
PLEX 2				
BMS4008	1	F:GAA GAG TGT GAG GGA AAG ACT G R:CGG CCC TAA GTG ATA TGT TG	HEX	160 – 190
D5S2	5	F:TAC TCG TAG GGC AGG CTG CCT G R:GAG ACC TCA GGG TTG GTG ATC AG	6'FAM	194 – 210
OARFCB20	2	F:GGA AAA CCC CCA TAT ATA CCT ATA C R:AAA TGT GTT TAA GAT TCC ATA CAT GTG	6'FAM	100 – 130
MAF65	15	F:AAA GGC CAG AGT ATG CAA TTA GGA G R:CCA CTC CTC CTG AGA ATA TAA CAT G	TET	120 – 150
MCM527	5	F:GTC CAT TGC CTC AAA TCA ATT C R:AAA CCA CTT GAC TAC TCC CCA A	6'FAM	163 – 187
SPS113	10	F:AAA GTG ACA CAA CAG CTT CTC CAG R:AAC GAG TGT CCT AGT TTG GCT GTG	6'FAM	133 – 153

A total of 13 microsatellites were selected based on the degree of polymorphism and genome coverage as suggested by Barker *et al.* (2001). The selected microsatellites markers complied with the recommendations of the Food and Agricultural Organisation (FAO) and the International Society for Animal Genetics (ISAG) (FAO, 2011). As stipulated by the working group of FAO, microsatellite loci that can be used on several species such as cattle, sheep and goats are preferable. The markers were divided into two multiplexes based on product size and dye label (Table 10).

3.2.7 Polymerase Chain Reaction (PCR)

PCR reactions were prepared in two multiplexes (Table 10). This minimized the chances of non-specific amplification. The PCR amplification was performed in a total volume of 10µl for each multiplex. The amplification was performed using the Applied Biosystem StepOneplus^{TM} Real-Time PCR instrument (Plate 11). The amplification consisted of 15 minutes at 95ºC, 35 cycles of 45 seconds at 94ºC, 35 cycles of 45 seconds at 59ºC annealing temperature, 1 minute at 72ºC, and a final extension step at 72ºC for 60 minutes. An ovine control DNA sample was included in each PCR. The Ovine control DNA sample serves to indicate a problem with the PCR or with the sample DNA. It also allows for monitoring of the sizing accuracy since its sizing and labelling is known.

Table 4: PCR Conditions for Multiplex 1

DNA Mix		Quantity
DNA		100ng
Buffer 10X		1 µl
$MgCl_2$ (25mM)		1.2 µl
DNTPs (10mM)		0.4 µl
Taq Gold (5U/µl)		0.4 µl
Primers (10µM):		
	CSRD247	0.4+0.4 µl
	HSC	0.3+0.3 µl
	INRA63	0.2+0.2 µl
	MAF214	0.5+0.5 µl
	OARAE129	0.2+0.2 µl
	OARCP49	0.05+0.05 µl
	OARFCB304	0.3+0.3 µl
In a total volume of		10 µl

Table 5: PCR Condition for Multiplex 2

DNA Mix		Quantity
DNA		100ng
Buffer 10X		1 µl
$MgCl_2$ (25mM)		1.2 µl
DNTPs (10mM)		0.4 µl
Taq Gold (5U/µl)		0.4 µl
Primers (10µM):		
	D5S2	0.2+0.2 µl
	MAF65	0.4+0.4 µl
	MCM527	0.2+0.2 µl
	SPS113	0.1+0.1 µl
	OARFCB20	0.4+0.4 µl
	BMS4008	0.2+0.2 µl
In a total volume of		10 µl

Plate 11: Thermal cycler - Applied Biosystem StepOneplusTM Real-Time PCR

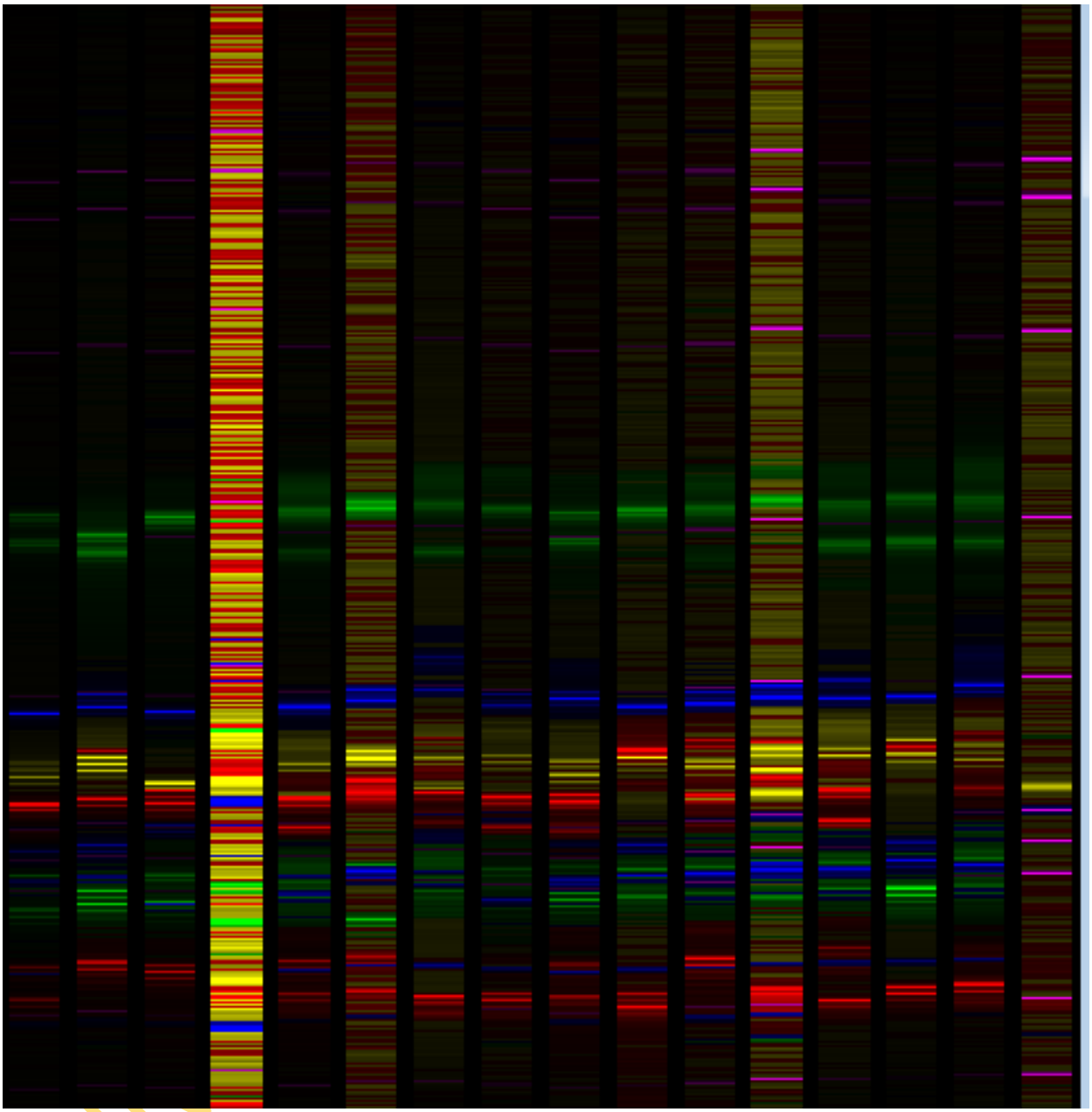


Plate 12: Sheep Virtual Gel Image showing migration lanes

3.2.8 Parameters Estimated

1. **Allele Frequency:** Allele frequency, or gene frequency, was calculated as the proportion of a particular allele (variant of a gene) among all allele copies being considered.

   If *f(AA)*, *f(Aa)* and *f(aa)* are the frequencies of the three genotypes at a locus with two alleles, then the frequency *p* of the *A*-allele and the frequency *q* of the *a*-allele were obtained by counting alleles. Because each homozygote *AA* consists only of *A*-alleles, and because half of the alleles of each heterozygote *Aa* are *A*-alleles, the total frequency *p* of *A*-alleles in the population is calculated as

   $$p = f(AA) + \frac{1}{2} f(Aa) = Frequency\ of\ A$$

   Similarly, the frequency *q* of the *a*-allele is given by

   $$q = f(aa) + \frac{1}{2} f(Aa) = Frequency\ of\ a$$

   Therefore:

   $$p + q = f(AA) + f(aa) + f(Aa) = 1$$

   and

   $$q = 1 - p\ and\ p = 1 - q$$

2. **Polymorphic Information Content:** The polymorphic informative content (*PIC*, Botstein *et al*., 1980) at both marker and population level was computed as

   $$PIC = 1 - \sum_{i} p_i^2 - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2p_i^2 p_j^2$$

   Where: *pi and pj* are the frequency of the alleles *i* and *j* of a given locus. The parameter PIC refers to the value of a marker for detecting polymorphism within a population, depending on the number of detectable alleles and the distribution of their frequency and has been proved to be a general measure of how

informative a marker is (Guo and Elston, 1999); the higher the PIC value, the more informative a marker.

3. **Hardy- Weinberg equilibrium:** Hardy-Weinberg principle is a model that relates allele frequencies to genotype frequencies. Like most models, Hardy-Weinberg is a simplification of real world complexities. Deviations from Hardy-Weinberg equilibrium (HWE) can indicate inbreeding, population stratification, and even problems in genotyping. Hardy-Weinberg equilibrium equation is given as $p^2 + 2pq + q^2 = 1$,

   Where p = the frequency of the dominant allele and

   q = the frequency of the recessive allele

   Tests of HWE was performed using χ^2 (Chi Square) goodness-of-fit test.

$$x^2 = \sum \frac{(O-E)^2}{E}$$

   Where x^2 = Chi Squared;

   O = Observed genotype frequency at a locus

   E = Expected genotype frequency at a locus

4. **Fixation Index:** The fixation index is a measure of how populations differ genetically. One derivation of the fixation index is F_{ST} This derivation measures the extent of genetic differentiation among subpopulations. The value of F_{ST} can theoretically range from 0.0 (no differentiation) to 1.0 (complete differentiation, in which subpopulations are fixed for different alleles).

   F_{ST} was measured as $F_{ST} = (H_T - H_S)/H_T$, in which H_T and H_S represent heterozygosity of the total population and of the subpopulation, respectively.

5. **Heterozygosity:** Heterozygosity is the percentage of heterozygotes in a population. Heterozygosity was estimated according to Nei (1987):

$$H1 = [2n/2n - 1][1 - iQ^{ml}(pL^2)]$$

Where: n = the number of individual sheep per population

ml= the number of alleles at locus 1

pL= the frequency of the I^{th} allele at locus 1

6. **Genetic Identity and Genetic Distance:** theoretically, the genetic distance between two populations is defined in terms of population gene frequencies for al loci in the genome. However, in practice we must estimate the genetic distance by sampling a certain number of individuals from the population and examining a certain number of loci.

   Genetic distance was calculated according to Nei (1978), unbiased standard genetic distance Ds:

   $Ds = (1 - J_{xy}) - ½\{(1 - J_x) + (1 - J_y)\}$

   $Ds = In\ (J_{xy}/\sqrt{J_x J_y})$

   Where: $J_x = (2n_x \Sigma x^2_i - 1) / (2n_x - 1)$

   $J_y = (2n_y \Sigma y^2_i - 1) / (2n_y - 1)$

   $J_{xy} = \Sigma\ xy$

   n = population size (number of individuals in the sample)

   $x_i y_i$ = allele frequencies for x^{th} allele in population x and y

7. **Phylogenetic Analysis:** A phylogeny is a tree that shows the evolutionary relationships among a group of organisms. There are many methods of constructing a phylogenetic tree from genetic distance data, but the neighbour-joining method was used in this study.
8. **Assignment Test:** The idea behind assignment tests is to use individual genotypes to assign individuals to populations or clusters. Given a set of populations, and the allele frequencies of those populations, what is the likelihood of a given individual's genotype in the population in which it was sampled versus its likelihood in the other populations in the set? An individual was assigned to the population for which it had the highest likelihood.
9. **Analysis of Molecular Variance:** Analysis of Molecular Variance (AMOVA) is a method of estimating population differentiation directly from molecular data

and testing hypotheses about such differentiation. A variety of molecular data – molecular marker data (for example, RFLP or AFLP), direct sequence data, or phylogenetic trees based on such molecular data – may be analyzed using this method (Excoffier, et al. 1992). The design and formulas used for the calculation of AMOVA for genotypic data with several groups of populations, within-individual level is as described by Excoffier *et al*.'s (2006).

10. **Principal Correspondence Analysis:** A method used for analysis of a set of variables, such as allele frequencies, by calculation of a new set of statistically independent coordinates that each correspond to a weighted combination of the original variables in such a way that each coordinate captures as much variation in the original variables as possible. In many data sets, a small number of coordinates may explain a large proportion of the initial variability, thus increasing efficiency. Plotting the distribution of individuals or breeds in a graph of the first two or three coordinates allows for simple visualization of the pattern of diversity. The coordinates potentially correspond to phylogeographic lines.
11. **Genetic Structure:** The population *structure* was investigated using multi-locus genotype data to. The structure programme uses include inferring the presence of distinct populations, assigning individuals to populations, studying hybrid zones, identifying migrants and admixed individuals, and estimating population allele frequencies in situations where many individuals are migrants or admixed.

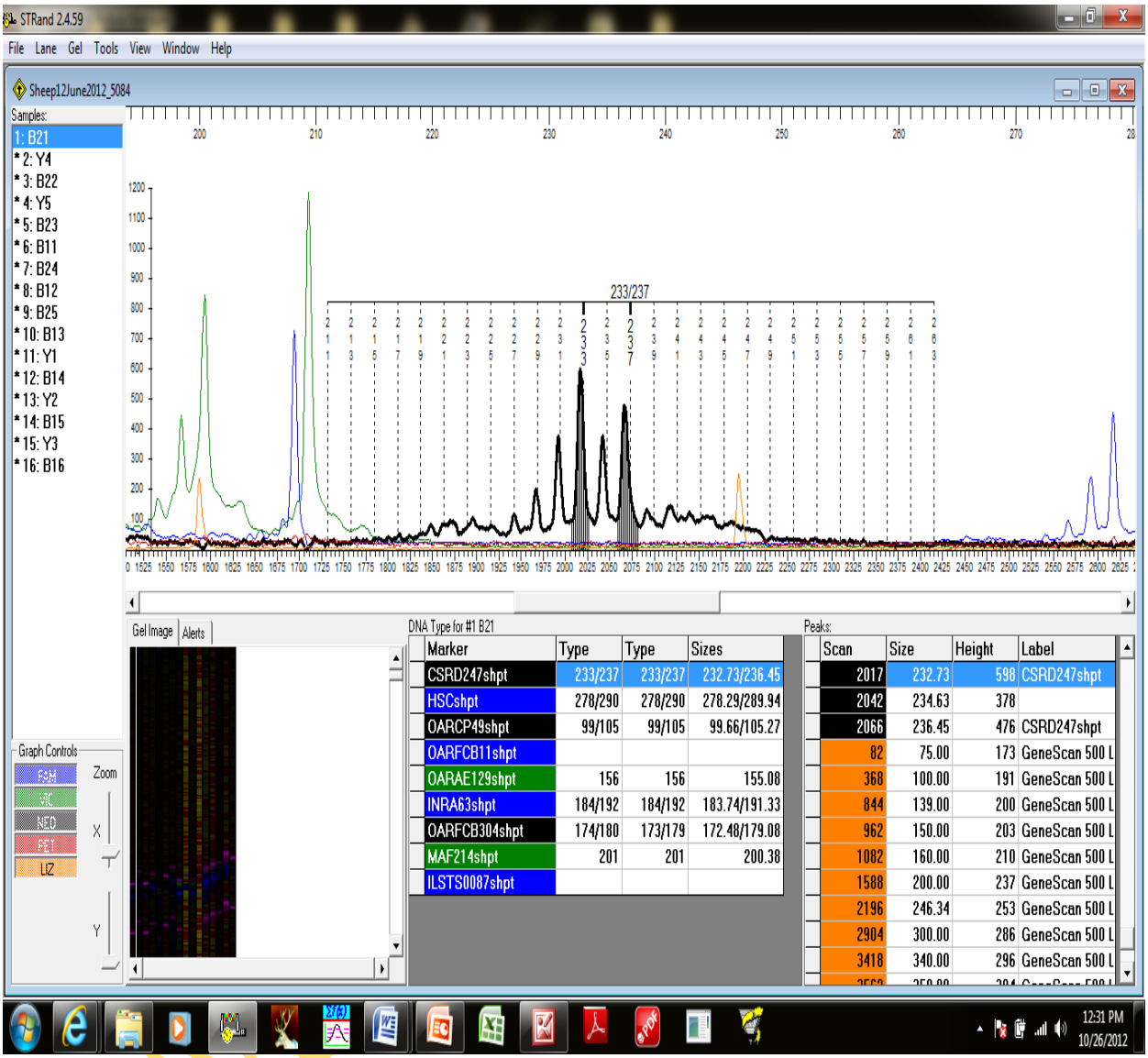


Plate 13: STRand Platform Showing Band Alignment from a Sheep Gel

3.2.9 Statistical Analysis: Software programmes and Analysis

1. **Allele Sizes:** STRand version 2.2.30 (Locke *et al.*, 2000) was used to determine allele sizes from the virtual gel image produced from the real time PCR.

2. **Data Editing:** Microsoft office excel spreadsheet (Office, 2007) was used to edit the data containing allele sizes before performing further analysis.

3. **Data Conversion:** the data was converted to applicable formats to perform statistical analysis with other software using the Programme CONVERT Version 1.31 (Glaubitz, 2005).

4. **Mean number of Alleles and Allele Frequency:** the programmes CERVUS version 3.0 (Marshall, 2007) and GENPOP version 4.1.3 (Rousset, 2008) were used to determine mean number of alleles and allele frequencies.

5. **Polymorphic Information Content, Hardy-Weinberg equilibrium, pair-wise Fst and gene flow:** the programme POPGENE version 1.31 (Yeh and Yong, 1999) was used to determine the Polymorphic Information Content (PIC), Hardy-Weinberg equilibrium (HWE) and whether there were any significant deviations from HWE, the genetic differentiation with the calculation of pair-wise F_{st} and gene flow (N_m) values and the genetic diversity within and between the breeds.

6. **Linkage disequilibrium**: Linkage disequilibrium was determined with GENPOP version 4.1.3 (Rousset, 2008).

7. **Genetic Structure:** The genetic structure of the populations was investigated using the software programme STRUCTURE (Pritchard *et al*., 2000). The software infers the number of populations into which the analysed genotypes can be divided, by estimating the natural logarithm of the probability that a given genotype (*G)* is part of a given population (*K)*: Ln Pr (G | *K*). In order to choose the appropriate number of inferred clusters (*K*), eleven independent runs were performed for each K, fitting *K* from 2 to 7 without prior information on breed of origin. All runs used a burn-in period of 10000 steps and followed by

100000 Markov Chain Monte Carlo (MCMC) iterations. The model used was based on an assumption of admixture and correlated allele frequencies as suggested by several authors (Pritchard *et al*., 2000; Vicente *et al*., 2008; Zuccaro *et al*., 2008).

8. **Factorial Correspondence Analysis (FCA):** GENETIX 4.05 (Belkhir *et al*., 2000) was used to run a correspondence analysis which was used to investigate further the differentiation of the breeds, taking into consideration a likely occurrence of admixture between some of the populations.

9. **Analysis of molecular variance (AMOVA)**: Analysis of molecular variance (AMOVA) considering groups and population as sources of variation was assessed by ARLEQUIN 3.0 (Excoffier *et al*., 2005).

10. **Phylogenetic Tree**: The PHYLIP 3.69 software programme (Felsentein, 2009) was used to build a phylogenetic tree based on Nei's genetic distance, using the neighbour-joining method (Saitou and Nei, 1987).

CHAPTER FOUR
RESULTS

4.1 STUDY 1: BLOOD PROTEIN POLYMORPHISM AND GENETIC VARIATION IN BALAMI, UDA YANKASSA AND WEST AFRICAN DWARF SHEEP POPULATIONS

4.1.1 Allele Frequency

The four studied loci were polymorphic in all breeds. Frequencies of observed alleles at the investigated loci are given in Table 6. Thirteen variants were found at 4 loci. The highest number of alleles occurred at the Tf locus (seven alleles) while two alleles were observed in the other loci. Except for the Tf locus which was very polymorphic, all the other alleles were present in all four breeds at different frequencies. The most frequent alleles were Hb^B for Balami, Uda, Yankassa and Hb^A for WAD; Ca^S and Al^A in Balami and Yankassa while Al^B was most frequent in Uda and WAD.

From twelve Transferrin alleles known in sheep breeds (Erhardt, 1986), only seven alleles were found in this present study. Six alleles were detected in each of the populations at the Tf locus. The Tf^B allele was most frequent in the Balami while the Tf^A was the most frequent in the Uda population. The Tf^A and Tf^C alleles had equal frequencies which was the highest value in the Yankassa population while the Tf^G was the most frequent in the WAD population. Tf^G was absent in the Balami population and present in all other breeds while Tf^P was present only in the Balami and absent in all other breeds.

Table 6: Allele Frequency for Polymorphic Loci

LOCUS	n	Allele	Observed no of alleles	POPULATIONS				
				BALAMI	**UDA**	**YANKASSA**	**WAD**	**Overall**
HB	25	A	91	0.2800	0.2800	0.4000	0.8600	0.4550
		B	109	0.7200	0.7200	0.6000	0.1400	0.5450
CA	25	F	39	0.0200	0.1600	0.1400	0.4600	0.1950
		S	161	0.9800	0.8400	0.8600	0.5400	0.8050
AL	25	A	101	0.7000	0.2400	0.8000	0.2800	0.5050
		B	99	0.3000	0.7600	0.2000	0.7200	0.4950
TF	25	A	42	0.1200	0.3000	0.3000	0.1200	0.2100
		B	35	0.3200	0.2200	0.0600	0.1000	0.1750
		C	41	0.2400	0.1400	0.3000	0.1400	0.2050
		D	24	0.0400	0.0400	0.2000	0.2000	0.1200
		E	40	0.2400	0.2800	0.1200	0.1600	0.2000
		G	16	-	0.0200	0.0200	0.2800	0.0800
		P	2	0.0400	-	-	-	0.1000

HB – Haemoglobin, CA – Carbonic Anhydrase, AL – Albumin, TF – Transferrin; A, B, C, D, E, F, G, P and S – alleles

4.1.2 Genotype Frequencies

The distribution of the genotypes and their frequencies are presented in Tables 7 and 8

4.1.2.1 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 population 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^{AB} 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}. Only the Yankassa had all three genotype present.

4.1.2.2 Carbonic Anhydrase

The Ca locus was polymorphic, having three genotypes controlled by two codominant alleles. The Ca^{FS} and Ca^{SS} genotypes occurred in the Balami, Yankassa and WAD population while the Uda alone had the Ca^{FF} genotype occurring with a frequency of 0.04. The frequency of Ca^{SS} was similar in the Balami, Uda and Yankassa, and ranged from 0.72 (Uda and Yankassa) to 0.96 (Balami), whereas the Ca^{FS} was most frequent (0.92) in the WAD.

4.1.2.3 Albumin

Three genotypes of albumin (Al^{AA}, Al^{AB} and Al^{BB}) determined by two codominant alleles were observed. The genotype heterozygous Al^{AB} had the highest value (0.60) among the Balami population while the homozygous Al^{BB} has the largest frequency (0.52) in the Uda population. The Homozygous Al^{AA} had the largest occurrence (0.60) in the Yankassa population while the heterozygous Al^{AB} and homozygous Al^{BB} had the same level of occurrence (0.48) in the WAD population. All three genotypes were observed in the WAD population while only the homozygous Al^{AA} and heterozygous

Al^{AB} were present in the Balami and Yankassa populations and the Uda population had only homozygous Al^{BB} and the heterozygous Al^{AB}.

4.1.2.4 Transferrin

The Tf locus was the most polymorphic with twenty-three genotypes (Tf^{AA}, Tf^{AB}, Tf^{BB}, Tf^{AC}, Tf^{BC}, Tf^{CC}, Tf^{AD}, Tf^{BD}, Tf^{CD}, Tf^{DD} ,Tf^{AE}, Tf^{BE}, Tf^{CE}, Tf^{DE}, Tf^{EE}, Tf^{AG}, Tf^{BG}, Tf^{CG}, Tf^{DG}, Tf^{EG}, Tf^{GG}, Tf^{AP}, Tf^{CP}) controlled by seven codominant alleles. In Balami population, the most common genotypes were Tf^{BB} and Tf^{BC} with a value of 0.12; the most common genotype in the Uda population was Tf^{AC} with a frequency of 0.24 (highest genotype frequency); in the Yankassa populations, the Tf^{AD}, Tf^{CD} and Tf^{DE} were the most frequent genotypes with the same value of 0.12 while in the WAD population, the genotypes Tf^{BG}, Tf^{CG} and Tf^{EG} were most frequent with a constant value of 0.12. Among all the breeds studied, the Tf^{AP}, Tf^{CC}, Tf^{CP}, Tf^{DG}, and Tf^{GG} genotypes were the rare genotypes. All other genotypes were observed with varying frequencies.

Table 7: Genotype Frequencies at the Haemoglobin, Carbonic Anhydrase and Albumin Loci in Balami, Uda, Yankassa and WAD Sheep Breeds

Locus	genotype	Balami	Uda	Yankassa	WAD	Total
Haemoglobin	AA	0	0	0.04	0.72	0.19
	AB	0.56	0.56	0.72	0.28	0.53
	BB	0.44	0.44	0.24	0	0.28
	Chi-square	3.467	3.467	5.773	0.558	0.405
	Probability	0.063	0.063	0.016	0.455	0.525
Carbonic Anhydrase	FF	0	0.04	0	0	0.01
	FS	0.04	0.24	0.28	0.92	0.37
	SS	0.96	0.72	0.72	0.08	0.62
	Chi-square	0.000	0.439	0.558	17.299	3.047
	Probability	1.000	0.508	0.455	0.000	0.081
Albumin	AA	0.4	0	0.6	0.04	0.26
	AB	0.6	0.48	0.4	0.48	0.49
	BB	0	0.52	0	0.48	0.25
	Chi-square	4.235	2.253	1.385	0.739	0.062
	Probability	0.040	0.133	0.239	0.390	0.803

WAD= West African Dwarf

Table 8: Genotype frequency at the Transferrin Locus in Balami, Uda, Yankassa and WAD Sheep Breeds

Locus	genotype	Balami	Uda	Yankassa	WAD	Total
	AA	0	0.04	0	0.04	0.02
	AB	0.04	0.08	0	0	0.03
	AC	0.08	0.24	0.4	0	0.18
	AD	0	0.08	0.12	0.04	0.06
	AE	0.08	0.12	0.04	0.04	0.07
	AG	0	0	0.04	0.08	0.03
	AP	0.04	0	0	0	0.01
	BB	0.12	0.04	0.04	0	0.05
	BC	0.12	0.04	0	0	0.04
	BD	0.04	0	0.04	0.04	0.03
	BE	0.2	0.2	0	0.04	0.11
	BG	0	0.04	0	0.12	0.04
Transferrin	CC	0	0	0	0.04	0.01
	CD	0.04	0	0.12	0.08	0.06
	CE	0.2	0	0.08	0	0.07
	CG	0	0	0	0.12	0.03
	CP	0.04	0	0	0	0.01
	DD	0	0	0	0.08	0.02
	DE	0	0	0.12	0.04	0.04
	DG	0	0	0	0.04	0.01
	EE	0	0.12	0	0.04	0.04
	EG	0	0	0	0.12	0.03
	GG	0	0	0	0.04	0.01
	Chi-square	11.512	20.870	33.963	12.120	27.012
	Probability	0.716	0.141	0.004	0.670	0.171

WAD= West African Dwarf

4.1.3 Heterozygosity

The observed heterozygosity (H_o) values were between 0.52 (52.0%) for Balami and Uda populations, and 0.61 (61.0%) for WAD population (Table 9). The average observed heterozygosity for all populations was 0.56 (56.0%). The value for expected heterozygosity (H_e) was observed in the Balami population to be lowest (0.4151) while the highest value was recorded for the WAD population (0.4982). The mean value of H_e for all the populations was 0.5357 and this was higher than the values recorded for each of the population in the current study. The most diverse population recorded is the WAD with expected heterozygosity value of 49.8%.

Table 9: Mean Number of Alleles, Mean Heterozygosity, Polymorphic Information Content and Deviations from Hardy-Weinberg's Equilibrium per Breed across the Allozyme Loci

Breed/Population	n	MNA*	Heterozygosity*		PIC	DHWE
			Ho	*He*		
Balami	25	3.00 (2.00)	0.5200 (0.35)	0.4151 (0.30)	0.750	0
Uda	25	3.00 (2.00)	0.5200 (0.23)	0.4588 (0.22)	1.000	0
Yankassa	25	3.00 (2.00)	0.5900 (0.31)	0.4598 (0.24)	1.000	2
WAD	25	3.00 (2.00)	0.6100 (0.29)	0.4982 (0.25)	1.000	1
Mean	100	3.25 (2.50)	0.5600 (0.21)	0.5357 (0.21)	1.000	

n= Sample size; MNA= Mean Number of Alleles; *Ho* =Observed Heterozygosity;

He= Expected Heterozygosity; PIC= Polymorphic Information Content; DHWE= no of loci with deviations from Hardy-Weinberg's Equilibrium per breed; WAD = West African Dwarf; *= Standard deviation in Parenthesis

4.1.4 Hardy-Weinberg's Equilibrium

Hardy-Weinberg equilibrium test for single locus was conducted for the populations within the four allozyme markers. Results shown in Table 10 revealed that there were some genotypes with loci that deviated significantly from HWE ($P<0.05$). The locus Hb and Tf with P-Values of 0.008 and 0.033 were found not to be in HWE for Yankassa, while there was a deviation at the locus Ca with P-Value of 0.000 for WAD population. There were no deviations from Hard-Weinberg's equilibrium in any of the studied loci's for Balami and Uda sheep populations.

Table 10: Chi-squared Probability for Hardy-Weinberg Equilibrium for Four Nigerian Sheep Population at four Allozyme Loci

Allozyme Marker	Populations				
	Balami	**Uda**	**Yankassa**	**WAD**	**Entire Data Set**
HB	0.131(0.010)	0.130(0.009)	0.033(0.004)*	1.000(0.000)	0.556(0.016)
CA	1.000(0.000)	0.487(0.016)	1.000(0.000)	0.000(0.000)***	0.112(0.010)
ALB	0.062(0.005)	0.274(0.020)	0.529(0.021)	0.625(0.015)	0.847(0.014)
TF	0.558(0.011)	0.078(0.005)	0.008(0.003)**	0.654(0.015)	0.083(0.006)

HB = Haemoglobin; CA = Carbonic anhydrase; ALB = Albumin; TF = Transferrin

Standard errors are giving in parenthesis * = P < 0.05; ** = P < 0.01; *** = P < 0.001

4.1.5 Genetic Identity and Genetic Distance

The results of genetic identity are presented in Table 11. The results indicate that the Yankassa and Balami populations are more genetically alike (0.9519) while the Balami and WAD populations were the least genetically identical (0.6436). The distance between populations ranged from 0.0493 to 0.4407. The smallest genetic distance was observed between Yankassa and Balami populations while the farthest distance was observed between WAD and Balami populations.

Table 11: Genetic identity and genetic distance among four Nigerian breeds of sheep

Population	Balami	Uda	Yankassa	WAD
Balami	****	0.8873	0.9519	0.6436
Uda	0.1196	****	0.8274	0.7627
Yankassa	0.0493	0.1894	****	0.6947
WAD	0.4407	0.2709	0.3642	****-

Genetic Identity (above Diagonal) and Genetic Distance (below Diagonal) among Nigerian Sheep Populations

4.1.6 Gene Flow and F- Statistics

Population differentiation examined by fixation indices F_{is}, F_{it} and F_{st} for each of the four loci studied across four Nigerian sheep population are shown in Table 12. The global heterozygosity deficit (F_{it}) was estimated at -0.0506 and the within-breed deficit in heterozygote (F_{is}) ranged between -0.4155 (Ca) to -0.0968 (Tf) with a total mean of -0.2478 for all loci. Global breed differentiation evaluated by F_{st}, was estimated at 0.1581 with a range of 0.0576 (Tf) to 0.2459 (Alb). The gene flow values for each of the four loci studied ranged from 0.7666 for Alb to 4.0919 for Tf. The mean gene flow over all loci was recorded at 1.3318.

Table 12: F-Statistics and Gene Flow for four allozyme markers in Nigerian Sheep

Locus	Sample Size	F_{is}	F_{it}	F_{st}	Nm*
HB	200	-0.3882	-0.0687	0.2302	0.8362
ALB	200	-0.2997	0.0199	0.2459	0.7666
TF	200	-0.0968	-0.0336	0.0576	4.0919
Mean	200	-0.2478	-0.0506	0.1581	1.3318

*Nm = Gene flow estimated from $F_{st} = 0.25(1- F_{st})/ F_{st}$.

4.1.7 Dendogram

Dendogram of genetic distance between four Nigerian indigenous sheep populations at the Albumin locus divided the four breeds into two distinct clusters of Uda – WAD at node 1 (genetic distance of 0.0021) and Balami – Yankassa at node 2 (with a distance of 0.0128) and the distance at node 3 = 0.4949 (Including all populations) (Figure 7).The topology of the dendogram of genetic distance obtained at the Carbonic anhydrase locus can be used to differentiate two sub clusters of Uda – Yankassa at node 1 (with a distance of 0.0004) and Balami at node 2 (with a distance of 0.0121 including Balami, Uda and Yankassa). The WAD was totally separated from the above two sub clusters at node 3 with a distance of 0.1840 including all four populations (Figure 8). When the Dendogram of genetic distance was viewed at the haemoglobin locus, the topology differentiated two sub clusters as in the Ca locus, but this time the sub clusters were Balami – Uda with a distance of 0.0000 at node 1 and Yankassa with a distance of 0.0238 at node 2 (including Balami Uda and Yankassa). Again the WAD was totally separated from the two sub clusters with a distance of 0.5802 at node 3 (Including all four populations) (Figure 9). The tree topology of the dendogram of genetic distance between Balami, Uda, Yankassa and WAD sheep populations at the Transferrin locus revealed two sub clusters with Balami – Uda at node 1 with a distance of 0.1258, and Yankassa at node 2 with a distance of 0.3045 (including Balami, Uda and Yankassa) with the WAD separated completely at node 3 with a distance of 0.4347 (including all four populations) (Figure 10).

The Phylogenetic tree of the genetic distances pooled for the four loci studied supports the genetic distance estimates where the Balami population is the most genetically distant from the WAD population. The Balami and Yankassa 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 11).

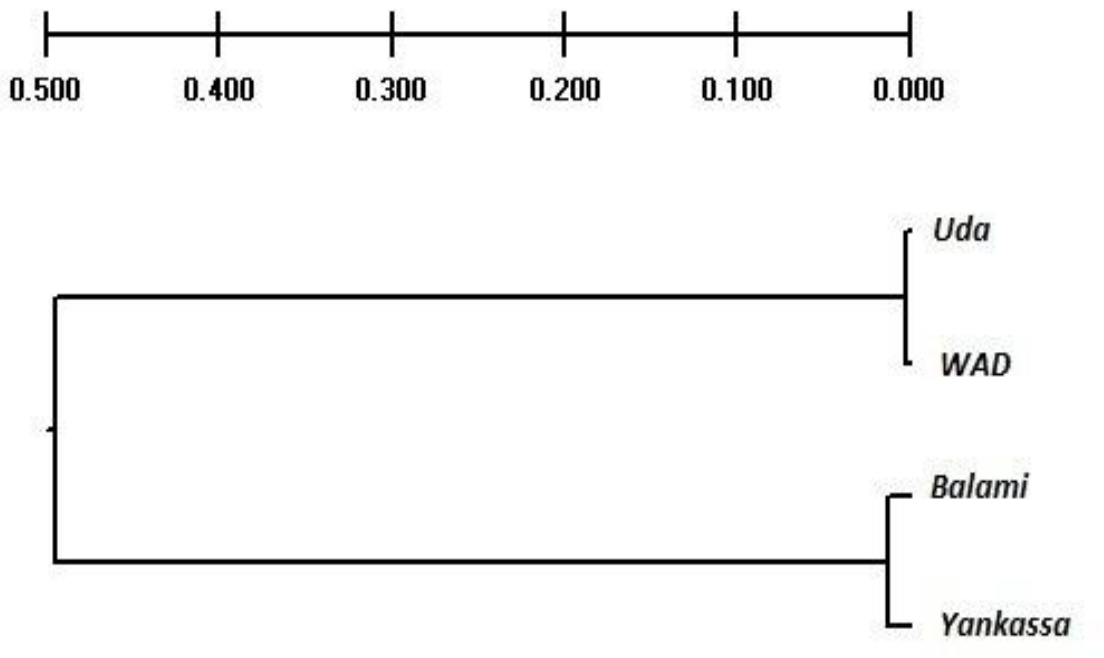


Figure 7: Dendogram of Genetic Distance among Four Nigerian Indigenous Sheep Populations at the Albumin Locus

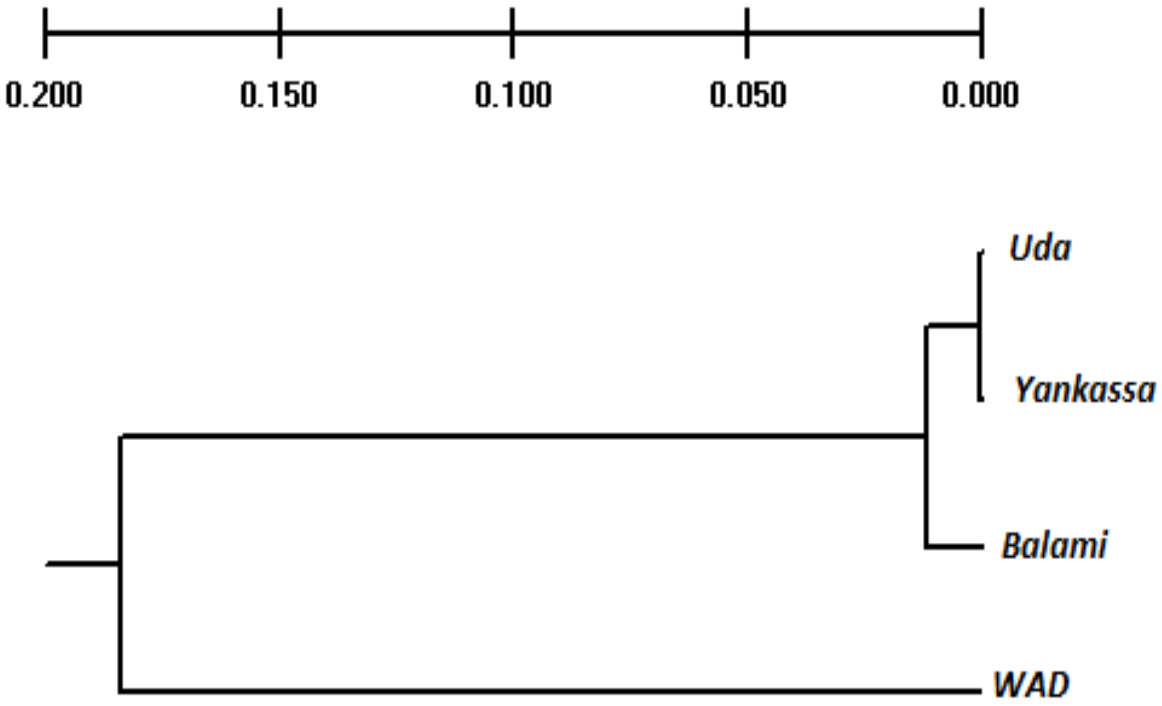


Figure 8: Dendogram of Genetic Distance among Four Nigerian Indigenous Sheep Populations at the Carbonic Anhydrase Locus

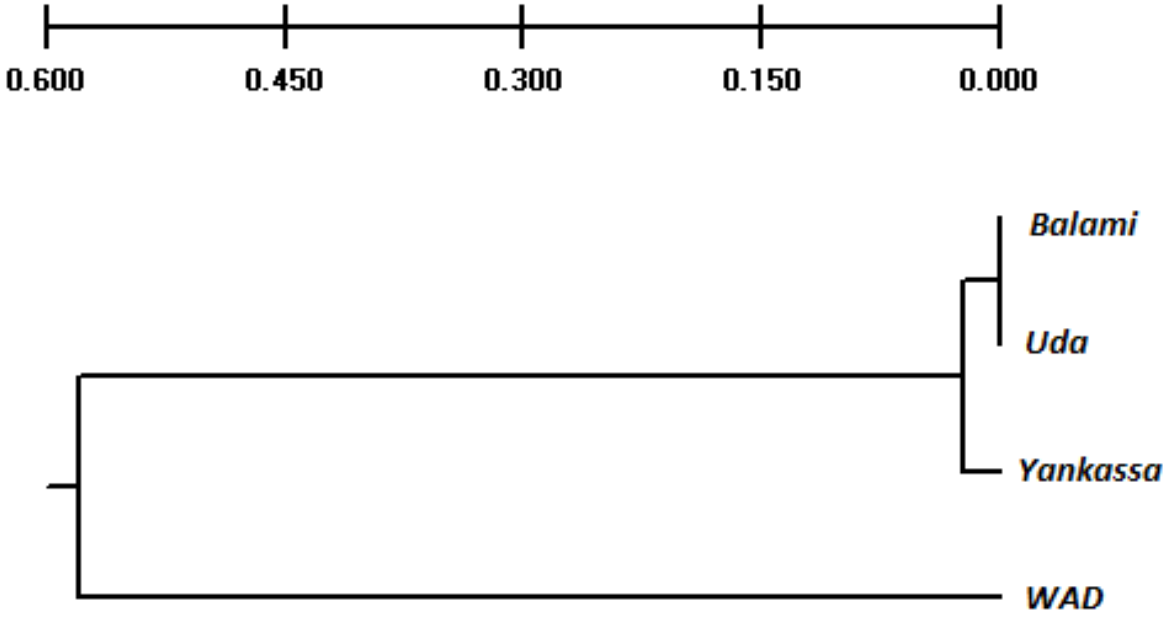


Figure 9: Dendogram of Genetic Distance among Four Nigerian Indigenous Sheep Populations at the Haemoglobin Locus

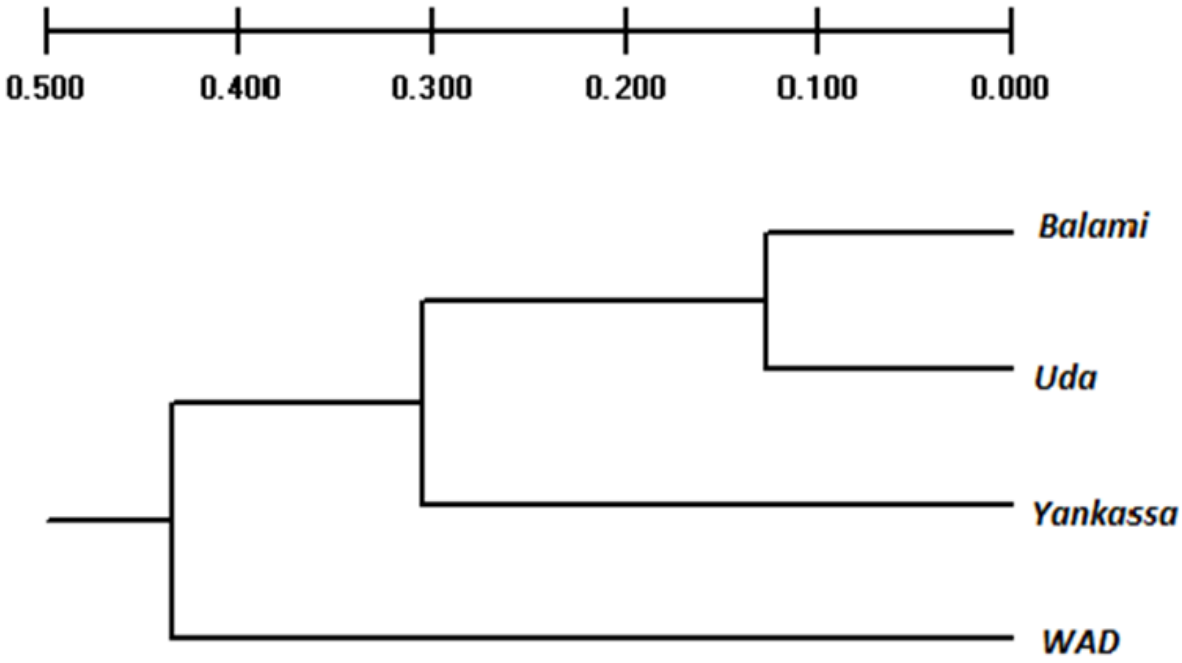


Figure 10: Dendogram of Genetic Distance among Four Nigerian Indigenous Sheep Populations at the Transferrin Locus

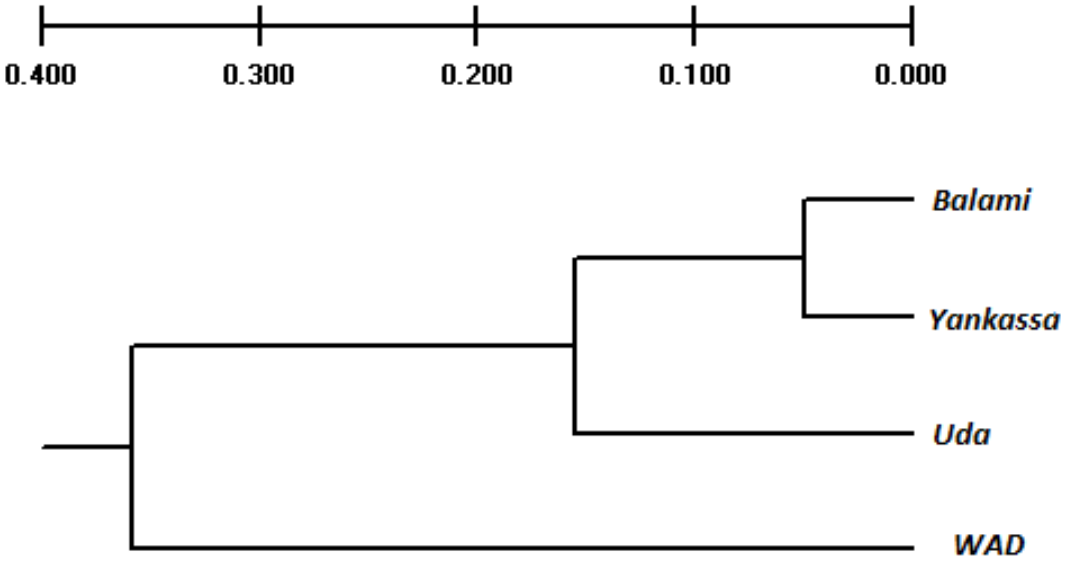


Figure 11: Dendogram of Genetic Distance among Four Nigerian Indigenous Sheep Populations Based on Four Allozyme Loci

4.2 STUDY 2: MICROSATELLITE VARIATIONS AMONG BALAMI, UDA, YANKASSA AND WEST AFRICAN DWARF SHEEP POPULATIONS OF NIGERIA

4.2.1 Characteristics of the Microsatellite Markers

The allelic frequencies in each population and marker are as shown in Tables 13 – 17. The total number of alleles found at the thirteen microsatellite loci studied in Nigerian indigenous sheep was 149. The mean number of alleles per locus was 11.46 with the lowest being 5 for MAF214 and the highest being 17 for OARCP49 (Table 13). Observed heterozygosity ranged from 0.260 for MAF214 to 0.802 for BMS4008. The number of unique alleles found for all the breed populations in all the loci were 45 (Tables 13 to 18).

4.2.2 Genetic Variability

Allele frequencies are summarized in Tables 13 - 17. Allele frequencies ranged from 0.0200 to 0.9090 for the studied microsatellites. Unique alleles were observed for each of the population studied and these are presented in Tables 13 - 18. In Balami population, 13 alleles (CSRD247- 2 alleles, HSC- 1 allele, OARE129- 2 alleles, OARCP49- 1 allele, OARFCB304- 2 alleles, BMS4008- 2 alleles, OARFCB20- 1 allele, and SPS113- 1 allele) were observed to be unique to the population with frequencies ranging from 0.0208 to 0.1000. Sixteen unique alleles (CSRD247- 1 allele, INRA63- 3 alleles, OARAE129- 1 allele, OARCP49- 3 alleles, OARFCB304- 2 alleles, BMS4008- 2 alleles, D5S2- 1 allele, OARFCB20- 1 allele, MAF65- 1 allele, MCM527- 1 allele) were observed in the Uda population with allele frequencies ranging from 0.0200 to 0.0600. Yankassa population had ten alleles (CSRD247- 3 alleles, OARCP49- 2 alleles, BMS4008- 2 alleles, MAF65- 2 alleles, SPS113- 1 allele) unique to the population with frequencies ranging from 0.0217 to 0.0652. West African Dwarf (WAD) sheep population had six unique alleles (CSRD247- 2 alleles, INRA63- 1 allele, MAF214- 1 allele, MAF65- 1 allele, SPS113- 1 allele) with frequencies ranging from 0.0263 to 0.0909. The presence of these unique alleles in the population can be a means of distinguishing between the populations.

Mean number of alleles (MNA) per population ranged from 5.85 in WAD to 8.54 in the Uda population (Table 18).

Table 13: Polymorphism at CSRD247 and HSC Loci in four Nigerian sheep breeds

Microsatellite/bp	Balami	Yankassa	Uda	WAD
CSRD247				
219				0.0294*
221		0.0227*		
223	0.0217		0.0400	
225		0.0227*		
227	0.0217		0.0600	0.0294
231				0.0588*
233	0.4783	0.4091	0.3400	0.7647
235	0.0217	0.1136	0.0200	0.0588
237	0.3696	0.3182	0.4400	0.0588
239	0.0217		0.0600	
241	0.0217*			
243	0.0217	0.0909	0.0200	
245			0.0200*	
251	0.0217*			
253		0.0227*		
HSC				
268	0.0217*			
270	0.0435	0.0682	0.0400	
274		0.0227	0.0400	0.1176
276	0.0435	0.0455	0.1200	0.1176
278	0.0870		0.0800	0.0294
280	0.1739	0.0682	0.1800	0.1765
282	0.1739	0.1136	0.1800	0.1417
284	0.1957	0.3409	0.1000	0.0294
286	0.0217		0.0400	0.0294
290	0.1522	0.0682	0.0400	
292		0.1818	0.0400	0.2647
294	0.0217		0.0200	
296	0.0217		0.0200	0.0294
298	0.0217	0.0227	0.0600	0.0294
300		0.0227	0.0200	0.0294
302	0.0217	0.0455	0.0200	

***Unique alleles

Table 14: Polymorphism at INRA63, MAF214 and OARAE129 Loci in four Nigerian sheep breeds

Microsatellite/bp	Balami	Yankassa	Uda	WAD
INRA63				
174			0.0200*	
176			0.0200*	
178		0.0870		0.3235
180	0.1739	0.0435	0.0400	0.1176
182				0.0588*
184	0.5000	0.1739	0.2200	0.2647
186	0.0217	0.0870	0.1200	0.1176
188		0.1304	0.0600	
190			0.0200*	
192	0.1739	0.4130	0.4200	0.1176
206	0.0652	0.0435	0.0400	
208	0.0652	0.0217	0.0400	
MAF214				
179	0.0455	0.0238		
197				0.0909*
199	0.1818	0.2619	0.3636	0.2727
201	0.6818	0.7143	0.6136	0.6364
209	0.909		0.0227	
OARAE129				
144	0.0250	0.0909	0.0909	
156	0.4750	0.3636	0.5909	0.3824
158	0.3500	0.5455	0.2727	0.6176
160	0.0250		0.0227	
166	0.0250*			
178	0.1000*			
180			0.0227*	

***Unique alleles

Table 15: Polymorphism at OARCP49 and OARFCB304 Loci in four Nigerian sheep breeds

Microsatellite/bp	Balami	Yankassa	Uda	WAD
OARCP49				
89	0.2083	0.1522	0.0600	0.0294
91		0.0217*		
93		0.0217*		
97	0.0417	0.0435	0.0200	
99	0.3125	0.2391	0.3400	0.4412
101	0.1250	0.0217	0.1800	0.0294
103	0.0208	0.0435	0.0200	
105	0.1875	0.1522	0.1600	0.2059
107		0.1957	0.0600	0.0294
109	0.0208	0.0217	0.0200	0.2059
111	0.0417*			
113	0.0208		0.0200	0.0588
115		0.0870	0.0200	
119			0.0200*	
123			0.0200*	
129			0.0200*	
133	0.0208		0.0400	
0ARFCB304				
157	0.0208	0.0870	0.1200	
171		0.0217		0.0313
173	0.3542	0.3696	0.3000	
175	0.0208	0.0435		
177			0.0600*	
179	0.3125	0.2174	0.2400	0.8125
181	0.1458	0.1087	0.0400	
183	0.0208*			
185	0.0417		0.0200	
187	0.0417	0.0870	0.0400	0.0938
189	0.0208*			
193	0.0208	0.0217	0.0200	
195		0.0435	0.1400	0.0625
197			0.0200*	

***Unique alleles

Table 16: Polymorphism at BMS4008, D5S2 and OARFCB20 Loci in four Nigerian sheep breeds

Microsatellite/bp	Balami	Yankassa	Uda	WAD
BMS4008				
160			0.0600*	
168	0.1739	0.1522	0.1400	0.4737
170		0.1739	0.0200	0.0789
172	0.0217	0.0870	0.0200	0.1053
174			0.0200*	
176	0.0435*			
178		0.0652*		
180	0.0870	0.0217	0.1000	0.0263
182	0.3261	0.3478	0.4800	0.2105
184	0.2391	0.1304	0.1600	0.1053
186		0.0217*		
188	0.0435*			
190	0.0652*			
D5S2				
196	0.5000	0.3913	0.3125	0.5526
198	0.1957	0.3261	0.2708	0.3158
200	0.1522	0.0652	0.1250	0.0789
208	0.1522	0.2174	0.2708	0.0526
210			0.0208*	
OARFCB20				
100	0.1957	0.0217	0.3958	0.0789
102	0.2391	0.1304	0.2083	0.1053
104	0.0652	0.1957	0.0833	0.3421
106	0.0217	0.1957	0.0625	0.0526
108			0.0208*	
110	0.0217*			
112	0.0652	0.0217	0.0208	0.0263
114	0.0652		0.0208	
116	0.0870	0.1957	0.0625	0.3158
118	0.0870			0.0789
120	0.1304	0.1087	0.0833	
128	0.0217	0.1087		
130		0.0217	0.0417	

***Unique alleles

Table 17: Polymorphism at MAF65, MCM527 and SPS113 Loci in four Nigerian sheep breeds

Microsatellite/bp	Balami	Yankassa	Uda	WAD
MAF65				
120			0.0208*	
122	0.1087			0.0263
126	0.1304		0.1042	0.0263
128		0.0217*		
130				0.0263*
132		0.0217	0.0208	
134	0.2391	0.1957	0.1250	0.1316
136	0.4565	0.4348	0.6458	0.2368
138	0.0217	0.1957	0.0208	0.1053
140		0.0870		0.1053
144		0.0217	0.0417	0.3421
148	0.0435		0.0208	
150		0.0217*		
MCM527				
163			0.0208*	
167	0.0217	0.0435	0.0417	
171	0.0870		0.0417	
173		0.0435	0.0208	0.0526
175	0.2174	0.2609	0.3333	0.0526
177	0.1087	0.0652	0.1042	0.1316
179	0.0870	0.0870	0.0208	0.3158
181	0.4348	0.4130	0.2500	0.4211
183		0.0870	0.0208	
187	0.0435		0.1458	0.0263
SPS113				
133	0.0217*			
135	0.1087	0.1304	0.0435	0.1316
137				0.0789*
143	0.6957	0.4348	0.7609	0.6053
145	0.0870	0.0652	0.0652	0.0263
147		0.0652*		
151	0.0652	0.0435	0.0870	0.0526
153	0.0217	0.2609	0.0435	0.1053

***Unique alleles

Table 18: Mean Number of Alleles (MNA), Mean Heterozygosity (Ho,He), Mean Polymorphic Information Content (PIC), Deviations from Hardy-Weinberg Equilibrium (DHWE) and Number of Unique Alleles (NUA) per Breed across the Microsatellite Loci

Breed/Population	N	MNA*	Heterozygosity*		PIC	NUA	DHWE
			H_o	H_e			
Balami	24	7.69(2.75)	0.6272(0.0281)	0.7159(0.0338)	0.6639	13	4
Uda	25	8.54(3.35)	0.6858(0.0262)	0.9631(0.0406)	0.6549	16	3
Yankassa	23	7.23(2.65)	0.6826(0.0271)	0.7400(0.0335)	0.6847	10	6
WAD	19	5.85(2.30)	0.6288(0.0321)	0.6478(0.0457)	0.5869	06	4
Total		11.38(4.03)	0.6596(0.0141)	0.7340(0.0336)	0.6476		

n= Number of samples used in this study. WAD = West African Dwarf

*= Standard deviation in Parenthesis

4.2.3 Heterozygosity

Heterozygosity values were calculated to determine the level of genetic variation within the populations (Table 18). The observed heterozygosity (H_o) values were between 0.6272 (62.7%) for Balami population and 0.6858 (68.6%) for Uda population. The average observed heterozygosity for all populations was 0.6596 (65.96%). The value for the expected heterozygosity (H_e) was observed in the WAD population to be lowest (0.6478) while the highest value was recorded for the Yankassa population (0.7400). The mean value for all the populations was 0.7340 and this was higher than the values recorded for the WAD population in the current study. The most diverse population recorded is the Yankassa with expected heterozygosity value of 74%.

4.2.4 Hardy-Weinberg's Equilibrium

Hardy-Weinberg equilibrium test for single locus was conducted for the populations using thirteen microsatellite markers. Results shown in Table 19 revealed that there were some genotypes with loci's that deviated significantly from HWE ($P<0.05$). The following markers were found not to be in HWE ($P<0.05$) for a specific population. For Balami CSRD247, MAF214, OARAE129 and OARCP49 with P-Values ranging from 0.000 to 0.036 had deviations from HWE. The locus OARFCB304, BMS4008, and MAF65 were found not to be in equilibrium ($P<0.05$) with P-Values ranging from 0.007 to 0.049 for Uda sheep population. The Yankassa population had CSRD247, HSC, INRA63, MAF214, OARAC129, and OARCP49 with P-Values ranging from 0.000 to 0.020 not in HWE while the West African Dwarf populations had CSRD247, MAF214, OARFCB304, and MCM527 with P-Values in the range of 0.000 to 0.0004 in disequilibrium.

Table 19: Chi-squared Probability for Test of Hardy-Weinberg Equilibrium at Thirteen microsatellite loci in Four Indigenous Sheep Populations

Microsatellite Marker	Nigerian Sheep Populations			
	Balami	**Uda**	**Yankassa**	**WAD**
CSRD247	0.036*	0.143	0.001**	0.004**
HSC	0.884	0.216	0.000***	0.970
INRA63	0.901	0.595	0.020*	0.081
MAF214	0.000***	0.315	0.004**	0.000***
OARAE129	0.000***	0.116	0.014*	0.708
OARCP49	0.002**	0.517	0.000***	0.990
OARFCB304	0.083	0.025*	0.557	0.000***
BMS4008	0.963	0.044*	0.247	0.088
D5S2	0.314	0.803	0.669	0.357
OARFCB20	0.159	0.387	0.894	0.153
MAF65	0.355	0.007**	0.672	0.492
MCM527	0.806	0.396	0.746	0.000***
SPS113	0.658	0.865	0.692	0.859

* = P < 0.05; ** = P < 0.01; *** = P < 0.001

Table 20: Mean Number of Alleles (MNA), Polymorphic Information Content (PIC), Allelic Richness (AR), Frequency of Null Alleles (F (Null)), Hardy-Weinberg Significance, Observed Heterozygosity and Expected Heterozygosity, in Four Nigerian Sheep Populations

Microsatellite Marker	MNA	PIC	AR	F(Null)	Significance of Hardy-Weinberg	Heterozygosity	
						H_o	H_e
CSRD247	7.50	0.617	5.648	-0.0339	0.9543(NS)	0.713	0.671
HSC	12.50	0.882	9.943	+0.0741	ND	0.770	0.897
INRA63	7.50	0.775	7.136	+0.0253	0.5000(NS)	0.761	0.805
MAF214	3.25	0.418	3.193	+0.3195	0.0000263(***)	0.250	0.487
OARAE129	4.00	0.512	3.712	+0.2679	0.00224(*)	0.346	0.599
OARCP49	10.50	0.812	8.395	+0.0714	0.1138(NS)	0.719	0.833
OARFCB304	8.25	0.747	7.242	+0.0388	0.3084(NS)	0.727	0.779
BMS4008	7.50	0.764	6.974	-0.0068	0.8810(NS)	0.800	0.794
D5S2	4.25	0.641	4.094	-0.0166	0.4868(NS)	0.719	0.697
OARFCB20	9.25	0.853	8.397	+0.0353	ND	0.809	0.872
MAF65	7.50	0.715	6.826	-0.0035	0.9509(NS)	0.742	0.743
MCM527	7.50	0.750	6.743	+0.0370	0.6090(NS)	0.719	0.781
SPS113	5.75	0.554	5.202	+0.0802	0.1260(NS)	0.500	0.582
Mean						0.658	0.734
St.Dev						0.178	0.121

F(Null) = Frequency of Null alleles; ND = Not Determined; NS = Not significant, *** = significant at P<0.001; * = Significant at P<0.01, AR= Allelic Richness

4.2.5 Genetic Identity and Genetic Distance

The genetic distance estimates of Nei (1972) were calculated using allele frequencies. The distance between populations ranged from 0.0930 to 0.3138 (Table 21). The smallest genetic distance was observed between Uda and Balami populations while the farthest distance was observed between WAD population and the Uda population.

The results of genetic identity (Table 21) indicates that the Uda and Balami populations are more genetically alike (0.9112) while the Uda and the WAD populations were the least genetically identical (0.7307).

4.2.6 Genetic Differentiation

Population differentiation examined by fixation indices F_{it}, F_{is} and F_{st} for each of the thirteen loci studied across four Nigerian sheep population are given in Table 22. The global heterozygosity deficit (F_{it}) was estimated at 0.1022 and the within-breed deficit in heterozygote as evaluated by F_{is} parameter ranged between -0.1317 (CSRD247) to 0.4983 (MAF214) having a total mean of 0.0481 for all loci. Global breed differentiation evaluated by F_{st} was estimated at 0.0568 with a range of 0.0184 (MAF214) to 0.1007 (OARFCB304). The gene flow values for each of the thirteen loci studied ranged from 2.2332 for OARFCB304 to 13.3303 for MAF214.

The F_{st} values (Table 23) determined for Nigerian sheep population ranged from the lowest genetically different populations 0.0150 (Balami and Uda) to the highest genetically different populations with values of 0.0994 (WAD and Uda). The result indicates that the WAD and Uda are the most genetically distant among the Nigerian sheep population.

Table 21: Genetic Identity and Distance between Nigerian Sheep Populations

Population	Balami	Uda	WAD	Yankassa
Balami	-	0.9112	0.7966	0.8731
Uda	0.0930	-	0.7307	0.8642
WAD	0.2274	0.3138	-	0.7963
Yankassa	0.1357	0.1459	0.2278	-

Nei's (1972) original measure of genetic identity and genetic distance

*Genetic Identity (above Diagonal) and **Genetic Distance (below Diagonal)

Table 22: F-Statistics and Gene Flow for All Loci's Studied

Locus	F_{is}	F_{it}	F_{st}	Nm*
CSRD247	-0.1317	-0.0421	0.0792	2.9053
HSC	0.1025	0.1402	0.0420	5.7090
INRA63	-0.0396	0.0399	0.0765	3.0184
MAF214	0.4983	0.5075	0.0184	13.3303
OARAE129	0.3704	0.4040	0.0534	4.4311
OARCP49	0.0924	0.1260	0.0370	6.5020
OARFCB304	0.0170	0.1160	0.1007	2.2332
BMS4008	-0.0577	-0.0025	0.0521	4.5446
D5S2	-0.0671	-0.0373	0.0280	8.6834
OARFCB20	0.0021	0.0688	0.0668	3.4909
MAF65	-0.0870	-0.0056	0.0749	3.0890
MCM527	0.0268	0.0717	0.0462	5.1671
SPS113	0.0880	0.1318	0.0480	4.9579
Mean	0.0481	0.1022	0.0568	4.1536

*Nm = Gene flow estimated from $F_{st} = 0.25(1- F_{st})/ F_{st}$.

Table 23: Pair Wise Fst and Gene Flow (Nm) Estimates for Four indigenous Sheep Populations

Population	Balami	Uda	WAD	Yankassa
Balami	-	16.62	3.51	9.58
Uda	0.0150	-	2.33	7.59
WAD	0.0682	0.0994	-	3.66
Yankassa	0.0256	0.0316	0.0640	-

Nm = (1- F_{st})/4 F_{st};

*Fst (below Diagonal) and **Gene Flow (Nm, above Diagonal)

4.2.7 Phylogenetic Analysis

The genetic distance estimates were used to construct the Phylogenetic tree (Figure 12). The Phylogenetic tree supports the genetic distance estimates where the Balami population is the most genetically distant from the WAD population. The Uda and Balami populations formed a different cluster, indicating a closer relationship between the two populations.

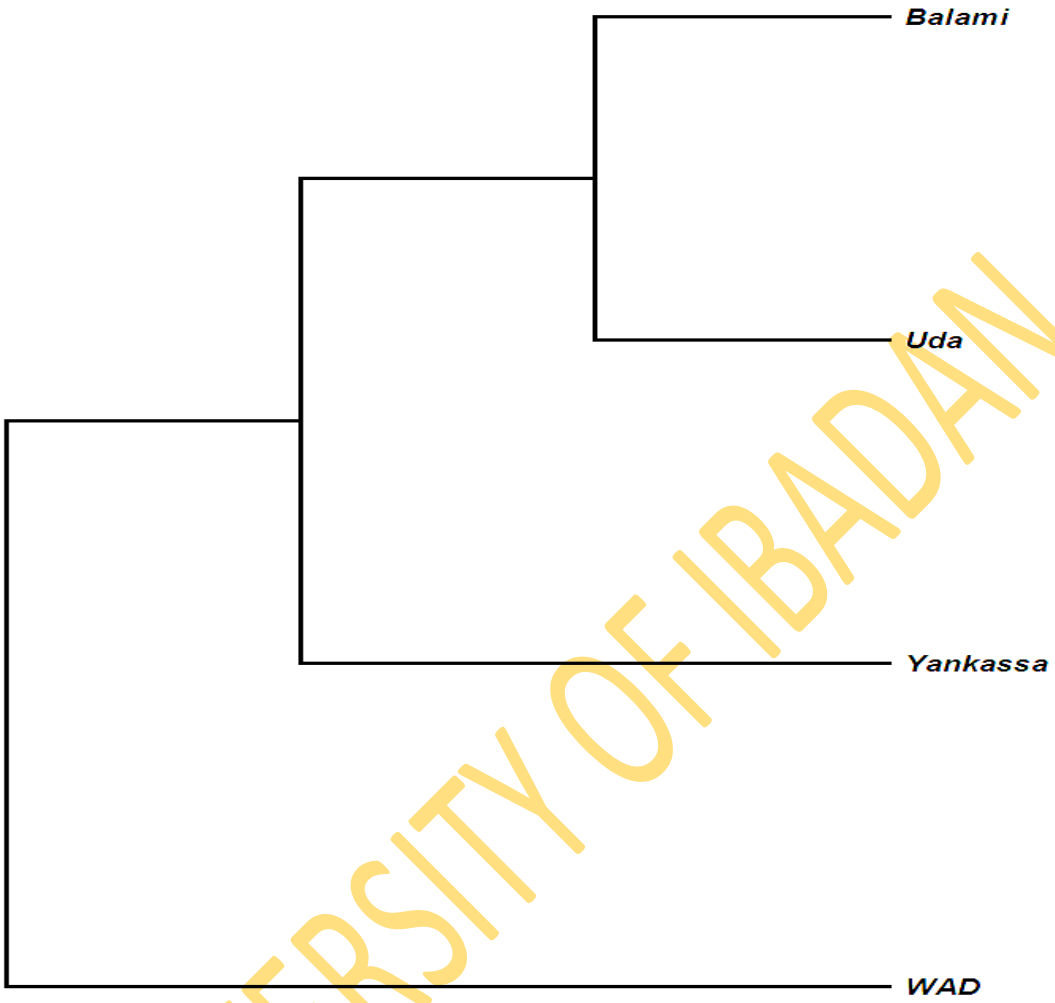


Figure 12: Dendogram of Genetic Distance between Four Nigerian Indigenous Sheep Populations

4.2.8 Assignment Test

Assignment test was performed using the program STRUCTURE with the number of expected population (k) ranging from 2 to 7. The Ln Pr(G/K) had the best value at K = 3 (-3848.3) with a variance of 359.7 . Therefore, it was assumed that K = 3 is the most likely number of clusters. In Table 23, the proportion of membership of each of the 4 sheep populations in the third cluster is presented. For K = 3, the following clusters were produced: Balami and Uda; WAD, and Yankassa. Individuals from the Yankassa breed were assigned to the cluster of Balami and Uda, while individuals from the Uda and Balami breeds were assigned to the Yankassa cluster.

Table 24: Proportion of Membership of Each Pre-defined Population in Each of the 3 Clusters Inferred in the Most Likely Run of the STRUCTURE Program

	Inferred Clusters			
Breeds	**1**	**2**	**3**	**N**
Balami	0.657	0.078	0.265	24
Uda	0.608	0.052	0.340	25
WAD	0.130	0.764	0.106	19
Yankassa	0.192	0.130	0.678	23

WAD = West African Dwarf; n = Number of animals per breed.

4.2.9 Analysis of Molecular Variance (AMOVA)

The AMOVA (Table 25) revealed that the percentage of variation among populations was 4.53% and within individuals was 92.91% while the remaining 2.56% was variation among individuals within populations.

Table 25: AMOVA Results of Nigerian Sheep Breeds Based on 13 Microsatellite Markers

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among populations	3	40.565	0.20192 Va	4.53
Among individuals within populations	87	379.770	0.11391 Vb	2.56
Within individuals	91	376.500	4.13736 Vc	92.91
Total	181	796.835	4.45318	

4.2.10 Factorial Correspondence Analysis (FCA)

The principal component analysis also referred to as the factorial correspondence analysis (FCA) was performed including all breeds and loci using corresponding allele frequencies (Figure 13). The Factorial Correspondence Analysis (FCA) was used to visualize the individuals in multidimensional space and to discover the relationships within and among the breeds. The first Axis explains 45.38% of the total variation and separates the WAD from the others; 32.01% of the total variation is explained by Axis 2 which separates the Yankassa breed; and 22.61% of the total variation is explained by Axis 3 which grouped the Balami and Uda together.

Individuals from the Balami breed were assigned to the Uda cluster, while individuals from the Yankassa breed were assigned to the Balami and Uda clusters (Figure 1).

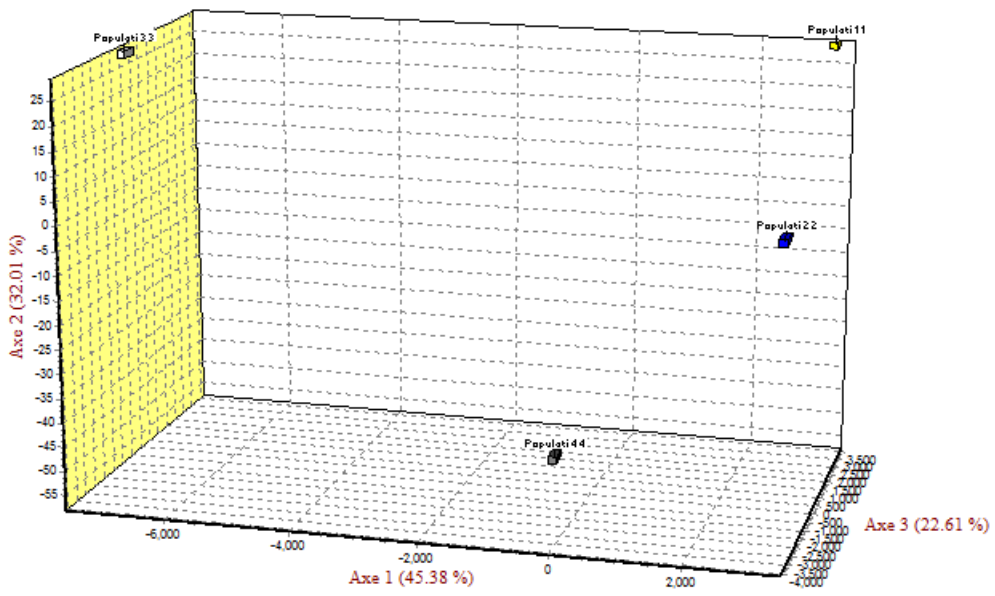


Figure 13: Spatial Representation of the Breeds as Defined by the Factorial Correspondence Analysis

Population 11 = Balami, Population 22 = Uda, Population 33 = West African Dwarf (WAD), Population 44 = Yankassa

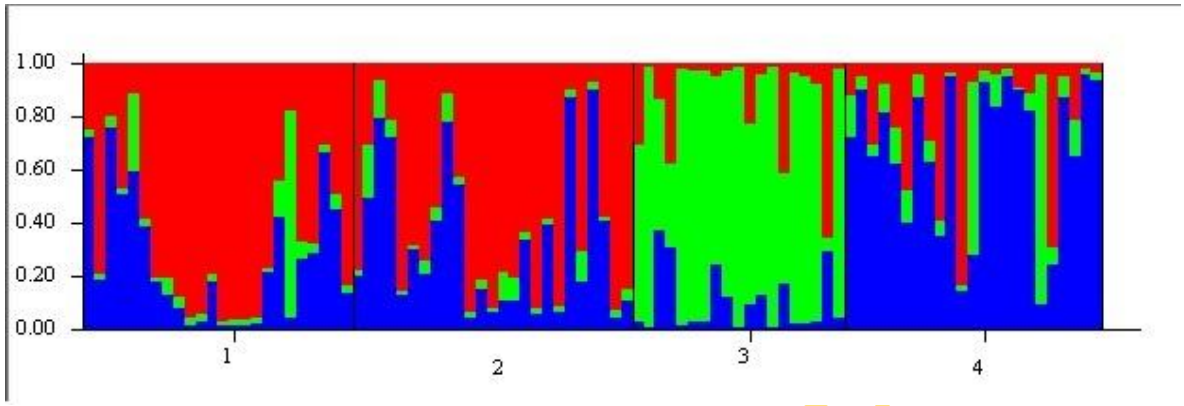


Figure 14: Structure Bar Plot Based of Similarity between Breeds

WAD = West African Dwarf Sheep

CHAPTER FIVE
DISCUSSIONS

5.1 STUDY 1: BLOOD PROTEIN POLYMORPHISM AND GENETIC VARIATION IN BALAMI, UDA YANKASSA AND WEST AFRICAN DWARF SHEEP POPULATIONS

5.1.1 Allele Frequency

5.1.1.1 Haemoglobin

All of the breeds in this study were polymorphic for Hb with frequencies of Hb^{B} considerably higher than those of Hb^{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^{B} is generally the most occurring allele in most sheep breeds. However, in contrast, the Hb^{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^{A} conferred on it for survival, while the Balami and Uda breeds may have need of Hb^{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^{B} while the other 40.0% were found to have Hb^{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^{A} as found in this study had been reported for other sheep populations. Buis and Tucker (1983) found that in some Dutch breeds (Friesian, Schoonebeker, Drente and Kempen), Hb^{A} was the more common allele compared to Hb^{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^A occur at higher frequency in the two breeds with Hb^A occurring

in 98.8% of the WAD population and 78.78% of the Yankassa population sampled. Schillhorn and Folaranmi (1978) reported that haemoglobin allele types have selective advantages in different geographical regions, while Hb^A has been shown to have advantage in sheep at higher altitudes; Hb^B occurs more commonly in lowland breeds. In Nigeria, Hb^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 fit. Animals with Hb^B have decreased haematocrit values, lower blood viscosity and higher availability of water compared to Hb^A types. This seems to be of adaptive significance in habitats characterized by the aridity of the climate such as the northern zone of Nigeria. Tsunoda *et al*. (2006) stated that Hb^A allele has a high affinity for oxygen and is important for survival in mountain areas at altitudes over 3000 m. this claim was buttressed by Ordas (2004) who reported that Hb^A has a higher affinity for molecular oxygen than Hb^B because of differences in oxygen dissociation rates. The higher availability of molecular oxygen in erythrocytes with Hb^A may be responsible for the higher incidence of parasitism. This may be due to the fact that the Hb^A erythrocytes may support parasite establishment and propagation more than those with Hb^B which have lower diffusible intra erythrocytic oxygen. Thus, Allonby (1976), Altaif and Dargie (1978,) and Buvanendram *et al.* (1981) reported a possible correlation between haemoglobin polymorphism and genetic resistance to helminth infection in sheep and goats. In the biological respect, the allele Hb^A is characterised by a great selection advantage in comparison with the allele Hb^B. In a great measure, the selective advantage of Hb^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 Kamenek, 1978).

5.1.1.2 Transferrin

The seven alleles present 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^{P} allele is a rare allele exclusively found in the Balami breed at very low frequency and the Tf^{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^{P} in Balami breed and the same was also reported for Sardi and Beni 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 report obtained in this study showed that highest frequency of Tf^{A} and Tf^{C} in Yankassa is supported by the report of Ibeagha-Awemu and Erhardt (2004) on the same breed, where Tf^{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^{B} Tf^{D} and Tf^{E} alleles in this study was also reported by Ibeagha-Awemu and Erhardt (2004). The occurrence of Tf^{G} 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^{G} and Tf^{P} 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 (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.

5.1.1.3 Carbonic Anhydrase

The four breeds in this current study were polymorphic at the Ca Iocus. The Ca^{s} was the most common in all of the breeds. A study by Ordas and Primitivo (1983) on Churra sheep breeds was compared with the current investigation. They discovered a new allele (Ca^{M}) at the red cell carbonic anhydrase locus in Churra sheep. They found the Ca^{S} allele to be present in all of the breeds studied (Churra, Lacha and Manchega), and this allele was fixed (monotype) in most of the breeds.

Mila Savic *et al.* (2000) reported the predominance of Ca^{S} allele (0.98) in Yugoslavia Tsigai sheep. Sargent *et al.* (1999) reported the presence of Ca^{B} allele in all of the breeds studied and speculates that the latter allele may be the same as the Ca^{S} allele reported by other authors.

Bius and Tucker (1983) and Zanotti *et al*. (1990) reported that Ca^{S} is generally the most occurring allele in sheep breeds. However, Ibeagha-Awemu and Erhardt (2004) reported Ca^{S} as monomorphioc in Yankassa, Uda, Mbororo and West African Dwarf sheep breeds and in Merino, East Friesian Milk and German Grey Heath sheep breeds. The monomorpic occurrence of Ca^{S} was contradicted by the report of Ca^{M} and Ca^{S} in similar breeds (Missohou, 1999). Akinyemi and Salako (2012) also reported Ca^{S} as the most frequent allele in Balami, Uda and Yankassa sheep breed while adding that data on the polymorphism of this blood enzyme and the physiological advantages it confers is however scarce.

5.1.1.4 Albumin

All samples tested were polymorphic at the Alb locus. This however was not the case in the report of Mwacharo *et al.* (2002) who reported that Albumin was monomorphic for Alb^{S} allele in all the fat tailed sheep studied. The authors however recorded polymorphism in the Merino sheep with the frequency of Alb^{F} being very low (0.0063). The allele Alb^{A} in this current study is speculated to be the same as the Alb^{S} while Alb^{B} is most likely the same as the Alb^{F} allele reported in Mwacharo *et al.* (2002).

Mila Savic *et al.* (2000) reported that no albumin polymorphism in Tsigai sheep has been observed so far and so all samples tested were identified as Alb^S phenotypes, which commonly occurs in other sheep breeds. However, Mila Savic *et al.* (2000) mentioned that the Alb^F is most often found in native Norwegian sheep. The observed polymorphism in this current study may be due to the electroperentic membrane used. In this study, cellulose acetate electrophoresis was used while in the report of Mila Savic *et al.* (2000), blood protein separation was carried out by Horizontal electrophoresis and in Mwacharo *et al.* (2002), blood typing was done using horizontal Starch gel electrophoresis.

In a study of 19 sheep breeds from Southern Africa, Sargent *et al.* (1999) reported Alb^B as being fixed in all the breeds' stidied, except the South African meat Merino and the Van Rooy breeds. The authors also noted that the frequencies of the Alb^A alleles were low (0.019 and 0.006, respectively) in the two breeds that were reported to be polymorphic at the Alb system. The result from this present study however, does not agree with most of previous authors as the four breeds in this study were polymorphic and had frequencies ranging from 0.24 to 0.80 for Alb^A and 0.20 to 0.76 for Alb^B. The overall allele frequency at the Alb locus were 0.5050 for Alb^A and 0.4950 for Alb^B. It will be difficult to compare the results of this present study with other reports as data on cellulose acetate electrophoresis for this locus is scarce.

5.1.2 Heterozygosity

Genetic diversity can be measured as the amount of actual or potential heterozygosity and diversity within the breed is measured as the estimate of heterozygosity. Heterozygosity coefficients which are measures of genetic diversity were similar in the breeds studied. This is an indication of the similar mean number of allele occurring in each breed (3.0±2.0). The values were within the range of 0.3 - 0.8 in a population (Takezaki and Nei, 1996) recommended for markers to be useful for genetic variation. These values indicate that there is sufficient genetic variability within the populations studied. The values observed in this study were in the range of values reported by Kowalska and Zaton-Dobrowolska (2008), who reported heterozygosity coefficients in

the range of 0.591 and 0.703 with an overall value of 0.655. This result was however higher than those reported by other authors (Akinyemi and Salako, 2012; Shahrbabak *et al.*, 2010; Mwacharo *et al.*, 2002; Clarke *et al.*, 1989). The comparison of average observed and expected heterozygosity values did not show significant differences ($P<0.05$) between and within breeds. However, all breeds showed numerically smaller expected than observed heterozygosities.

5.1.3 Hardy-Weinberg's Equilibrium

The significant deviations from HWE ($P< 0.05$, $P<0.01$, and $P<0.001$) observed for some of the loci within the breeds 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 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 various 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.

5.1.4 Gene Flow and F- Statistics

The negative values of F_{IT} observed at 3 of the four loci in the four breeds studied and the overall negative value of -0.0506 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. A large part of the total genetic diversity can be explained by the variation within breeds (0.8419) and to a smaller extent by the variation among breeds (0.1581).

This result indicates that genetic diversity quantified by allozyme markers shows little genetic differentiation among Nigerian sheep breeds studied.

5.1.5 Genetic Distance, Genetic Identity and Dendogram

Nei (1972) standard genetic distance (D) obtained in this study ranged between 0.0493 and 0.4407 which indicates little genetic differentiation between the breeds. According to Nei (1976) the D values for local breeds is between 0.000 and 0.058. 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 genetic distance between Spanish dairy sheep breeds in the range of 0.0094 and 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 - 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. Boujenane *et al*. (2008) reported a genetic distance range of 0.006-0.026 among six Moroccan local sheep, namely – D'man, Beni Ahsen, Sardi, Timahdite, Beni Guil and Boujaad. Distances obtained in this study between breeds were higher than those by Akinyemi and Salako (2012) who reported a range of 0.003 – 0.015. The Balami seemed closer to Yankassa than Uda in this study. This was supported by the results of Akinyami and Salako (2012).

The Phylogenetic tree constructed separated the WAD from other indigenous Nigerian sheep, suggesting either early prehistoric separation of the WAD sheep or separate historical origin. The close genetic relationship between the Balami and Yankassa breed may be attributed to possible interbreeding between these two populations which are predominantly white in colour to form a homogenous population separated by no physical geographic 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 (0.4407), 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 is expected to produce vigour (positive heterosis) in the hybrid.

5.2 STUDY 2: MICROSATELLITE VARIATIONS AMONG BALAMI, UDA, YANKASSA AND WEST AFRICAN DWARF SHEEP POPULATIONS OF NIGERIA

5.2.1 Allele Polymorphism

The number of alleles observed at a locus is an indication of the genetic variability at that locus. The Food and Agricultural Organization (FAO, 1998) have recommended that for genetic diversity studies microsatellite loci should have not less than four alleles. The total number of alleles per locus in this study ranged from 5 (MAF214) to 17 (OARCP49), with a mean value of 11.46, indicating that all the microsatellite loci's studied were sufficiently polymorphic and are appropriate for analysis of genetic diversity. The value observed in this study was smaller than 17.9 recorded for four Romanian sheep breeds with 11 microsatellite markers (Kevorkian *et al.,* 2010), 15.4 in Italian native sheep breeds with 19 markers (Dalvit *et al.,* 2009), 19.9 in European sheep with 23 markers (Handley *et al.,* 2007) and 12.33 alleles recorded for North American thinhorn sheep (*Ovis dalli)* population (Worley *et al.,* 2004) while Baumung *et al.* (2006) reported an average of 15 alleles per locus when 25 microsatellite markers were surveyed in 717 animals of 11 Austrian sheep breeds. Values observed in this study were higher than 8.6 alleles observed in each of the population studied with 10 markers in four Chinese sheep populations (Chen, *et al.,* 2011), 6.65 reported for Bhutanese sheep breeds (Dorji *et al.,* 2010), 9.68 recorded for Gentile di Puglia sheep breeds with 19 markers (d'Angelo *et al.,* 2008). Glowatzki-Mullis *et al.* (2007) analyzed 10 diverse breeds with a 19- microsatellite multiplex and found an average maximum of 7.79 alleles for Red Engadin sheep and Adebambo *et al.* (2003) reported a range of 2.88 to 11.5 alleles for Nigerian sheep breeds and their crosses with 30 bovine microsatellite markers. Although estimates of average number of alleles will vary with the specific markers employed and possibly with the accuracy of the genotyping system used, the results of this current study indicate that the Nigerian breed displays relatively high levels of genetic diversity.

5.2.2 Unique/Private Alleles

Grigaliunaite *et al*. (2003) showed that when a unique allele has a frequency below 0.1 it might be an allele that is present in several populations at low frequency and could be found also in other breeds, if greater fraction of the total population would be screened. A high number (45) of private alleles were observed in Nigerian sheep breeds but only one allele in Balami population had frequency equal to 10% (Marker OARAE129 at allele 178). In the study of desert big horn, Gustavo *et al.* (2000) reported alleles unique to single populations (MAF65-121 in Castle Dome, OarFCB128-112 in San Ysidro, MAF65-133 and MAF209-111 in old Dad and OarFCB128-118, MAF48-134, and MAF65-119 in Alberta). In Bhutanese and foreign sheep, Dorji *et al.* (2010) reported 11 and 16 unique alleles, respectively. Peter (2005), in the study of 57 European and Middle Eastern sheep breeds, showed the presence of 2 private alleles in Bardhoka, only one in Ruda and none in Shkodrane. In this current study, though there were many private alleles, only one was informative while all other private allele data obtained were not informative.

5.2.3 Hardy-Weinberg Equilibrium

The majority of the markers were highly polymorphic and generally in Hardy–Weinberg's equilibrium (HWE) with 3 to 6 markers deviating from HWE for all the breeds in the study. The significant deviations from HWE ($P< 0.05$, $P<0.01$, and $P<0.001$) observed for some of the loci within the breeds could be attributed to unobserved null alleles, excess of heterozygote individuals than homozygote individuals, migration, high mutation rate in microsatellite and artificial selection in the breeds (Aminafshar *et al*., 2008). These differences may also be attributed to some level of non-random mating due to preferential use of specific rams. Deviations from HWE at microsatellites loci have also been reported in various studies such as in Jalauni breed (Arora *et al*., 2008), Pag Island sheep breed (Ivankovic et al., 2005), Turkish sheep breeds (Soysal *et al*., 2005), West African Djallonke breed (Wafula *et al*., 2005), sheep breeds of Northern Spain (Alvarez *et al*., 2004), Baltic sheep breeds (Grigaliunaite *et al*., 2003), Sasi sheep (Rendo *et al*., 2003), and Uruguayan Corriedale (Tomasco *et al*., 2002). Despite these deviations, there seems to be no major issue with

any of the 13 microsatellite markers, taken individually, to preclude their potential use for adequate genotyping of Nigerian sheep populations. The issue remains that of selecting those that provide the highest information content for individual identification and parentage testing.

5.2.4 Gene Diversity

Takezaki and Nei (1996) have determined that gene diversity should be in the range of 0.3 to 0.8 in a population in order for markers to be useful for measuring genetic variation. Gene diversity for each breed ranged from 0.6478 to 0.7400, with an average of 0.7340. This confirmed that the markers used in this study were appropriate for measuring genetic variation. The values of gene diversity estimates were comparable with values detected for Baltik (0.57 – 0.76, Grigaliunaite *et al*., 2003), Austrian (0.67 – 0.78, Baumung *et al*., 2006), Bulgarian (0.73 – 0.80, Kusza *et al*., 2010), Romanian (0.67 – 0.79, Kevorkian *et al*., 2010), Italian native breeds (0.70 – 0.80, Dalvit *et al*., 2009). These gene diversity estimates are smaller than those detected for Egyptian sheep breeds (0.81 – 0.86, El Nahas *et al*., 2010), Iranian sheep breeds (0.83, Nanekarani *et al*., 2010), but higher than values found for Indian sheep breeds (0.59-0.65, Mukesh *et al*., 2006), Slovak Tsigai populations (0.46 – 0.61, Kusza *et al*., 2009), and West Balkan Pramenka sheep types (0.739 – 0.830, Cinkulov *et al*., 2008).

5.2.5 Heterozygosity Estimate

The average direct count of heterozygosity over all loci in the population was less than the expected heterozygosity. The observed heterozygosity (H_o) values varied between 0.250 (MAF214) to 0.800 (BMS4008), while the expected values (H_e) ranged from 0.487 (MAF214) to 0.897 (HSC). The mean values were 0.658 ± 0.178 and 0.734 ± 0.121 for H_o and H_e, respectively. When viewed in relation with figures for other studies, the observed heterozygosity of 0.658 in Nigerian sheep was lower than that for Santa Ines Sheep (Souza *et al.,* 2012). The expected heterozygosity value of 0.734 for this current study was comparable with that of Nilagiri (Girish *et al*., 2007), Swiss sheep breeds (Stahlberger-Saitbekova *et al*., 2001), Austrian sheep breeds (Baumung *et al*., 2006), Spanish breeds (Alvarez *et al*., 2004), Alpine sheep breeds (Dalvit *et al*., 2008), and Sarda sheep flocks (Pariset *et al*., 2003) and higher than that reported for

Afshari sheep breed of Iran (Qanbari *et al.*, 2007) but lower than both Madras Red and Mecheri (Prema *et al*., 2008; Selvam *et al*., 2009). The comparison of average observed and expected heterozygosity values did not show great differences between breeds. All breeds showed smaller observed than expected heterozygosities. Estimates of observed heterozygosity confirm the high level of diversity evidenced in the Nigerian sheep breeds. High value of average expected heterozygosity (0.734) within the breed could be attributed to the large allele numbers detected in the tested loci (Kalinwski, 2002).

Several factors can contribute to less than expected heterozygosity in a population. One reason might be inbreeding, i.e. mating between relatives. In case of inbreeding, the deficit affects all or most of the loci in a similar way. Other factors that can also cause a deficit of heterozygotes in the population might be the presence of "null alleles" (non-amplifying alleles). This may cause a false observation of homozygotes excess. Peter *et al*. (2005) have indicated the presence of null alleles in locus OarAe129. Finally, the presence of population substructure within the breed may lead to a Wahlund effect.

The high values of heterozygosities and allelic richness obtained in this study confirm that native breeds of sheep represent an important reservoir of genetic diversity, even though the level of differentiation among closely located breeds is small. This is in accordance with the prediction of (Handley *et al*., 2007) of a higher within-breed diversity and lower genetic differentiation in southern than in northern European sheep breeds. Peter *et al*. (2007) observed a higher genetic diversity of Middle Eastern, Turkish, Greek, Albanian and Romanian sheep breeds compared with northwestern European breeds.

5.2.6 Polymorphic Information Content

The polymorphic information content (PIC) is a parameter indicative of the degree of informativeness of a marker. All markers in this study had values higher than 0.5, indicating that they are highly informative. Only MAF214 had a PIC value away from 0.5 (0.4181). This further confirms that the set of microsatellite markers were effective for genetic diversity estimation in Nigerian sheep breeds. The values of PIC reported in

this study were comparable with values of 0.7223 to 0.8385 detected for 4 sheep populations with 10 microsatellites (Chen, *et al*,. 2011), 0.63 – 0.87 for Uruguayan sheep population with 10 markers (Ivanna *et al.,* 2002), 0.67 in Afshari sheep of Iran (Qanbari *et al.,* 2007), 0.65 for fat tailed-sheep in southern region of Jordan (Al-Atiyat *et al.,* 2012), 0.543 to 0.929 with an average of 0.775 for sheep breeds from Southern peninsular and Eastern region of India (Arora *et al.,* 2011).

5.2.7 Genetic Differentiation

The positive values of F_{it} (between populations and between the 13 loci in the four breeds) and F_{is} showed the deficiency of heterozygotes in the populations and that mates were more related in comparison with the average relationship of the population. This observed deficiency of heterozygotes could also be due to non–random sampling (Selective breeding).

The mean F_{is} value observed was 0.0481 indicating the homozygous/inbreeding status of the population. F_{is} estimates reported in most literature indicates various levels of inbreeding, such as 0.19 in Sarda sheep (Pariset *et al*., 2003), 0.066 in Spanish breeds (Alvarez *et al*., 2004), and 0.033 in Turkish sheep breeds (Soysal *et al*., 2005). Among the Indian sheep breeds, Arora and Bhatia (2004) reported a mean F_{is} value of 0.058 in Muzzafarnagri indicating a very low rate of inbreeding in that population. However, a high rate of inbreeding was reported in Nali and Chokla (0.397 and 0.299, respectively; Sodhi *et al*., 2005) and Magra (0.159; Arora and Bhatia, 2006) sheep breeds.

According to Hartl (1980), pair wise F_{st} values up to 0.05 is indicative for moderate differentiation between populations. The pair wise F_{st} values reported in this current study between all pairs of the tested breed ranged from 0.0150 between Uda and Balami to 0.0994 between Uda and WAD. The gene flow between populations ranged from 2.33 (between WAD and Uda) to 16.63 (between Balami and Uda) with an average estimate of 4.15. The WAD breed showed the highest values of Pair wise F_{st} and lowest estimated values of gene flow with other breeds in the study, indicating that this population has maintained an important genetic isolation from all other breeds. This isolation may be due largely to the distance created by geography between the WAD

which is predominantly a Southern breed and the others which are predominantly northern breeds. The estimated F_{st} value, which corresponds to the proportion of genetic variability accounted for by the differences among breeds, was 0.0568. Thus, a large part of the total genetic diversity can be explained by the variation within breeds (0.9432) and to a smaller extent by the variation among breeds (0.0568). This result indicates that genetic diversity quantified by microsatellite markers shows moderate differentiation among Nigerian sheep breeds. The degree of differentiation observed between the Nigerian sheep breeds could be due to the amount of gene flow among them. These estimates of genetic differentiation compared with those from other genetic diversity studies, e.g. 18.3% for Indian sheep (Mukesh *et al*., 2006), 13.3% Slovak Tcigai populations (Kusza *et al*., 2009), 8.2% Romanian breeds (Kevorkian *et al*., 2010), 8.3% Bulgarian breeds (Kusza *et al*., 2009), 8% Austrian (Baumung *et al*., 2006), 6.1% Six Indian Sheep Breeds, (Arora *et al*., 2010), 5.7% for Alpine sheep (Dalvit *et al*., 2008) and European and Middle-Eastern breeds including the Albanian sheep breeds (Peter *et al*., 2007), 5% for Pramenka types (Cinkulov *et al*., 2008), 4.6% for Ethiopian sheep (Gizaw *et al*., 2007), 3.7% in three Egyptian sheep (El Nahas *et al*., 2010) and Manchega sheep (Calvo *et al*., 2006). The results were higher compared to those reported by Nanekarani *et al*. (2010) for pelt sheep breeds of Iran (0.018). Low F_{st} value were also reported for Portuguese native sheep (0.29, Calvo *et al*., 2006) and northern Spanish breeds (F = 0.029, Rendo *et al*., 2004).

5.2.8 Genetic Distance Estimation

The analysis of Nei genetic distance indicated that the four Nigerian sheep breeds are considerably distinct. The distance between breeds ranged from 0.0930 (Balami and Uda) to 0.3138 (Uda and WAD). The Yankassa and WAD (0.2278) and the Balami and WAD (0.2274) had similar distance values indicating that they are equally close to the WAD breed. The WAD is a Southern dwarf breed, adapted to the deciduous rainforest zones; the Yankassa breed is widely adapted and as such distributed around the middle-belt of Nigeria with pockets of its populations isolated in the guinea and derived savannah zones while the Balami and Uda are predominantly located in the core North where they are adapted phenotypically for long distance treks in the hot savannah zone

of the country. The genetic distance estimates reported in this current study were lower than values reported in an earlier study of Nigerian sheep breeds. Adebambo *et al.* (2003) reported that the genetic distance was 0.432, 0.534 and 0.665 between the West African Dwarf (WAD) and the Yankassa, WAD and Uda and WAD and Balami, respectively. The differences in the reported values of genetic distance may be due to the difference in the microsatellite markers used in both studies.

5.2.9 Cluster Analysis

The unrooted neighbour-joining dendogram showed two clear clusters in accordance with the phylogenetic relationships among breeds. The WAD was the first to be seen branching away from the other breeds, while the Yankassa in turn branched from the two predominant Northern breeds. The genetic proximity between Balami and Uda breeds could be due to the presence of a common breeding system and geographical husbandry area, which might have led to genetic exchange between them. This seems to be partially confirmed by the structure analysis, showing that Balami and Uda breeds shared a cluster. However, similar relationships were observed where the Yankassa and the WAD were more closely related compared to the Uda and Balami (Adebambo *et al.*, 2003) which are essentially Northern breeds. This confirms further the evolutionary divergence of the breeds which makes them distinct from the others. The result of the current study further supports the phenotypic adaptation of each of the breeds in the study population. The Balami and Uda breeds are animals with long legs as compared to the WAD or the Yankassa breeds. This adaptive feature explains the survivability of these breeds in the Northern savannah regions where they are required to trek very long distances in search of pasture in the heat. On the other hand the WAD is adapted with short legs and stocky body which is indicative of the geographic distribution of the breed around the Southwest of the country in the rainforest regions where the grasses are green almost all year round. The Yankassa is a breed whose phenotype is in-between the two extremes which explains its ability to survive in both geographical regions and in the middle belt.

5.2.10 Factorial Correspondence Analysis (FCA)

According to the correspondence analysis, the first axis explains 45.38% of the total variation and separates the WAD from the rest. The second axis representing 32.01% of the total variation, showed the isolation of the Yankassa breed, while the third axis which represents 22.61% of the total variation grouped the Balami and Uda breeds together.

The FCA results corroborate the findings based on the Nei genetic distances, which also indicated the isolation and a greater genetic distance of the WAD breed. In fact, the least variable breeds are usually the most distinct ones (Hedrick, 1999). It is important to highlight that WAD is mainly reared in the south, unlike the other breeds reared exclusively in the North by nomads for meat production; therefore, there was less gene exchange between the former and the other breeds.

5.2.11 Population Structure

Population structure was investigated by varying K from 1 to 6. Although the analysis showed the highest probability of forming 5 clusters, K = 3 was chosen as the best value to describe the genetic structure of the breeds, since the increase of Ln Pr(G|K) from K = 3 to K = 5 was low if compared with the increase from K = 1 to K = 3. However, as suggested by Pritchard *et al*. (2000), the inferred clusters are not necessarily the corresponding real ancestral population and they can be determined by sampling schemes.

Assuming K = 3 the WAD formed distinct group suggesting that admixture was nearly zero for the breed. The high average percentage of assignment of individuals for the WAD breed pointed out the existence of clear genetic differences compared to other breeds and this result is also confirmed by the geographic distribution of this breed as well as the different breeding system. The Uda, Balami and Yankassa breeds exhibited the presence of admixture. The Uda and Balami clustered together up to K = 4, confirming the genetic closeness between these breeds. For K = 4, Balami, Uda and Yankassa breeds formed three distinct clusters but with proportion of membership split in two or more clusters. This result could be probably due to the phylogenetic

relationships among these breeds and/or to the migration of individuals among the several farms present in the area.

5.2.12 Analysis of Molecular Variance (AMOVA)

Analysis of molecular variance (AMOVA) provided an estimate of the measure of population genetic differentiation within and between populations. The AMOVA showed that 4.53 percent of the genetic variation among Nigerian sheep breeds is attributed to among populations compared with 2.56 percent due to variation among individuals within populations and 92.91 percent due to variation within individuals. The hierarchical analysis of variance revealed a value of 0.04534 for F_{ST} (Variance among populations). The value obtained in this study was low but significant ($P<0.001$) indicating the presence of breed differences. In the same way the variance components within individuals were highly significant ($P<0.001$). Dashab *et al.* (*2011*) showed that about 2.4% of the total genetic variation was explained by population differences and 97.6 percent corresponded to differences among individuals. Paiva *et al*. (2011) showed that 12.84% of observed variation was explained by differences between breed among populations while the difference observed within populations explained 87.16%. According to McClean *et al.* (2011), the percentage of molecular variance among the individuals was 21%, while there was a 49% molecular variance within individuals. Qing *et al.* (2009) reported that genetic variations within populations were over 19 times (data from structural loci) or 37 times (data from microsatellite loci) than those among populations. The high and significant within individuals source of variation suggests that breeds exhibited distinct genetic variation useful in utilizing these genetic resources as breeders strive to increase productivity and the breeds' competitive advantage.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

Traditionally, classifications of breeds were based on phenotypic traits. In some cases, recent genetic studies have found differences in the structure proposed. In sheep, one has to bear in mind that morphological changes (e.g. horns or coat colour) were not the result of adaptations to the environment, but have a social significance and thus may not be indicative of the genetic relationships. In recent years, microsatellites have proven to be very useful for the determination of genetic relationships among populations.

By this study, biochemical and molecular genetic diversity for four sheep breeds of Nigeria (Balami, Uda, Yankassa and West African Dwarf), based on four protein loci and 13 microsatellite loci, was determined and analysed.

Allozyme studies can mesh genetic and ecological information to strengthen inferences about specific aspects of population structure, especially breeding structure and effective gene flow. The results of this study can, therefore, be used in breeding programs, and present a study of the current genetic structure of the different sheep breeds found in Nigeria. Furthermore, the results for the sheep breeds are important for the future monitoring of gene flow in populations, to determine levels of inbreeding and crossbreeding in each breed, and to enhance the global information on domestic animal diversity.

The estimates of heterozygosity obtained using information from the four loci studied were within the recommended range for measuring genetic variability among sheep populations and as such the estimates were useful in the study of four breeds of sheep indigenous to Nigeria. The genetic distance estimate of Nei (1972) in this study using protein loci indicated that the Balami and Yankassa were the most genetically similar

while the farthest distance was recorded between Balami and West African Dwarf indicating that these may have had different historical origins or prehistoric migration route.

This study was carried out to provide a basis for ensuring that any future conservation, utilization and genetic improvement programs maintain the genetic diversity of the Nigerian indigenous sheep breeds. However, this study only analysed a small sample of the total population of sheep in Nigeria using four loci out of about 30 that can be used in this type of study. Thus, it is recommended that this study be continued to cover more animals per populations and number of loci so as to capture correctly the genetic diversity of Nigerian sheep populations before making genetic improvement and conservation decision. The use of DNA microsatellite markers for this type of study provides more information, hence the need for the study with 13 microsatellite loci.

Microsatellite analysis revealed that the Nigerian sheep genotypes have more within breed variation than between breed variation. The Uda showed the highest heterozygosity level, while the Balami had the least heterozygosity value. However, all of the breeds in this study had above average heterozygosity values. The high genetic distance between the West African Dwarf and the Uda indicates separate development, while the close similarity of the Balami and the Uda indicates high gene flow and likely common ancestors. The panel of microsatellite markers used for this study was useful in determining the purity of the breeds and can contribute to the integrity of sheep stud breeding in Nigeria.

The large genetic distance between the WAD and Uda was expected. These two breeds should be conserved as they are genetically distinct breeds with geographical boundaries as barriers preventing gene flow between them. The close relationship between Balami and Uda indicated that these breeds although phenotypically very different, have more in common genetically and may only be considered as types or varieties for conservation purposes.

It is concluded that Nigerian sheep breeds are significantly different from one another. All of them exhibit high within group genetic diversity and they are admixed to various

degrees. Data and results of the present study can be used in deciding the conservation priorities of the breeds. However, it must be emphasized that samples and studied breeds may affect the observed patterns of genetic diversity. Hence, more independent studies must be carried out before the major steps in conservation of sheep breeds will be taken.

As genotyping platforms are converting from microsatellites to Single nucleotide polymorphisms (SNPs) and whole-genome analysis, it is recommended that these breeds be further evaluated using these new genotyping platforms and the results compared with results from Microsatellite, Protein and traditional typing methods so as to reach a valid decision as to breed differences for the purpose of conservation, use and management.

REFERENCES

Achilli, A., Olivieri, A., Pellecchia, M., Uboldi, C., Colli, L., Al-Zahery, N., Accetturo, M., Pala, M., Kashani, B.H., Perego, U.A., Battaglia, V., Fornarino, S., Kalamati, J., Houshmand, M., Negrini, R., Semino, O., Richards, M., Macaulay, V., Ferretti, L., Bandelt, H.J., Ajmone-Marsan, P. & Torroni, A. (2008). Mitochondrial genomes of extinct aurochs survive in domestic cattle. Current Biology, 18: R157-R158.

Adebambo Olufunmilayo, J. L. Williams, Sara Blott& B. Urquhart. (2003). Genetic relationships between Native Sheep breeds in Nigeria based on microsatellite DNA polymorphisms. Animal Genetic Resource Information. 34: 27-39

Ademosun A. A. Benyi, K., Chiboka, O. and Manyabuntu, C.M. (1983). Review of the hair sheep in the South Western Nigeria. In: Hair sheep of the West Africa and the Americas, Ed. Fitzhugh, H.A and G.E. Bradford, pp 219-226

Adu, I.F. and Ngere, L.O. (1979). The indigenous sheep of Nigeria. World Review of Animal Production 15, 3: 51–62.

Aganga, A.A., Umunna, N.N., Oyedipe, E.O. and Okoh, P.N. (1988) Seasonal variations in water requirement and influence of intermittent watering on grazing Yankasa sheep.Small Ruminant Research 1: 381–386.

Akinyemi, M. O and Salako A. E. (2012). Genetic relationship among Nigerian indigenous sheep populations using blood protein polymorphism. Agricultural Science and Technology, Vol. 4, No 2, pp 107 - 112, 2012

Akinyemi, M. O. (2010). Morphological and Biochemical Diversity In: Balami, Uda And Yankassa Sheep Of Nigeria. In: Dissertation. University of Ibadan, Ibadan, Nigeria

Akinyemi, M.O., Salako, A.E., Osaiyuwu, O. H., and Ige, A.O. (2010).Haemoglobin phenotypes in Balami and Uda sheep of Nigeria. Proceedings of 15th annual conference of Animal Science Association of Nigeria (ASAN), Theme: Diversifying Nigeria's Economy: Animal Production option. Ed: O.J. Ifut, U.A. Inyang, I.P. Akpan and I.E. Ebeso. University of Uyo, 13th – 16th September, 2010. Pp 16-17.

Al-Atiyat, R. M., Tabbaa, M. J., Salameh, N. M., Tarawneh, K. A., Al-Shmayla, L. and Al-Tamimie, H. J. (2012). Analysis of genetic variation of fat tailed-sheep in southern region of Jordan. Asian Journal of Animal and Veterinary Advances 7 (5): 376 – 389.

Allendorf, F. W., and G. Luikart (2007). Conservation and the Genetics of Populations. First Edition, Wiley-Blackwell, MA, USA.

Allomby E.W. and Urquhart G.M. (1976). A possible relationship between haemonchosis and haemoglobin polymorphism in Merino sheep in Kenya. Res. Vet. Sci., 20,212–214.

Altaif K.I.and Dargie, J.D. (1978). Genetic resistance to Helminths: comparison of the development of Ostertagia circumcinta infection in Scottish blackface sheep of different types. Res. Vet. Sci. 24:391-395

Altshuler, D., Pollara, V.J., Cowles, C.R., Van Etten, W.J., Baldwin, J., Linton, L and Lander, E.S. (2000). A SNP map of the human genome generated by reduced representation shotgun sequencing. Nature 407: 513-516.

Alvarez, I., Royo, L. J., Fernandez, I., Gutierrez, J. P., Gomez, E., and Goyache, F. (2004). Genetic relationships and admixture among sheep breeds from Northern Spain assessed using microsatellites. Journal of Animal Science 84:2246 – 2252.

Aminafshar M, Amirinia C, Torshizi RV (2008). Genetic diversity in buffalo population of guilan using microsatellite marker. Journal of Animal and Veterinary Advances, 7(11): 1499-1502.

Aquadro, C.F., Jennings, R.M., Bland, M.M., Laurie, C.C. and Langley, C.H. (1992). Patterns of naturally occuring restriction map variation, dopa decarboxylase activity variation and linkage disequilibrium in the Ddc gene region of Drosophilia melanogaster. Genetics 132: 443-452.

Arora R and Bhatia S. (2004) Genetic structure of Muzzafarnagri sheep based on microsatellite analysis. Small Ruminant Res. 54, 227-230.

Arora R and Bhatia S. (2006) Genetic diversity of Magra sheep from India using microsatellite analysis. Asian-Australian Journal of Animal Science. 19, 938-942.

Arora R, Bhatia S, Sehrawat A, Maity S.B and Kundu S.S. (2008) Genetic variability in Jalauni sheep of India inferred from microsatellite data. Article no. 4 retrieved from www.cipav.org.co/lrrrd20/1/ aror20004.htm on 2/5/2008.

Arora, R. J., Bhatia, S., Mishra, B. P., Jain, A. and Prakash, B. (2011). Diversity analysis of sheep breeds from Southern peninsular and Eastern region of India. Tropical Animal Health Production 43: 402 – 408.

Arora, R.; S. Bhatia, and B. P. Mishra. (2010). Genetic Variation and Relationship of Six Indian Sheep Breeds Adapted to the Northwestern Arid Zone of Rajasthan. Biochemical Genetics: 1-9.

Arranz J.J., Bayo´n Y., San Primitivo F. (1998). Genetic relationships among Spanish sheep using microsatellites. Anim. Genet., 29, 435–440.

Avise, J.C., Arnold, J., Ball, R.M., Bermingham, E., Lamb, T., Neigel, J.E., Reeb, C.A., and Saunders, N.C. (1987). Intraspecific phyleogeography: The mitochondrial DNA Bridge between population genetics and systematics. Annual Review of Ecology and Systematics 18: 489-522.

Ayalew, W., van Dorland, A. &Rowlands, J. (2004). Design, execution and analysis of the livestock breed survey in Oromia Regional State, Ethiopia. Addis Ababa and Nairobi.OADB (Oromia Agricultural Development Bureau) and ILRI (International Livestock Research Institute).

Baker, C. M. A and Manwell. C. (1980).Chemical classification of cattle. I. Breed group. Animal Blood Groups and Biochemical Genetics 11, 127-150.

Baker, C.M.A., Manwell, C., Labrisky, R.F. and Harper, J.A. (1966). Molecular genetics of avian proteins – V. Egg, blood and tissue proteins of the Ringnecked pheasant, Phasianus colchicus L. Comp. Biochem. Physiol. 17: 467-499.

Barbour, K.M., Oguntoyinbo, J.S., Onyemelukwe, J.O.C. and Nwafor J.C. (eds) (1982) Nigeria in Maps. Hodder and Stoughton, London, England.

Barker, J.S.F., Tan, S.G., Moore, S.S., Mukherjee, T.K., Matheson, J.L. & Selvara, O.S. (2001). Genetic variation within and relationships among populations of Asian goats (Capra hircus). Journal of Animal Breeding Genetics 118: 213-233

Baumung R., Simianer H., Hoffmann I. (2004). Genetic diversity studies in farm animals – a survey. Journal Animal Breed and Genetics, 121, 361–373.

Baumung, R.; V. Cubric-Curik, K. Schwend, R. Achmann, and J. Slkner. (2006). Genetic characterisation and breed assignment in Austrian sheep breeds using microsatellite marker information. Journal of Animal Breeding and Genetics 123: 265-271.

Beaumont, M.A. and Bruford, M.W. (1999). Microsatellites in conservation genetics. In "Microsatellites: Evolution and Application" (D.B. Goldstein and C. Schlotterer, Eds.), pp. 165-183, Oxford University Press, New York.

Belkhir, K., Borsa, P., Goudet, J, Chikhi, L., Bonhomme, F., (2000). GENETIX logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, Populations, Interactions CNRS UMR 5000, Université de Montpellier II, Montpellier (France).

Bindu,K. A. and Raghavan, K. C. (2010). Haemoglobin Polymorphism in Malabari Goats. Veterinary World Vol.3 (2): 74-75.

Bjornstad, G. and Roed, K.H. (2001). Breed demarcation and potential for breed allocation of horses assessed by microsatellite markers. Animal Genetics 32: 59-65.

Blench, R and Kevin C MacDonald (1999). The Origins and Development of African Livestock.Routledge.ISBN 1-84142-018-2.

Bostein D., White R. L., Sckonick M., Davis R. W. (1980). Construction of genetic linkage map in man using restriction fragment length polymorphism. American Journal of Human Genetics, 32, 314 – 316.

Botha, K. (2001). The genetic relationship of seven horse breeds in South Africa using DNA markers. MSc thesis, Department of Plant Sciences and Genetics, University of the Free State, Bloemfontein, South Africa.

Boujenane, I., Ouragh, L., Benlamlih, S., Aarab, B., Miftah J., Oumrhar,H. (2008). Variation at post-albumin, transferrin and haemoglobin proteins in Moroccan local sheep. Small Ruminant Research (79) 113-117

Bradford G.E. and Fitzhugh H.A. (1983). Hair sheep: a general descriptionIn: hair sheep of West Africa and the Americas. A genetic resource for the tropics. Edited by H.A. Fithugh and G.E.Bradford. Wurak International Study

British Coloured Sheep Breeders Association (BCSBA),"An introduction to coloured sheep" http://www.bcsba.org.uk/coloured-sheep/coloured-sheep.html. Retrieved 2012-06-05.

Brown, W.M. (1985). The mitochondrial genome of animals. In "Molecular Evolutionary Genetics" (R.J. Macintyre, Ed.), pp. 95-130, Plenum Press, New York.

Bruford, M.W., Bradley, D.G. & Luikart, G. (2003). DNA markers reveal the complexity of livestock domestication. Nature Reviews Genetics, 4: 900-910.

Budiansky, Stephen (1999). The Covenant of the Wild: Why animals chose domestication. Yale University Press.ISBN 0-300-07993-1.

Buduram, P., (2004). Genetic characterization of Southern African sheep breeds using DNA narkers. In: Dissertation. University of the Free State, Bloemfontein, South Africa.

Buis, R. C., and Tucker, E.M. (1983). Relationships between rare breeds of sheep in the Netherlands as based on blood-typing. Animal Blood Groups and Biochemical Genetics 14:17-26.

Bunch, T. D. and Foote, W. C. (1976). Chromosomes, hemoglobins, and transferrins of Iranian domestic sheep. Journal of Hered 67:167-170.

Bunge, R., Thomas, D. L., and Stookey, J.M. (1990). Factors affecting the productivity of Ramboillet ewes mated to rams lambs. J. Anim. Sci. (68) 2253-2262

Buvanenedran, V., Umoh, J.E. and Abubakar B.V. (1980). An evaluation of body size as related to weight of three West African breeds of cattle in Nigeria. J. Agric Sci Camb.95:219-224

Calvo, J. H.; J. A. Bouzada, J. J. Jurado, and M. Serrano. (2006). Genetic substructure of the Spanish Manchega sheep breed. Small Ruminant Research 64: 116-125.

Canatan, T. and Boztepe, S. (2000). The polymorphism of haemoglobin and transferring in Turkish hair goat. Indian vet. J. 77: 966 – 968

Carter, A.H. and Cox, E.H. (1982). Sheep breeds in New Zealand In: Wickman G.A and McDonald M.F. (eds.) Sheep Production Vol.1 Breeding and Reproduction Ray Richards Auckland pp11-38

Chen, R. J., Yang Z. P., Zhu, X. R., Zhang, Y. Q., Ji, D. J., Mao, Y. J., Wang, X. L. and Li, Y. L. (2011). Analysis of genetic polymorphism and genetic distance among four sheep populations. African Journal of Biotechnology, Vol 10 (73), pp.16697 – 16702.

Chenyambuga, S.W. (2002). Genetic characterization of indigenous goat populations of sub-Saharan Africa using microsatellite DNA markers. PhD Thesis, Department of Animal Science and Production, Sokoine University of Agriculture, Sokoine, Tanzania.

Cinkulov, M. Ćinkulov M., Popovski Z., Porcu K., Tanaskovska B., Hodžić A., Bytyqi H., Mehmeti H., Margeta V., Djedović R., Hoda A., Trailović R., Brka M., Marković B., Važić B., Vegara M., Olsaker I., Kantanen J.. (2008). Genetic diversity and structure of the West Balkan Pramenka sheep types as revealed by microsatellite and mitochondrial DNA analysis. Journal of Animal Breeding and Genetics 125: 417-426.

Clarke, S.W., E.M. Turker and Osterhoff, D.R. (1989). Blood groups and biochemical polymorphisms in the Namaqua sheep breed. Animal Blood Groups and Biochemical Genetics 20:279-286.

Cornall, R.J., T.J. Aitman, C.M.Hearne& J.A. Todd. (1991). The generation of a library of PCR analyses microsatellite variants for genetic mapping of the mouse genome. Genomics 10: 874-881.

Crawford, A.M. and Littlejohn, R.P. (1998). The use of DNA markers in deciding conservation priorities in sheep and other livestock. Animal Genetic Resources Information 23: 21-26.

Crawford, A.M., K.G. Dodds, A.J. Ede, C.A. Pierson, G.W. Montgomery, H.G. Garmonsway, A.E. Beattie, K. Davies & J.F. Maddox. (1995). An autosomal genetic Linkage map of the sheep genome. Genetics 140: 703-724.

D' Angelo, F., Albenzio,M., Sevi, A., Ciampolini, R., Cecchi, F., Ciani, E. and Muscio, A. (2008). Genetic variability of the Gentile di Puglia sheep breed based on microsatellite polymorphism. Journal of Animal Science 87:1205-1209. http://jas.fass.org/cgi/ content/full /87/4/1205. Downloaded from jas.fass.org May 6, 2011.

DAGRIS. (2004). Domestic Animal Genetic Resources Information System (DAGRIS). J.E.O. Rege, W. Ayalew& E. Getahun, eds. Addis Ababa. International Livestock Research Institute.

Dalvit C, Sacca E, Cassandro M, Gervaso M, Pastore E, Piasentier E. (2008). Genetic diversity and variability in Alpine sheep breeds. Small Ruminant Research 80: 45-51.

Dalvit, C.; M. De Marchi, E. Zanetti, and M. Cassandro (2009). Genetic variation and population structure of Italian native sheep breeds undergoing in situ conservation. Journal of animal science 87: 3837.

Devendra, C. and McLeroy, G.B. (1982) Goat and sheep production in the tropics. London: Longman.

Di Stasio, L. (1995). Biochemical Genetics In: Genetics of sheep eds Piper, L. Ruvinsky,A. Pp 133-147

Diez-Tascon C., Littlejohn R.P., Imeida P.A.R., Crawford A.M. (2000). Genetic variation within the Merino sheep breed: analysis of closely related populations using microsatellites. Anim. Genet., 31, 43–51.

Dobzhansky, T. (1951). Genetics of the origin of species. 3rd edn. Columbia University Press New York 364pp

Dorji, T., Jianlin, H., Wafula, P., Yamamoto, Y., Sasazaki, S., Oyama, K., Hanotte, O., Lin, B. and Mannen, H. (2010). Sheep genetic diversity in Bhutan using microsatellite markers. Animal Science Journal 81: 145 – 151.

Dowling, T.E., Moritz, C. and Palmer, J.D. (1990). Nucleic acids ii: Restriction site analysis. In "molecular systematics" (D.M. Hillis and C. Moritz Eds.), pp. 250317, Sinauer, Sunderland, Massachusetts.

Drury, D. W., Ashley L. S, Michael, J. W. (2009). Genetic Differentiation among Wild Populations of Tribolium castaneum Estimated Using Microsatellite Markers. Journal of Heredity: sp077.

El Nahas, S.M, Hassan, A.A, Abou Mossallam, A.A, Mahfouz, E.R, Bibars, M.A, Oraby, H.A.S, de Hondt HA (2010). Analysis of genetic variation in different sheep breeds using microsatellites. African Journal of Biotechnology 7(8): 1060-1068.

Ensminger, M.E. and Parker, R. O. (1986). Sheep and Goat Science, Fifth Edition. Danville, Illinois: The Interstate Printers and Publishers Inc. ISBN 0-8134-2464-X.

Epstein, H (1971). The origin of the domesticated animals of Africa. Africana Publishing Corporation, New York

Esharatkhah, B., Safi, S., Khaki, Z., Khazraei N. P. and Sadaghian M. (2007). Study on haemoglobin polymorphism in two breeds of Iranian sheep. Journal of Animal and Veterinary Advances, 6, 12, 1426-142.

Estoup, A., Piesa, P., Krieg, F., Vaiman, D. and Guypmard, R. (1993). (CT) and (GT) microsatellites: a new class of genetic markers for Salmotrutta L. (Brown trout). Heredity 71: 488-496.

Evanno, G., S. Regnaut, and J. Goudet (2005). Detecting the number of clusters of individuals using the softwarestructure: a simulation study. Molecular Ecology 14:2611-2620.

Excoffier L., Laval G., Schneider S. (2005). Arlequin ver. 3.0.An integrated software package for population genetics data analysis. Evolutionary Bioinformatics, 1:47 – 50.

Excoffier, L., G. Laval, S. Schneider (2006) ARLEQUIN version 3.01: an integrated software package for population genetics data analysis. University of Bern, Institute of Zoology, Switzerland. Available from http://cmpg.unibe.ch/software/arlequin3

Excoffier, L., P. E. Smouse, J. M. Quattro (1992). Analysis of Molecular Variance Inferred From Metric Distances Among DNA Haplotypes: Application to Human Mitochondrial DNA Restriction Data. Genetics 131(2):479-491.

Falconer, D. S. (1989). Introduction to quantitative Genetics. Third edition Longman Scientific and Technical, England.

Falush, D., Matthew, S, and Pritchard, J. K. (2003). Inference of Population Structure Using Multilocus Genotype Data: Linked Loci and Correlated Allele Frequencies. Genetics 164(4):1567-1587.

Falush, D., Matthew, S, and Pritchard, J. K. (2007). Inference of population structure using multilocus genotype data: dominant markers and null alleles. Molecular Ecology Notes 7(4):574-578.

FAO (1998). Secondary Guidelines for Development of National Farm Animal Genetic Resources Management Plans. Measurement of Domestic Animal Diversity (MoDAD): Original Working Group Report. Home page address: http://dad.fao.org/en/refer/library/guidelin/workgrp.pdf.

FAO (2000).World Watch List for domestic animal diversity. FAO, Rome.

FAO. (1986a). Animal genetic resources data banks - 1. Computer systems study for regional data banks. Animal Production and Health Paper No. 59, Volume 1. Rome.

FAO. (1986b). Animal genetic resources data banks - 2. Descriptor lists for cattle, buffalo, pigs, sheep and goats. Animal Production and Health Paper No. 59, Volume 2. Rome.

FAO. (1986c). Animal genetic resources data banks - 3. Descriptor lists for poultry. Animal Production and Health Paper No. 59, Volume 3. Rome.

FAO. (1992). The management of global animal genetic resources. Proceedings of an Expert Consultation, Rome, Italy, April 1992. Edited by J. Hodges. Animal Production and Health Paper No.104. Rome.

FAO. (2005). Genetic characterization of livestock populations and its use in conservation decision making, by O. Hannotte& H. Jianlin. In J. Ruane& A. Sonnino, eds. The role of biotechnology in exploring and protecting agricultural genetic resources, pp. 89–96. Rome. (Available at www.fao.org/docrep/009/a0399e/a0399e00.htm).

FAO. (2006). A system of integrated agricultural censuses and surveys, volume 1, World Programme for the Census of Agriculture 2010. Statistical Development Series No. 11. (Also available at www.fao.org/es/ess/census/default.asp)

FAO. (2007). The state of the world's animal genetic resources for food and agriculture ftp://ftp.fao.org/docrep/fao/010/a1250e/a1250e.pdf. Accessed 30/05/2012 @ 1: 00 pm

FAO. (2011) Draft Guidelines On Molecular Genetic Characterization Of Animal Genetic Resources. Rome, 18 – 22 July 2011

FAO/UNEP. (1998). Primary guidelines for development of national farm animal genetic resources management plans. Rome.

Felsentein, J. (2009). Phylogeny Inference Package PHYLIP, Version 3.69. Department of Genome Sciences and Department of Biology, University of Washington, Seattle, WA, USA.

Food and Agriculture Organization of the United Nations (FAO) (1984). Animal genetic resource conservation by management, databanks and training. Animal Production and Health Paper No. 44/1. Rome.

FOS (1989). Facts and Figures about Nigeria. Lagos: Federal Office of Statistics.

Frandson, R. D. and Spurgeon. T. L. (1992). Anatomy and physiology of farm animals.5th ed. Lippincott, Williams and Wilkins. 572 pp

Gagneux, P., Boesch, C. and Woodruff, D.S. (1997). Microsatellite scoring errors associated with noninvasive genotyping based on nuclear DNA amplified from shed hair. Molecular Ecology 6: 861-868.

Gibson, J.P., Ayalew, W. and Hanotte, O. (2007). Measures of diversity as inputs for decisions in conservation of livestock genetic resources.In D.I. Jarvis, C. Padoch& D. Cooper, eds. Managing biodiversity in agroecosystems. New York, USA. Columbia University Press.

Girish, H., Sivaselvam S. N., Karthikeyan, S.M.K. and Saravanan, R. (2007). Molecular characterisation of Nilagiri sheep (Ovis aries) of south India based on microsatellites. Asian-Aust. J. Anim. Sci. 20(5), 633-637

Gizaw, S.; J. A. Van Arendonk, H. Komen, J. J. Windig, and O. Hanotte. (2007). Population structure, genetic variation and morphological diversity in indigenous sheep of Ethiopia. Animal Genetics 38: 621-628.

Glaubitz, J. (2005). Convert Version 1.31. http://www.agriculture.purdue.edu/fnr/html/faculty/Rhodes/Students%20and%20Staff/glaubitz/software.htm. (Downloaded May, 2012)

Glowatzki-Mullis, M.; C. Gaillard, G. Wigger, and R. Fries. (2007). Microsatellite-based parentage control in cattle. Animal Genetics 26: 7-12.

Gorman, G. C., Renzi, J. Jr. (1979). Genetic Distance and Heterozygosity Estimates in Electrophoretic Studies: Effects of Sample Size. Copeia (2): 242-249.

Grigaliunaite I, Tapio M, Viinalass H, Grislis Z, Kantanen J and Miceikiene I. (2003) Microsatellite variation in the Baltic sheep breeds. Veterinarija ir zootechnika T. 21 (43), 66-73.

Groeneveld, L.F., Lenstra, J.A., Eding. H., Toro, M.A., Scherf, B., Pilling, D., Negrini, R., Jianlin, H., Finlay, E.K., Groeneveld, E., Weigend, S. (2010). Genetic diversity in livestock breeds. Animal Genetics, 41 (suppl. 1): 6-31.

Guo X. and Elston R. C., (1999). Linkage informative content of polymorphic genetic markers. Hum Hered 49:112–118.

Gustavo, A. G., Steven, T. K., Walter, M. B. and Philip, W. H. (2000). Genetic variation and population structure in desert bighorn sheep: implication for conservation. Conservation Genetics 1: 3 – 15.

Gyllensten, U., Wharton, D. and Wilson, A.C. (1985). Maternal inheritance of mitochondrial DNA during backcrossing of two species of mice. Journal of Heredity 76: 321-324.

Hall, S.J.G. (1991). Body dimensions of Nigeria cattle, sheep and goats. Anim. Prod. 53:61-69

Handley, L. J., Byrne, K., Santucci, F., Townsend, S., Taylor, M., Bruford, M. W. and Hewitt, G. M. . (2007). Genetic structure of European sheep breeds. Heredity 99: 620-631.

Hanotte, O., Tawah, C.L., Bradley, D.G., Okomo, M., Verjee, Y., Ochieng, J.W. and Rege, J.E.O. (2000). Geographic distribution and frequency of a taurine Bos Taurus and an indicine Bos indicus Y specific allele amongst sub-Saharan African cattle breeds. Molecular Ecology 9: 387-396.

Hartl, D. L., (1980) Principles of Population Genetics. Sinauer Associates Inc., Sunderland, Massachusetts USA.

Hasselholt, M. (1969). Serum protein polymorphism's in swine electrophoretic identification. Genetics and Application. Munksgaard, Copenhagen pp. 48-52.

Haumesser, J.B. and Gerbaldi, P. (1980) Observations sur la reproduction etl'élevage du mouton Oudahnigérien. REMVT 33, 2: 205–213.

Hiendleder S, Kaupe B, Wassmuth R, and Janke, A. (2002). "Molecular analysis of wild and domestic sheep questions current nomenclature and provides evidence for domestication from two different subspecies" (PDF). Proc. Biol. Sci.269 (1494): 893.DOI:10.1098/rspb.2002.1975.PMC1690972.PMID12028771.http://www.pubmedcentral.nih.gov/picrender.fcgi?artid=1690972&blobtype=pdf. Retrieved 2008-01-12.

Hiendleder, S., Kaupe, B., Wassmuth, R. and Janke, A. (2002). Molecular anlysis of wild and domestic sheep questions current nomenclature and provides evidence for domestication from two different subspecies. Proceedings of the Royal Society of London. Series B, Biological Sciences 269: 893-904.

Hiendleder, S., Mainz, K., Plante, Y. & Lewaiski, H. (1998). Analysis of Mitochondrial DNA indicates that domestic sheep are derived from two different ancestral maternal sources: No evidence for contribution from Urial and Argali. Journal of Heredity 89: 113-120.

Hiendleder, S.; K. Mainz, Y. Plante, H. Lewalski (2007). "Analysis of mitochondrial DNA indicates that domestic sheep are derived from two different ancestral maternal sources: no evidence for contributions from urial and argali sheep.".The Journal of Heredity 89 (2): p. 113. DOI:10.1093/jhered/89.2.113. PMID 9542158.

Hoeh, C., Dowling, T.E. and and Brown, W.M. (1991). Heteroplasmy suggests limited biparental inheritance of Mytilus mitochondrial DNA. Science 251: 1488-1490.

Ibeagha-Awemu E.M. and Erhardt G. (2004). Genetic variations between African and German sheep breeds and description of a new variant of vitamin D-binding protein. Small Ruminant Resource 55, 33–43

ILCA (1979). Systems Study 3. Small Ruminant Production in the Humid Tropics. Addis Ababa.

Imumorin, I. G., Ologun,A.G., and Oyeyemi, M.O. (1999). Preliminary observations on effect of haemoglobin genotype and estimate on genetic distance at the Hb locus in West African Dwarf and red Sokoto goats. Trop. J.Anim.Sci.1:1-9

ISAG/FAO Standing Committee. (2004). Secondary Guidelines for Development of National Animal Genetic Resources Management Plans. Measurement of Domestic Animal Diversity (Mo-DAD): Recommended Microsatellite Markers. Recommendations of joint ISAG/FAO Standing Committee.http://dad.fao.org/ Retrieved Jan. 26, 2009.

Ivankovic A. P., Dove P., Kavar P., Caput P., Mioe B., Pavie V., Stuhee V. and Leto J. (2005) Genetic characterization of the Pag Island sheep breed based on microsatellite and mtDNA data. Small Ruminant Res. 57, 167-174.

Ivanna, T., Wlasiuk, G. and Lessa, E. P. (2002).evaluation of polymorphism in 10 microsatellite loci in Uruguayan sheep flocks. Genetic and Molecular Biology, 25, 1, 37 – 41

J. G. Ordas and F. S. Primitivo (1983). A new allele at the red cell carbonic anhydrase locus in sheep. Animal Blood Groups and Biochemical Generics I4: 233-234

Kalinwski, S. T. (2002). How many alleles per locus should be used to estimate genetic distances. Heredity, 88: 62-65.

Kevorkian, S. E. M., Georgescu, S. E., Manea, M. A., Zaulet, M., Hermenean, A. O. and Costache, M. (2010). Genetic diversity using microsatellite markers in four Romanian autochthonous sheep breeds. Romanian Biotechnological Letters, Volume 15, No. 1:5060

Kilgour L., Dixon S.C., Tucker E.M. (1990). Two new sheep haemoglobins, one of which is replaced by haemoglobin C in anaemia. Animal Genetics, 21, 115–121.

Kohn, M.H., Murphy, W.J., Ostrander, E.A. & Wayne, R.K. (2006). Genomics and conservation genetics. Trends in Ecology and Evolution, 21: 629-637.

Kowalska and Zaton- Dobrowolska (2008). Genetic distance between different breeds of sheep. Journ of Agrobiology, 25: 23-26

Kusza, S., Gyarmathy, E., Dubravska, J., Nagy I., Jávor1, A., Kukovics, S. (2009). Study of genetic differences among Slovak Tsigai populations using microsatellite markers. Czech Journal of Animal Science 54: 468-474.

Kusza, S.D, Dimov I, Nagy Z, Bosze A, Javor S, Kukovics. (2010). Microsatellite analysis to estimate genetic relationships among five bulgarian sheep breeds. Genetics and Molecular Biology 33: 51-56.

Kwatu,A.S., Umuna,N. N. and Chineme, C.N. (1982). Feedlot performance and carcass traits of Yankassa ram: Effect of varying the concentrate to roughage ratio. Nigerian Journal of Animal Production 10:76-81

Landegren U., Nilsson M. and Kwok, P. Y. (1998). Reading bits of genetic information: Methods for single nucleotide polymorphism analysis. Genome research 8, 769 – 776

Lebart L., Morineau A., Warwick K. (1984) - Multivariate Descriptive Statistical Analysis, J.Wiley, New-York.

Locke, M., Baack, E. and Toonen, R. (2000). Veterinary Genetics Lab, University of California at Davis.

Loftus, R., and Scherf, B. (1993). (eds) World Watch List for Domestic Animal Diversity. FAO, Rome, 376pp

Lowe1 W., Allendorf, F. (2010). What can genetics tell us about population connectivity? Molecular Ecology, Volume 19, Issue 15, pages 3038–3051.

Luikart G, MP Biju-Duval, O Ertugrul, Y Zagdsuren, C Maudt, P Taberlet. (1999). Power of 22 microsatellite markers in fluorescent multiplex for parentage testing in goats (Capra hircus). Anim Genet 30, 431-438.

Lush, J.L. (1945). Animal Breeding Plans, 3rd Ed. Iowa State University Press, Ames Iowa, 443pp

MacHugh, D. E., R. T. Loftus, D. G. Barley, P. M. Sharp, and P. Cunningham, (1994). Microsatellite DNA variation within and among European cattle breeds. Proc. R. Soc. Lond. B 256:25–31.

MacHugh, D.E., Shriver, M.D., Loftus, R.T., Cinningham, P. & Bradley, D.G. (1997). Microsatellite DNA variation and the evolution, domestication and phylogeography of taurine and zebu cattle (Bos Taurus and Bos indicus). Genetics 31: 31-38.

Manwell, C., Baker, C.M.A., (1977). Genetic distance between the Australian Merino and the Poll Dorset sheep. Genet. Res. Camb. 29, 239–253.

Marshall, T. (2007). Field Genetics Ltd.

Mason, l. L. (1996). A world Dictionary of Livestock Breeds, types and varieties. Fourth edition. C.A.B. International. 273 pp.

Mattiussi, C., Waibel, M. and Floreano, D. (2004). Measures of Diversity for Populations and Distances between Individuals with Highly Reorganizable Genomes. Evolutionary Computation 12(4): 495-515

McClean, L., Waterman, L., and Roberts, C. (2011). Genetic Analysis of Three Populations of Barbados Blackbelly Sheep at Microsatellite Loci. Journal of Agricultural Science and Technology A 1 1187-1191

Meadows, J.R.; Cemal, I. and Karaca, O. (2007). "Five ovine mitochondrial lineages identified from sheep breeds of the near East.".Genetics 175 (3): p. 1371. DOI:10.1534/genetics.106.068353.PMC1840082.PMID17194773.http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1840082. Retrieved 2010-02-10.

Melinda J. B., (2004). "Sheep" World Book. Mackiev Professor Coordinator of Graduate Studies, Department of Animal and Veterinary Sciences, California State Polytechnic University.

Melissa, R. (2001). Dental Anatomy of Ruminants http://www.vivo.colostate.edu/hbooks /pathphys/digestion/pregastric/cowpage.html. Retrieved 2012-06-05.

Meuwissen, T.H. (1991). Reduction of selection differentials in finite populations with a nested full-half sib family structure.Biometrics. 47(1): pp.195-203.

Miller M. P (1997). Tool for Population Genetic Analysis (TFPGA) Version 1.3. A Windows program for the analysis of allozyme and molecular population genetic data. Department of Biological Science. Northern Arizona University. p. 30

Missohou, A., Nguyen, T.C., Sow, R. and Gueye, A. (1999). Blood Polymorphisms in West African Breeds of Sheep. Tropical Animal Health and Production 31:175-179.

Moioli, B., F. Napolitano, L. Orru, and G. Catillo. (2006). Analysis of the genetic diversity between Gentile di Puglia, Sopravissana and Sarda sheep breed using microsatellite markers. Italian Journal of Animal. Science. 5:73–78.

Morhi, M., Jannatabadi, A. A. and Aslani, M.R. (2005). Studies on haemoglobin polymorphism of two breeds and its relationship to concentrations of iron, copper, haemoglobin, haematocrit and RBC number. Vet. Res. Comm. 29, 305-312

Morin, P. A., Luikart G., Wayne R. K. and the SNP workshop group (2004). SNPs in ecology, evolution and conservation. Trends in Ecology and Evolution 19, 208 – 216

Mukesh, M.; M. Sodhi, and S. Bhatia. (2006). Microsatellite-based diversity analysis and genetic relationships of three Indian sheep breeds. Journal of Animal Breeding and Genetics 123: 258-264.

Mwacharo, J.M., Otieno, C.J., Okeyo, A.M. (2002). Genetic variations between indigenous fat tailed sheep populations in Kenya. Small Ruminant Research 44:173-178

Nanekarani, S.; C. Amirinia, N. Amirmozafari, R. V. Torshizi, and A. A. Gharahdaghi. (2010). Genetic variation among pelt sheep population using microsatellite markers. African Journal of Biotechnology 9: 7437-7445.

Ndamukong, K. J. N. (1995). Haemoglobin Polymorphism in Grassland Dwarf sheep and goat of the North West province of Cameroun. Bull Anim. Health Prod Africa 43, 53 -56

Nei, M. (1973). Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci, 70: 3321–3323.

Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89, 583-592.

Nei, M. (1987). Genetic distance and molecular phylogeny. In "Population Genetics and Fishery Management" (N. Ryman & F. Utter, Eds), Chapter 8, pp. 193-223, University of Washington Press, Seattle.

Ngere, L. O., Adu, I. F. and Mani, I. (1979).Report of Small Ruminant Breeding Sub Committee. NAPRI bulletin, No 1 Shika

Nguyen T. C., Morera. L., Lianes D. and Leger P. (1992). Sheep blood polymorphism and genetic divergence between French Rambouillet and Spanish Merino: role of genetic drift. Anim. Genet. 23: 325 – 332

Nolte, M. (2003). The genetic characterisation of Camelus dromedarius in Southern Africa. Unpublished PhD Thesis: Rand Afrikaans University, Africa.

O'Brien, S. J., Roelke, ME, Marker L., Newman, A., Winkler, C. A., Meltzer, D., Colly, L., Evermann, J. F., Bush, M. and Wildt, D. E. (1985). Genetic basis for species vulnerability in the cheetah. Science Vol. 227 no. 4693 pp. 1428-1434

Oklahoma State University. (2005). Breeds of livestock. Stillwater, Oklahoma, USA. Department of Animal Science, Oklahoma State University. (Available at http: //www .ansi. okstate .edu/breeds/).

Olusanya, S. K. (1975) Electrolyte concentration and haemoglobin types in the blood of Nigerian dwarf sheep. Journal of Nig. Vet.Med.Asso.4, 59-63

Oni, O.O. (2002).Breeds and genetic improvement of small ruminants. Small Ruminant Training Workshop, held at the National Animal Production Research Institute, Ahmadu Bello University, Shika, Zaria. 13th to 18th January, 2002. pp. 2.

Ordas J.G. (2004). Structure of European ovine populations from directional autocorrelations between proteins. Journal of Animal Breed and Genetics, 121, 229–241.

Ordas, J.G. and Primitivo, F.S. (1986). Genetic variations in blood proteins within and between Spanish dairy sheep breeds. Animal Genetics 17:255-266

Otchere E. O., Ahmed, H. U.,Adenowo, T. K., Kallah, M.S., Bawa, E. L. K., Olorunju, S. A. S. and Voh A. A. (1987).Northern Nigeria; sheep and goat production in the traditional Fulani agro-pastoral sector. World Anim. Rev.64:50-55

Paiva, S. R., Mariante, A. Da Silva, Blackburn, H. D. (2011). Combining US and Brazilian Microsatellite Data for a Meta-Analysis of Sheep (Ovis aries) Breed Diversity: Facilitating the FAO Global Plan of Action for Conserving Animal Genetic Resources. Journal of Heredity 102 (6):697–704

Pariset, L.; Savarese M. C., Cappuccio I., and. Valentini, A. (2003). Use of microsatellites for genetic variation and inbreeding analysis in Sarda sheep flocks of central Italy. Journal of Animal Breeding and Genetics 120: 425-432.

Peter C, Bruford M, Perez T, Dalamitra S, Hewitt G, Erhardt, G, The Econogene Consortium (2007). Genetic diversity and subdivision of 57 European and Middle Eastern sheep breeds. Animal Genetics 38: 37-44.

Peter, C.; Prinzenberg E. M., and Erhardt G.. (2005). Null allele at the OarAE129 locus and corresponding allele frequencies in five German sheep breeds. Animal Genetics 36: 92.

Petit, E., Balloux, F. and Excoffier, L. (2002). Mammalian population genetics: Why not Y? Trends in Ecology and Evolution, 17: 28-33.

Plug I. &Badenhorst, S. (2001). "The Distribution of Macro mammals in Southern Africa over the past 30 000 years" Transvaal Museum, Pretoria.

Prema S, Sivaselvam S. N. and Karthickeyan S. M. K. (2008) A note on genetic analysis in Madras Red sheep (Ovis aries) of India using microsatellite markers. Livestock Research for Rural Development. 20 (11), paper 181.

Pritchard, J.K., Stephens, M., Donnelly, P., (2000). Inference of population structure using multilocus genotype data. Genetics 155, 945–959.

Promega (2003). Magnesil® KF, Genomic system. Promega Corporation, 2800 Woods Hollow Road, Madison, Wl 53711-5399, USA

Qanbari, S., Osfoori, R. and Eskandari Nasab, M. P. (2007). A preliminary study of marker data applicability in gene inrogression program for Afshari sheep breed. Biotechnology, 6: 513 – 519

Qing, G. R., Ping, L. W., Hong, C. and Tsunodak, K. (2009). Genetic differentiation among four Chinese sheep breed. Journal of Animal and Veterinary Advances 8 (7): 1381 – 1384

Raushenbach Yu O and Kamenek VM (1978). The role of biochemical polymorphism in ecogenetic differentiation of animals and its significance for selection for resistance to extreme environmental conditions. XVIth Intern, Conference of Animal Blood Groups and Biochemical Polymorphism, Leningrad 128.

Reed, C.A. (1984). The Beginnings of Animal Domestication. In Mason I.L. (ed.) Evolution of Domesticated Animals. Longman, London 1-6

Rege, J.E.O. (1992). Background to ILCA's animal genetic resources characterization project, objectives and agenda for the research planning workshop.In J.E.O. Rege& M.E. Lipner, eds. Animal genetic resources: their characterization, conservation and utilization. Research planning workshop, ILCA, Addis Ababa, Ethiopia, 19-21 February, 1992, pp. 55–59. Addis Ababa. International Livestock Centre for Africa.

Rendo F, Iriondo M, Jugo BM, Mazón LI, Aguirre A, Vicario A, Estonba A (2004). Tracking diversity and differentiation in six sheep breeds from the North Iberian Peninsula through DNA variation. Small Ruminant Research 52: 196-202.

Rendo F., Iriondo I., Jugo B. M., Mazon L. I., Aguirre A., Vicario A. and Estonba E. (2003) Tracking diversity and differentiation in six sheep breeds from the North Iberian Peninsula through DNA variation. Small Ruminant Research. 52, 195-202.

Reynolds, L. (1986). Small Ruminant Production. The present situation and possible nutritional intervention for improvement. ILCA Bulletin 25:13

RIKEN (2006). Genetic Quality Monitoring by Biochemical Isozymes. RIKEN Bioresource Center.

RIM (1992). Nigerian Livestock Resources. Four Volume Report to the Federal Government of Nigeria by Resource Inventory and Management Limited: 1. Executive Summary and Atlas; 11. National Synthesis; III, State Reports IV. Urban reports and Commercially Managed Livestock Survey Reports.

RMNCSBA. (2007). Rocky Mountain Natural Colored Sheep Breeders Association. "Natural Colored Sheep".Rare Breeds Watchlist. January 2007. http://www.rmncsba.org/. Retrieved 2012-06-05.

Roger, B. (1999). Traditional livestock breeds: Geographical distribution and dynamics in relation to ecology of West Africa. Overseas Development Institute, Portland House, Stage Place, London, SW1E 5DP. FAO. Working Paper 122.

Rousset, F. (2008). GENEPOP 4.1.3 for Windows/Linux/Mac OS X. http:// www. cecill. info/ index.en.html

Rowlands, J., Nagda, S., Rege, E., Mhlanga, F., Dzama, K., Gandiya, F., Hamudikwanda, H., Makuza, S., Moyo, S., Matika, O., Nangomasha, E. and Sikosana, J. (2003). The design, execution and analysis of livestock breed surveys - a case study in Zimbabwe. A report to FAO. Nairobi. International Livestock Research Institute.

Rychlik, T., Janik, A., Samborska, M., Parys, A. (1996) Polimorfizm grup krwi, ransferyny i hemoglobiny u owiec ras mięsnych hodowanych w Polsce. Rocz. Nauk. Zoot. 23, 3, 27-41

Ryder, M. L. (1964). The history of sheep breeds in Britain. Agricultural history review 12, 1-12

Ryder, M. L. (1983). Sheep and Man. Duckworth Press, London.

Ryder, M. L. (1984). Sheep In: Mason I.L. (ed) Evolution of Domestic Animals, Longman, London, 63-85

Saitou, N., Nei, M., (1987). The neighbour-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology Evolution 4, 406–425.

Salako A.E. and Ngere, L.O. (2002). Application of Multifactorial discriminant analysis in the morphometric structural differentiation of West African dwarf (WAD) and Yankassa sheep in south west Nigeria. Nig. J. Anim.Prod. 29(2):163-167

Salako, A. E., Ijadunola, T. O. and Agbesola, Y. O. (2007). Hemoglobin polymorphism in Nigerian indigenous small ruminant populations - preliminary investigation. Intentional Journal of Morphology Vol. 6 (22), pp. 2636-2638

Sargent, J., van der Bank F.H. and Kotze, A. (1999) .Genetic variation in blood protein within and between 19 sheep breeds from South Africa. South Africa Journal of Animal Science 29 (3)

Selvam, R., Rahumathulla, P.S., Sivaselvam, S.N., Karthickeyan, S.M.K. and Rajendran, R. (2009). Molecular genetic characterization of Madras Red sheep in Tamil Nadu, India using microsatellite markers. Livestock Research for Rural Development 21 (4) paper 50.

Shahrbabak, H. M., Farahani, A. K., Shahrbabak, M. M., and Yeganeh, H. M. (2010). Genetic variations between indigenous fat-tailed sheep populations. African Journal of Biotechnology. 9 (36), 5993-5996.

Shamsuddin, A. K., Nandakumaran, B. and Mukundan, G. (1986). Genetic studies on haemoglobin, albumin and amylase polymorphism in Malabari goats and its exotic cross breds. Kerala J. vet. Sci. 17: 1-6

Sibley, C.G. & Ahlquist, J.E. (1990). "Phylogeny and classification of birds: A study in molecular evolution" Yale University Press, New Haven and London.

Simianer H. (2002) Molekulargenetische Differenzierung verschiedener Rotviehpopulationen. Schriftenreihe des Bundesministeriums fur Verbraucherschutz, Ernahrung und Landwirtschaft. Heft 493. Landwirtschaftsverlag GmbH. Munster-Hiltrup, Germany.

Simmons, P. and Ekarius, C., (2001). Storey's Guide to Raising Sheep. North Adams, MA: Storey Publishing LLC. ISBN 978-1-58017-262-2.

Slatkin, M., (1985). Rare alleles as indicators of gene flow. Evolution. 39(1): 53-65.

Slavov, R., Slavova, P. and Laleva, S. (2004). Genetic structure of Ile de France sheep breed in Bulgaria according to the Transferrin and Haemoglobin polymorphous systems. Trakia Journal .of Sciences 2 (2) 38-40

Smith M.S., Barbara, A., Aseltine, M., Gerald K. (1997). Beginning Shepherd's Manual, Second Edition. Ames, Iowa: Iowa State University Press. ISBN 0-8138-2799-X

Sodhi M, Mukesh M and Bhatia S (2005). Characterizing Nali and Chokla sheep differentiation with microsatellite markers. Small Ruminant Research 65(3), 185-92.

Souza, C.A., Paiva, S.R., McManus, C.M., Azevedo, H.C., Mariante, A.S. and Grattapaglia, D. (2012). Genetic diversity and assessment of 23 microsatellite markers for parentage testing of Santa Ines hair sheep in Brazil. Genetic and Molecular Research 11 (2): 1217 – 1229. www.Funpecrp.com.br. Retrieved 5/12/2012

Soysal, M. I., Koban, E., Ozkan, E., Altunok, V., Bulut, Z., Nizamlioglu, M. and Togan, I. (2005). Evolutionary relationship among three native, two crossbreed sheep breeds of Turkey: Preliminary results. Rev. Med. Vet., 156: 289 – 293.

Stahlberger-Saitbekova, N.; J. Schlpfer, G. Dolf, and C. Gaillard. (2001). Genetic relationships in Swiss sheep breeds based on microsatellite analysis. Journal of Animal Breeding and Genetics 118: 379-387

Stoneking, M., Hedgecock D, Higuchi, R.G., Vigilante, L. & Erlich, H.A. (1991). Population variation of human mtDNA control region sequences detecte d by enzymatic amplification and sequence-specific oligonucleotide probes. American Journal of Human Genetics 48: 370-382.

Takezaki, N., Nei, M. (1996). Genetic Distances and Reconstruction of Phylogenetic Trees from Microsatellite DNA. Genetics 144 (1): 389-399.

Tanabe, S., Sugo, T. and Matsuda, M. (1991). Synthesis of protein C in human umbilical vein endothelial cells. Journal of Biochemistry 109(6): 924-928.

Tapio, I., Tapio, M., Grislis, Z., Holm, L.E., Jeppsson, S., Kantanen, J., Miceikiene, I., Olsaker, I., Viinalass, H., Eythorsdottir, E. (2005). Unfolding of population structure in Baltic sheep breeds using microsatellite analysis. Heridity 94.448–456.

Tapio, M., Ozerov, M, Tapio, I., Toro, M. A., Marzanov, N., Cinkulov, M., Goncharenko, G., Kiselyova, T., Murawski, M. and Kantanen, J. (2010). Microsatellite-based genetic diversity and population structure of domestic sheep in northern Eurasia. BMC genetics 11: 76.

Tella, M. A., Taiwo, V. O., Agbede, S.A. and Alonge, O.D. (2000). The influence of hemoglobin types on the incidence of Babesiosis and Anaplasmosis in West African Dwarf and Yankassa sheep. Trop. Vet.18: 121-127.

Templin, M.F., Stoll, D., Schrenk, M., Traub, P.C., Vöhringer, C.F. and Joos, T.O (2002). Protein microarray technology. Trends in Biotechnology 20: 160-166.

Terrill, C. E. and L. N. Hazel. (1946). Heritability of neck folds and face covering in range Rambouillet lambs as evaluated by scoring. J. Anim. Sci. 5: 170-179

Thermo Scientific (2006). KingFisher 96 Instrument. ThermoFisher Scientific, Retastie 2, P.O.Box 100 FIN-01621 Vantaa Finland

Tomasco I, Wlasiuk G and Lessa EP (2002) Evaluation of polymorphism in ten microsatellite loci in Uruguayan sheep flocks. Gen. Mol. Biol. 25(1), 37-41.

Tsunoda K., Hong C., Wei S., Hasnath M. A., Nyunt M. M., Rajbhandary H. B., Dorji T., Tumennasan, H. and Sato K. (2006). Phylogenetic relationships among indigenous sheep populations in East Asia based on five informative blood protein and non-protein polymorphisms. Biochemical Genetics, 44, 287-306.

Turton J. (1974). The collection, storage and dissemination of information on breeds of livestock. Proc. Ist World Congress on Genetics. Applied Livestock Production Vol. 2: 61-74.

Udo, R.K. (1970). Geographical regions of Nigeria. London: Heinemann Educational Books.

Vicente, A.A., Carolino, M.I., Sousa, M.C., Ginja, C., Silva, F.S., Martinez, A.M., Vega-Pla, J.L., Carolino, N., Gama, L.T., (2008). Genetic diversity in native and commercial breeds of pigs in Portugal assessed by microsatellites. Journal of Animal Science 86, 2496–2507.

Vignal, A., Milan, D., SanCristobal, M., and Eggen, A. (2002). A review on SNP and other types of molecular markers and their use in animal genetics. Genetic Selection and Evolution 34, 275 – 305.

Wafula P. O, Jianlin H. Sangare H., Sowe J. M., Coly R., Diallo B. and Hanotte O. (2005) Genetic characterization of West African Djallonke sheep using microsatellite markers. The Role of Biotechnol. 5- 7, 177-178.

Wallace, D.C., (1986). Mitochondrial genes and disease. Hosp. Pract., 21: 77- 92

Wang, D. G., Fan, J. B., Siao, C. J., Berno, A., Young, P., Sapolsky, R., Ghandour, G., Perkins, N., Winchester, E., Spencer, J., Kruglyak, L., Stein, L., Hsie, L., Topaloglou, T., Hubbell, E., Robinson, E., Mittmann, M., Morris, M. S., Shen, N., Kilburn, D., Rioux, J., Nusbaum, C., Rozen,S., Hudson, T. J., Lipshutz, R., Chee' M. and Lander E S. (1998). Large-scale identification, mapping, and genotyping of single-nucleotide polymorphisms in the human genome. Science. 280: 1077–1082.

Weaver, S., (2005). Sheep: small-scale sheep keeping for pleasure and profit. 3 Burroughs Irvine, CA 92618: Hobby Farm Press, an imprint of Bow Tie Press, a division of Bow Tie Inc. ISBN 1-931993-49-1.

Weir, B. S. and Cockerham, C. C. (1984). Estimating F-Statistics for the Analysis of Population Structure. Evolution 38 (6):1358-1370.

Weller, J. I. (2001). "Quantitative trait loci analysis in animals" CABI Publishing.

William, P. S. (2005). Sheep Care Guide. American Sheep Industry Association.http://www.sheepusa.org/index.phtml?page=site/get_file&print=1&file_id=9a0f14975be048606228762fc2d2a7ff. Retrieved 2012-06-06

Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A. and Tingey, S. V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res., 18: 6531–6535.

Wilson, A. C., Cann, R. L., Carr, S. M., George, M., Gyllensten, U. B., Helm-Bychowski, K. M., Higuchi, R. J., Palumbi, S. R., Prager, E. M., Sage, R. D. and Stoneking, M., (1985). Mitochondrial DNA and two perspectives on evolutionary genetics. J. Biol., 26: 375–400.

Wilson, R.T. (1991).Small Ruminant Production and the Small Ruminant Genetic Resource in Tropical Africa. FAO Animal Production Health Paper 88, FAO Rome, 231 pp

Wilson, R.T. and Durkin, J.W. (1983a) Livestock production in central Mali: weight at first conception and ages at first and second parturition in traditionally managed goats and sheep. Journal of Agricultural Science, Cambridge 100: 625–628.

Wilson, R.T. and Durkin, J.W. (1983b) Livestock production in central Mali: reproductive components in traditionally managed sheep and goats. Livestock Production Science 19: 523–529.

Wilson, R.T. and Light, D. (1986) Livestock production in central Mali: economic characters and productivity indices for traditionally managed goats and sheep. Journal of Animal Science 62,3: 567–575.

Worley, K., Strobeck, C., Arthur, S., Carey, J., Schwantje, H., Veitch, A., and Coltman, D. W. (2004). Population genetic structure of North American thinhorn sheep (Ovis dalli). Molecular Ecology 13, 2545 – 2556

Wright, S. (1965). The Interpretation of Population Structure by F-Statistics with Special Regard to Systems of Mating. Evolution 19(3):395-420.

Wright, S. (1978). Evolution and the Genetics of Populations, vol. 4. Variability Within and Among Natural Populations. University of Chicago Press, Chicago.

Yeh, F.C. & Yong, R. (1999). POPGENE version 1.31: Microsoft-based Freeware for Population Genetic Analysis. University of Alberta, Edmonton, Canada.

Zanotti, C., Gandini, G.C., Leone, P. (1990). Genetic variation and distances of five Italian native sheep breeds. Animal Genetics 21:87-92

Zanotti, C., Gandini, G.C., Leone,P., Rongnoni,G. (1988). Genetic relationships among four sheep breeds of the Italian Alpine Ark. J. Anim. Breed. Genet. 105: 135-142.

Zeuner, F.E. (1963). A History of Domesticated Animals. Hutchinson and Co. Ltd., London.

Zhang, D.X. and Hewitt, G.M. (2003). Nuclear DNA analyses in genetic studies of populations: practice, problems and prospects. Mol. Ecol., 12, 563–584.

Zuccaro, A., Bordonaro, S., Criscione, A., Guastella, M., Perrotta, G., Blasi, M., D'Urso, G., Marletta, D., (2008). Genetic diversity and admixture analysis of Sanfratellano and three other Italian horse breeds assessed by microsatellite markers. Animal 2, 991–998.